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**CONSEQUENCES OF INTRASPECIFIC GENETIC VARIATION FOR
POPULATION DYNAMICS AND NICHE EXPANSION**

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CONSEQUENCES OF INTRASPECIFIC GENETIC VARIATION FOR POPULATION
DYNAMICS AND NICHE EXPANSION

by

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CONSEQUENCES OF INTRASPECIFIC GENETIC VARIATION FOR POPULATION DYNAMICS AND NICHE EXPANSION

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Intraspecific genetic diversity is an important attribute of natural populations and is deemed critical for their adaptive potential and persistence. However, we have limited empirical understanding of the impact of genetic diversity on population performance under different conditions. For my dissertation, I conducted long-term laboratory experiments with populations of the flour beetle *Tribolium castaneum* to test the consequences of genetic variation for population dynamic stability and niche evolution. In Chapter 1, I show that genetic variation prevented population extinction in a novel habitat. In addition, genetically diverse populations were more stable, both in a novel heterogeneous habitat and in their ancestral habitat. In the ancestral habitat, alleles from a single founding lineage dominated the dynamics, leading to increased stability of genetically diverse populations. However, such a selective effect was not observed in the novel heterogeneous habitat. Therefore, while genetic variation within populations increased their stability and persistence, the magnitude of the impact and its mechanism depended on the selective habitat.

In Chapter 2, I ask whether genetic variation also facilitates resource niche expansion, i.e., use of a novel resource. Using stable carbon isotopes, I analyzed diets of beetles sampled from the above experiment and quantified the rate of change in resource use. Contrary to theoretical predictions, I found that genetic variation for resource use had no effect on the rate of niche evolution. Furthermore, behavioral niche expansion accounted for most of the adaptation to the novel resource, and the behavioral change hindered subsequent evolutionary change in resource use. It is thus apparent that in the short term, behavioral plasticity in niche use may impose far greater constraints on niche evolution than the amount of standing genetic variation.

Mathematical models predict that intraspecific competition generates selection for niche evolution, and that genetic variation increases the response to selection. Therefore, I hypothesized that the impact of genetic variation on resource niche evolution may depend on the degree of intraspecific competition. In the final chapter of this thesis, I describe results of an experiment to test this hypothesis. I found that genetic variation and competition indeed interacted to increase the rate of niche expansion in *T. castaneum*, but that their impacts were temporally variable. Furthermore, the two factors acted on different components of niche evolution: while competition only affected the degree of niche expansion, genetic variation also promoted maintenance of individual variation in resource use.

In summary, my thesis describes experiments to test for the ecological and evolutionary impacts of intraspecific genetic variation; and its interaction with behavioral plasticity, intraspecific competition, and resource availability. Genetic diversity and behavioral plasticity are common features of living organisms, and therefore it is vital to understand their combined consequences for population ecological and evolutionary dynamics. In addition, natural populations often face intense competition for limited resources. Hence the experimental results presented here can help us to better understand how populations overcome these resource constraints, given their specific genetic composition.

Biologists are increasingly aware that the intricate connection between ecological and evolutionary dynamics is important to gain a more complete understanding of population biology. The work described here represents one of the few experiments providing such detailed mechanistic understanding of the interactions between- and consequences of - key ecological and evolutionary parameters. Finally, the results have important implications for conservation biology, because they show that the effects of genetic diversity can vary greatly depending on a number of population and environmental parameters.

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CHAPTER 1

THE STABILIZING EFFECT OF INTRASPECIFIC GENETIC VARIATION ON POPULATION DYNAMICS IN NOVEL AND ANCESTRAL HABITATS

ABSTRACT

Recent studies show that intraspecific genetic variation in asexual species may have large effects on population, community and ecosystem functions, increasing their stability, productivity, and species richness. However, major questions regarding its impact on populations remain empirically unanswered: (a) how does intraspecific genetic diversity affect the ecological characteristics of sexual species, where recombination can alter the outcome of causal mechanisms like selection and niche diversification? (b) Does genetic diversity increase population dynamic stability? (c) Is the impact of genetic diversity dependent on the selective environment? To answer these, I founded replicate flour beetle (*Tribolium castaneum*) populations with different degrees of ecologically relevant, heritable trait variation, and monitored their dynamics for ~8 generations. I show that population stability and persistence increased with greater genetic variation, but this stabilizing effect was independent of habitat (different proportions of ancestral and novel resources). Alleles from a single founding strain underwent a selective sweep in the homogeneous ancestral habitat but not in the novel heterogeneous habitat. These results expand current understanding of the ecological impacts of genetic diversity by showing that genetically more diverse sexual populations persist longer and are more stable, but that the selective environment determines the mechanistic basis of the increased stability.

INTRODUCTION

Biologists have long known that heritable trait variation within populations is critical for evolutionary processes such as adaptation. While the evolutionary impacts of genetic variation are well understood, it is only during the past decade that we have begun to document the pervasive ecological impacts of standing intraspecific genetic variation (reviewed in Hughes *et al.* 2008). For instance, intraspecific genetic diversity enhances population persistence (Newman and Pilson 1997; Vilas *et al.* 2006), colonization success (Gamfeldt *et al.* 2005), growth (Pelletier *et al.* 2007), productivity (Bell 1991) and resistance to invasion (Crutsinger *et al.* 2007); it enhances group performance in social insects (Jones *et al.* 2004; Mattila and Seeley 2007); it allows for coexistence in predator-prey systems (Imura *et al.* 2003; Yoshida *et al.* 2003); and increases growth rates of metapopulations (Hanski and Saccheri 2006). Finally, genetic diversity enhances many important community and ecosystem functions (Booth and Grime 2003; Crutsinger *et al.* 2006; Hajjar *et al.* 2008; Hughes and Stachowicz 2004; Johnson *et al.* 2006; Lankau and Strauss 2007; Reusch *et al.* 2005).

Drawing on community diversity-stability concepts (Loreau 2000; Tilman 1999), two types of mechanisms have been proposed to account for these observed positive effects of genetic diversity (Hughes *et al.* 2008). Additive effects are said to occur when measured population functions can be attributed directly to the properties and proportions of constituent genotypes in monoculture. For example, the mean productivity of high-diversity populations (containing more genotypes) may be greater because they are more likely to contain more productive genotypes (the “sampling effect”; note that this is a statistical effect, independent of similar consequences of natural selection described next). Genetic diversity may also have non-additive effects, where population functions are an emergent property of interactions between constituent genotypes. One example is natural selection, which can drive a particular high-fitness genotype to fixation in a population and thereby increase the mean productivity of high-diversity assemblages. Another non-additive effect can arise from niche complementarity, whereby genotypes either occupy different niches or facilitate growth of other genotypes, increasing the net productivity of genotype mixtures. Empirical evidence (reviewed in Hughes *et al.* 2008) supports all these mechanisms, which are not necessarily mutually exclusive.

To date, studies of the ecological impacts of genetic variation (cited above) have used different clones or unique non-recombining genotypes- often plants- to create different levels of

genetic diversity in experimental populations. These studies thus effectively tested whether the community- and ecosystem-level impacts of intraspecific diversity are analogous to the previously documented positive consequences of species diversity. Their limitation, therefore, is that they fail to consider a critical feature that distinguishes intra- and inter-specific variation: sexual recombination among individuals generates novel allele combinations. Indeed, a recent review of the ecological effects of intraspecific diversity notes that the ecological consequences of genetic variation within sexual species remains an open question (Hughes *et al.* 2008). Mechanisms responsible for impacts of increased genetic diversity in sexual species may thus include selection on novel allele combinations in addition to selection for specific alleles. On the one hand, novel beneficial allele combinations arise from sexual reproduction; on the other hand, sexual recombination breaks up existing advantageous combinations, altering the efficacy and speed of selection. It is thus unclear, *a priori*, whether the ecological consequences of intraspecific diversity would be more or less strong than those observed in asexual species.

Previous studies have also largely neglected the impact of intraspecific genetic variation on population stability, which is an important predictor of population persistence. Recent theoretical models of population dynamics show that population stability can be enhanced by heritable trait variation among individuals (e.g. Bjornstad and Hansen 1994; Doebeli and de Jong 1999; Fox 2005; Jager 2001). Specifically, a one-locus two-allele population genetic model incorporated genotype-specific responses to population density to simulate genotype-specific variation in density dependence. In each generation, the relative fitness of genotypes was used to determine the distribution of the heritable trait (response to population density) (Doebeli and de Jong 1999). The model shows that incorporating such heritable variation in sensitivity to density dependence can simplify population dynamics and increase population stability. This result makes intuitive sense: consider population size fluctuations caused by over- and under-shooting of the carrying capacity due to strong density dependence. If individuals vary in the degree to which high population density affects their fecundity, populations are less likely to strongly over- or under-shoot their carrying capacity, mitigating extreme fluctuations in population size. However, we lack empirical tests of this theoretical prediction that heritable intraspecific variation stabilizes population dynamics.

Expression of heritable variation often varies with the selective environment in both laboratory and natural populations (for reviews, see Charmantier and Garant 2005; Hoffmann and Merila 1999), and the impacts of reduced genetic variance due to inbreeding are also more severe

in stressful habitats (Armbruster and Reed 2005). Hence, the impact of intraspecific genetic variation on population characteristics such as population stability is likely to be habitat-dependent. Furthermore, the likelihood of niche diversification increases with temporal or spatial environmental variation (e.g. Day 2000; Kassen 2002 and references therein; Rainey and Travisano 1998), and therefore the mechanistic basis of observed impacts of genetic diversity may also be habitat-dependent. Previous studies did not explicitly address the environmental context or test for an interaction effect of genetic variation and environment on the measured population or community properties (but see Gamfeldt and Kallstrom 2007, where population size predictability across replicates was a function of both the degree of environmental perturbation and genetic diversity). Thus, we have limited empirical evidence to evaluate whether the selective environment shapes the ecological impacts of genetic diversity.

Here I present the results of an experiment to address the aforementioned gaps in our understanding of the population-level ecological impacts of genetic variation. I manipulated founding genetic variation in replicate laboratory populations composed of freely interbreeding strains of the red flour beetle, *Tribolium castaneum*. Populations were maintained in three different habitat treatments: one containing only the ancestral resource (wheat flour); a second containing adjacent patches of the ancestral and a novel resource (corn flour), and the third containing only the novel resource. Flour beetle populations are regulated by negative density dependence (Sokoloff 1977), and exhibit additive genetic variation and gene-by-habitat interaction for critical traits such as resource use, degree of egg cannibalism, and fecundity and egg-to-adult viability (Via 1991; Via and Conner 1995). I found substantial among-strain phenotypic variation measured for fitness-related traits on corn and wheat flour (Table 1.1; Methods), indicating that genetic variation could have a detectable impact on population growth and dynamics in different habitats. As described below, the results show that genetic diversity increases population stability in all selective environments. However, the mechanistic basis of this stabilizing effect is dependent on the habitat.

METHODS

Beetle stocks and maintenance

The red flour beetle is a global pest that both inhabits and eats various cereals. The Beeman lab (Biological Research Unit, Grain Marketing and Production Research Center, Kansas) has

maintained numerous stock populations of this species primarily on wheat flour under laboratory conditions for over 20 years (~250 generations). I obtained four of these strains, originally collected from geographically different source populations: Col-2, Pak-3, Z-7 and Tiw-5, for use in my experiments. I maintained my stock and experimental populations in laboratory incubators, in conditions similar to the Beeman lab: 95% wheat flour + 5% yeast at 33⁰C ($\pm 1^0$ C) and 70% relative humidity. Organically produced flour was obtained from a single supplier throughout the experiment. I allowed stock populations to adjust to my lab conditions for six months before starting my experiments.

Among-strain trait variation

While geographically and temporally isolated populations accumulate neutral genetic variation, they could potentially retain common ancestral trait values and lack heritable variation for ecologically important traits. To test whether the four experimental strains differed in traits relevant to population growth in different habitats, I quantified three traits for each strain: fecundity and egg-to-adult survival in wheat and in corn flour, and behavioral preference for flour type. To quantify fecundity, I randomly picked sexually mature, mated females from stock populations of each strain to assay them. Females were confined singly in 1 ml plastic vials (to prevent confounding effects of egg cannibalism) in equal amounts of either wheat or corn flour. Fecundity was measured as the number of eggs laid per female per day, averaged over 3 days. To measure strain-specific survivorship, eggs from females of each strain were pooled and individual eggs were isolated in 0.5 ml vials containing either wheat or corn flour. Each vial was checked weekly, adding fresh flour each time, until eggs/larvae were dead or had eclosed successfully into adults. To measure behavioral resource preference, approximately 3-week old larvae were picked randomly from stocks and placed in the center of test Petri dishes (1 1/8th inch diameter), with one larva per Petri dish. Each Petri dish contained adjacent patches of equal amounts of wheat and corn flour. The presence/absence of the larva in each flour patch was noted after 24 hours as an indicator of behavioral resource choice. Visible burrows made by larvae while moving in the flour showed that larvae generally sampled both flour patches before settling in one patch.

Experimental populations

I initiated experimental populations in a staggered manner over three months, with 120 randomly picked adult beetles from stocks (Table 1.2). All stocks had an ~1:1 sex ratio (based on sexing 50

adults per stock); hence all experimental populations likely received equivalent numbers of females. I created four different levels of genetic variation using varying proportions of each of the four strains. Replicate populations with the lowest level genetic variation (single-strain populations) were founded using 120 adults of Col-2, Pak-3, Z-7 or Tiw-5 strains. Populations with the next level of genetic variation (two-strain populations) were founded using 60 adults per strain for each of the six possible two-strain combinations. Populations with the third highest level of variation (three-strain populations) were founded using 40 individuals per strain for each of the four possible three-strain combinations. Populations with the highest level of genetic variation were founded by mixing 30 individuals from each of the four strains. While greater accuracy of measured response variables required multiple replicates for each strain combination, I was constrained by the total number of populations I could maintain and census. I chose to maximize accuracy at the two extreme levels of variation with 5 replicates per strain combination, and 2 replicates for the intermediate levels (Table 1.2). Three different resource/habitat treatments were created: one containing 50g wheat flour, one with 25g each of wheat and corn flour in adjacent patches allowing free movement between resources, and the third with 50g corn flour. Two single-strain populations in the wheat treatment, one in the wheat + corn treatment, and two from the corn treatment were lost during the study due to incubator failure, decreasing the number of replicates of the relevant strains to three or four instead of five.

I censused each population every two weeks for a total of 16 census points, or ~8 overlapping generations. During each census, I counted larvae, pupae and adults in each flour patch within a container and discarded dead beetles. I replaced the used flour with fresh flour and returned larvae and pupae to the same flour type in which they were found (wheat or corn). I froze dead beetles and used flour at -80°C for 24 hours before discarding to prevent accidental spread of the pest species.

DATA ANALYSIS

Population persistence

Counts of extinct populations were analyzed as a function of genetic variation and habitat using a generalized linear model (*Proportion extinct populations = Genetic variation * Habitat*) with binomial errors in R (R Development Core Team 2008). Akaike's Information Criterion (AIC) was used to evaluate model fit and find the minimal model that best described the data. In

addition, I used Fisher's exact tests to test for a significant effect of genetic variation on the proportion of populations that went extinct during the study in each habitat.

Various measures and definitions of population stability have been used in the ecological literature (Gaston and McArdle 1994; Grimm and Wissel 1997). I quantified temporal variability in population size as a measure of stability as the most noninvasive and direct measure of stability in my experimental. Quantifying other stability measures such as resistance to perturbation would have interfered with my aim of analyzing the impact of founding genetic variation. Since all populations in the corn-only treatment either went extinct or declined severely during the experiment, data from this habitat was not analyzed for stability. All stability analyses therefore refer only to the wheat and wheat + corn treatments.

Population size and stability: Monte-Carlo Permutations

Population size and stability data for replicates of each strain combination were averaged (excluding extinct populations) to obtain a single value for each of the 15 strain combinations that comprised the four levels of genetic variation (Table 1.1). Parametric analysis of this study may be unreliable for two reasons: first, the experimental design was unbalanced due to the combinatorics inherent in manipulating genetic variation (4, 6, 4, and 1 data points for each level of genetic variation; Table 1.2), and second, the distribution of error variances was not normal. I therefore conducted Monte Carlo permutations of the data to test for significant effects of habitat, genetic variation, and their interaction. To test for an effect of genetic variation alone, I randomly shuffled values of population CV within each habitat, breaking up any association between strain combination and CV. I used the shuffled dataset to calculate estimates of regression slopes for the dependence of CV on genetic variation. 50,000 permutations were run to obtain a distribution of simulated regression slopes. A p value for the observed regression slope (obtained by fitting a linear model to the original dataset) was calculated as the probability of finding a simulated slope at least as extreme as that observed. Similarly, to test for a significant effect of habitat, I grouped data for each strain combination and randomly assigned habitat, breaking up the association between habitat and CV for each strain combination. I then calculated the overall difference in mean CV in both habitats and used the distribution of differences from 50,000 permutations to test whether the observed difference between habitats was significantly greater than zero. To test for an interaction between genetic variation and habitat, I shuffled values of CV across the entire dataset and calculated the difference in the regression slope of the dependence of CV on genetic

variation for both habitats. Similar permutations were carried out with mean population size as the response variable. All permutations were done in R (R Development Core Team 2008).

Elucidating mechanism: testing for a selective sweep

For each habitat, I used two separate tests to determine whether selective sweep of allele(s) from a single founding strain was responsible for increased population stability and size. First, for each strain in turn, I categorized all experimental populations based on the fraction of founding adults belonging to that strain (Table 1.2, column 3). Excluding populations with no individuals of the focal strain, each population was thus composed of 25%, 33%, 50%, or 100% individuals of each strain. I used a linear model to test for a significant regression between CV/population size and fraction of individuals of the focal strain. A significant regression with a positive slope would indicate a selective sweep of alleles of the focal strain. Second, I calculated pair wise Tukey HSD contrasts for population CV and size between all single-strain and high-diversity populations in wheat and wheat + corn habitats. The number of replicates in each case was similar (five initially, or three after extinction) and there was no significant heteroscedasticity. A selective sweep of alleles from a founding strain would be indicated if the size and stability of one of the single-strain populations were higher than other single-strain populations and equivalent to that of the high-diversity populations.

RESULTS

Among-strain trait variation

Results from assays of three different traits show significant among-strain variation for fecundity and survival in wheat and corn flour, as well as behavioral preference for corn flour (Table 1.1). Strains with greater fitness in wheat flour (Pak-3 and Tiw-5) performed relatively poorly in corn flour, while about 50% of the larvae of strains Col-2 preferred corn over wheat flour and 85% Col-2 eggs survived to adulthood in corn. On the other hand, Col-2 females had very low fecundity in corn. These results highlight two important points. First, the experimental strains varied in fitness components (survival and fecundity, both of which are highly heritable traits in *T. castaneum*) and behavioral preferences in both resources (with unknown heritability), all of which can affect population dynamics. Second, no single strain had the highest fitness in both

resources, and hence, measured trait variation could have a significant impact on population processes in different habitats.

Population persistence

Almost all populations that went extinct during the study were single-strain populations with very low founding genetic variation (Figure 1.1; in wheat: six out of a total of seven extinct populations were single-strain; in wheat + corn: four of four; in corn: 16 of 20). On the other hand, none of the highest-diversity (four-strain) populations went extinct in any habitat (five replicate populations per habitat). While genetic variation generally tended to increase population persistence in all three habitats, the interaction term between habitat and genetic variation for the proportion of extinct populations was not significant (Figure 1.1; GLM with binomial error, model AIC = 26.11; effect of genetic variation: $p < 0.001$; effect of habitat: $p = 2.9 \times 10^{-6}$). Habitat-specific analyses show that the positive effect of genetic variation was statistically significant only in the corn treatment (Fisher's exact test; $p = 5.2 \times 10^{-6}$), with no significant association between genetic variation and proportion of extinct populations in wheat ($p = 0.1$) and wheat + corn habitats ($p = 0.19$). These results indicate that genetic variation generally increased population persistence, but that the effect size was greatest in the novel selective habitat.

Population stability

Excluding extinct populations, genetically more diverse populations were more stable in both wheat and wheat + corn habitats (Figure 1.1). Note that CV is inversely related to stability; thus a higher CV indicates greater size fluctuations, and hence, lower stability. Permutation tests show that genetic variation significantly increased population stability (Table 1.3). Habitat also had a significant effect on population stability, with populations in the novel (wheat + corn) habitat being less stable.

In the wheat habitat, high-diversity populations did not have maximum stability: single-strain populations of Pak-3 as well as some intermediate-diversity populations were more stable, although high-diversity populations were *on average* most stable (Figure 1.1). On the other hand, in the wheat + corn habitat, high-diversity populations founded with all four strains had the highest stability (Figure 1.1), which was also equivalent to their stability in wheat. Thus, while low-diversity populations responded negatively to the novel habitat, high-diversity populations showed a *lack* of response, leading to a perceptible difference in the slopes of regression lines in

the two habitats (Figure 1.1). However, this difference is not statistically significant (interaction term, Table 1.3). Hence, contrary to expectation, the effect of genetic variation on population stability was independent of habitat, although both genetic diversity and habitat independently affected population stability.

Population size

As expected, population size in the wheat-only habitat was greater than that in the novel wheat + corn habitat which contained only half the amount of the ancestral wheat resource (Figure 1.2B). Mean population size also increased with increasing genetic variation, but there was no significant interaction effect of genetic variation and habitat on population size (Table 1.3; Figure 1.2B). Thus, genetic variation and habitat independently affected mean population size during the study, reflecting the patterns observed for population stability.

Elucidating mechanism: testing for selective sweeps

In both wheat and wheat + corn habitats, high-diversity (four-strain) populations had higher stability and size than the mean values for single-strain populations (horizontal dashed lines in Figure 1.3), indicating that the effects of genetic diversity were not additive in either habitat. Potential non-additive mechanisms responsible for the observed increase in population size and stability of more diverse populations include (a) selective sweep of alleles from a single high-fitness founding strain (b) selective sweep of a recombinant genotype with novel allele combinations (c) individual niche complementarity without a selective sweep (d) a combination of one or more of these mechanisms.

The first case leads to a number of predictions that can be used to test whether allele(s) from a single founding strain underwent a selective sweep in high-diversity populations leading to increased size and stability. First, the size and stability of high-diversity populations would match those of single-strain populations carrying the selected allele. In the ancestral wheat habitat, single-strain populations had lower stability and size compared to four-strain populations, with the exception of Pak-3 populations whose size and stability exceeded that of four-strain populations (Figures 1.3A & 1.3C; Table 1.4). This enhanced performance in monoculture, while not statistically significant (Table 1.4), suggests that Pak-3 alleles could have undergone a selective sweep in high-diversity populations due to their high fecundity and survivorship in wheat (Table 1.1). Second, we would expect that population stability and size would be positively

correlated to the initial frequency of the selected allele(s). This prediction is largely borne out when population size and stability are regressed against the fraction of Pak-3 individuals (Figure 1.4A): population size increases significantly with the fraction of the founding population composed of Pak-3 individuals (linear regression: $R^2 = 0.22$; $t = 2.4$, $p = 0.026$), and population CV appears to decrease with the fraction of Pak-3 individuals, although this decrease is not significant ($R^2 = 0.14$; $t = -1.8$; $p = 0.087$). A similar analysis for the other three strains shows no significant correlation between the fraction of the founding population composed of the focal strain and population size or stability ($p > 0.1$ in all cases). These results show that Pak-3 alleles were selectively favored in wheat flour populations, increasing their size and stability.

In the wheat + corn habitat, all single-strain populations performed better in combination with other strains than by themselves (Figures 1.3B & 1.3D; Table 1.4), including Pak-3 populations. The observation that Pak-3 monocultures had a substantially poorer performance than four-strain populations in wheat + corn, in conjunction with their relatively poor individual survivorship in corn flour (Table 1.1), renders it unlikely that a selective sweep of Pak-3 alleles could lead to the observed better performance of four-strain populations. Furthermore, regressing population size and stability against the fraction of Pak-3 individuals shows that while population size did not change as a function of fraction of Pak-3 founders (linear regression: $R^2 = 0.006$; $t = -0.34$, $p = 0.74$), population CV significantly increased (i.e. population stability decreased) with the fraction of Pak-3 individuals ($R^2 = 0.4$; $t = 3.68$, $p = 0.001$) (Figure 1.4). Therefore, increasing the number of initial Pak-3 individuals had no effect on the mean population size, but it decreased population stability in wheat + corn, indicating that a selective sweep of Pak-3 alleles could not have led to the increased size and stability of high-diversity populations in this habitat. In light of known trait variation in corn use (Table 1.1), plausible alternative mechanisms include selection of a high-fitness, novel recombinant genotype, or persistent among-individual niche differences without dominance by a single genotype (mechanisms b and c above).

DISCUSSION

This experiment aimed to quantify the impact of intraspecific genetic variation in a sexual species on population stability in different selective environments. Results show that the stability of single-species populations increases as a function of founding intraspecific genetic variation, both in novel and ancestral habitats. Contrary to expectation, the results indicate the lack of a

statistically significant interaction effect of genetic diversity and habitat on population stability. Analyzing the response of populations with various strain combinations in detail, however, shows that the mechanism(s) responsible for the stabilizing effect of genetic variation was different in the two habitats.

Distinguishing additive and non-additive impacts of genetic variation

If genetic diversity had an additive effect on population function, we would expect the size and stability of high-diversity populations to equal the weighted mean of all single-strain populations (since high-diversity populations were composed of all four strains in equal proportion, this is simply their arithmetic mean). However, high-diversity populations had far greater size and stability than the single-strain overall mean (Figure 1.3), indicating that the effects of genetic diversity were non-additive. Either the selective fixation of some alleles, coexistence of different alleles as novel recombinant genotypes in high-diversity populations, or among-individual variation in niche use could be responsible for the observed positive effects of genetic diversity. Below I discuss data from both habitats (wheat-only and wheat + corn) separately, to infer the causal mechanism in each case.

Non-additive effects in the ancestral habitat: selective sweeps

A selective sweep of beneficial alleles could be enhanced if such alleles imparted a large initial fitness advantage in high-diversity populations. Mating in *T. castaneum* occurs rapidly and multiply after sexual maturity (Pai and Yan 2003); hence most adults used to initiate the experimental populations had probably already mated within their parent stock population and eggs in the experimental populations would largely carry strain-specific allele combinations. Therefore, the next generation could have a higher representation of more fecund strains, allowing their alleles to rapidly increase in frequency. Pak-3 individuals had a significantly greater fecundity and survivorship in wheat (Table 1.1), and their single-strain population properties were equivalent to high-diversity populations in the wheat habitat (Figure 1.3A & 1.3C; Table 1.4). Post-hoc regression analysis shows that populations founded with a larger fraction of Pak-3 individuals were larger and more stable (Figure 1.4). Hence, it is likely that Pak-3 individuals largely dominated the dynamics of high-diversity populations in the wheat habitat, through a selective sweep of their alleles. Note that heritable variation in *any* trait that confers an initial, large relative fitness advantage at the beginning of the experiment could determine the

likelihood of a selective effect as well as the identity of traits under selection. For instance, if the experimental populations were initiated with juvenile stages instead of already mated adults, it is possible that competitive ability, relative survivorship, or degree of cannibalism would also determine whether and which alleles were selected. Hence, these results can probably be generalized for different organisms, experimental conditions, and for varying life histories.

Non-additive effects in the heterogeneous habitat and the role of sexual recombination

In the wheat + corn habitat, a selective sweep of alleles from a single founding strain is unlikely to have caused greater stability of more diverse populations for a number of reasons. First, low-fecundity founding strains that could be out-competed in wheat had a higher survival in (and behavioral acceptance of) corn flour (Table 1.1), which may be thought of as a refuge allowing individuals not carrying Pak-3 alleles to persist. Only half the available resource in this habitat offered a relative fitness advantage for Pak-3 alleles, while other strains had greater fitness than Pak-3 in corn flour; making it unlikely that Pak-3 alleles could sweep to fixation in this heterogeneous habitat. This conclusion is also supported by the observed negative correlation between population stability and fraction of founding individuals from Pak-3 stocks (Figure 1.4).

Thus, more diverse populations in the wheat + corn habitat had greater stability either due to individual niche complementarity (resource specialization) or due to a selective sweep of a recombinant, high-fitness genotype. An *a priori* hypothesis may predict that the former mechanism is likely to operate in a heterogeneous habitat such as the wheat + corn treatment, where broad resource niche diversification is possible and different alleles can coexist as a result of such diversification. Genetic variation for resource use (such as that demonstrated for *T. castaneum*) can lead to greater niche complementarity in more diverse populations, and such increased exploitation of novel niches can in turn lead to higher population size and stability. For sexual species, niche complementarity can be measured as the degree of individual variation in niche use (Bolnick et al. 2003). A positive correlation between niche complementarity and population size and stability would indicate that niche complementarity was responsible for increased stability of more diverse populations. However, stable carbon isotope analysis of beetles sampled periodically from experimental populations shows the lack of such a positive correlation; furthermore, these data indicate very little individual resource niche complementarity (see Chapter 2). Therefore, individual niche complementarity could not have imparted greater stability in the wheat + corn habitat.

A third non-additive mechanism that could explain greater stability of more diverse populations is a selective sweep by a recombinant genotype, which would be more likely in high-diversity populations with greater standing genetic variation. As mentioned above, stable isotope data indicate that individuals in all populations utilized equivalent amounts of corn flour in addition to wheat flour. Therefore, populations that maintained greater size and stability could only do so if they were more efficient in their use of one or both resources. A generalist genotype able to exploit both resources more efficiently than ancestral genotypes would have greater fitness and could increase in frequency in the population. To test this hypothesis, one would have to determine whether efficiency of wheat and corn flour utilization (quantified as, e.g., the number of viable offspring produced per g flour consumed by an individual) was positively correlated with population stability or size. I lack such data for these experimental populations, and thus the mechanism leading to increased population stability as a function of genetic diversity in the wheat + corn habitat remains to be explicitly tested. Based on the evidence presented above, however, I can reject the two commonly invoked causal mechanisms: selective sweeps of alleles from founding strains and niche complementarity.

Note that in the absence of assortative mating, sexual recombination would generate novel competing genotypes in each generation, in addition to breaking apart favorable allele combinations. Therefore, it is also possible that multiple recombinant genotypes led to the increased size and stability of more diverse populations; whether or how long such genotypes coexisted or if they outcompeted each other in rapid succession remains to be tested. In contrast, in asexual populations novel genotypes can only be generated via mutation, and the co-existence of multiple beneficial mutations is relatively rare. Compared to standing genetic variation, mutation also generally leads to lower rates of adaptation (see Barrett and Schluter 2008 for a review). Thus, in the heterogeneous habitat, sexual recombination appears to have played a major role in determining the mechanism through which intraspecific founding genetic variation affected population parameters.

Ideally a selective effect should be tested using the relative abundance of functionally important strain-specific alleles in the experimental populations; this was difficult due to the paucity of knowledge of specific loci associated with life-history traits in *Tribolium*. While many studies use variation in neutral molecular markers as an indicator of quantitative trait variation, the lack of concordance between the two is well established (see McKay and Latta 2002 for a review; for a meta-analysis, see Reed and Frankham 2001); also, sexual recombination can

rapidly dissociate correlations between neutral markers and positively selected alleles. Therefore, using molecular markers would not be informative in this case. Consequently, the stabilizing effect of genetic variation demonstrated here refers specifically to founding genetic variation. Finally, it is important to note that the three potential mechanisms discussed above need not be mutually exclusive. For example, selection for high fecundity on wheat could initially increase the frequency of Pak-3 alleles in wheat + corn populations, since egg-adult development time is longer and survivorship is lower in corn flour (Table 1.1). As the population size increased and wheat became a limiting resource, niche variation could maintain different alleles in the population. In this experiment, I lacked the ability to explicitly test for such combined or sequential effects. This issue may be resolved with long-term measurement of temporal changes in the relative abundance of functional alleles, coupled with measures of temporal change in individual variation in niche use efficiency. Such studies are possible in the future when cost-effective molecular resources are developed for more species, including knowledge of specific loci tightly linked to functional fitness traits under selection.

Genetic variation and population extinction

The positive impact of genetic diversity on population stability is also reflected in its effect on population extinction. In the habitat consisting solely of the novel corn resource, population persistence was significantly higher for populations with greater intraspecific variation. In habitats offering at least some amount of the ancestral resource, this positive effect of genetic diversity was not statistically significant, although it tended to the same direction (Figure 1.1). A combined analysis of all extinction data shows the lack of a significant genetic variation by habitat interaction, indicating that the power to detect the impact of genetic diversity on extinction is greatly enhanced in novel selective environments. This pattern suggests that future studies of ecological impacts of genetic diversity should explicitly test for environmental effects on measured response variables. As discussed above, the inclusion of environment as an explanatory variable in such experiments is also necessary to differentiate and determine the mechanistic basis for the observed impacts of genetic diversity.

Conclusions

Recent literature has emphasized the intimate connection between ecological and evolutionary dynamics via feedbacks, and the pressing need for studies that examine the mechanistic basis of

such concurrent dynamics (Carroll et al. 2007; Fussmann et al. 2007; Hairston et al. 2005; Ives and Carpenter 2007). As a step in this direction, I show here that an “evolutionary” parameter (genetic variation) can greatly alter critical “ecological” characteristics (population stability and persistence), and that the mechanisms causing this effect are habitat-dependent. The latter observation also suggests that heritable variation, which is important for subsequent evolution, may in turn be maintained in the long term due to greater population stability in novel heterogeneous habitats. Further experiments are required to specifically test this hypothesis and establish the mechanism(s) responsible for the outcome.

While this study shows that the dynamics and persistence of a density-regulated pest species are positively affected by intraspecific genetic variation, studies on other organisms under different selective environments are needed to evaluate the impacts of genetic diversity in a broader context. Because the speed and outcome of evolutionary change may differ greatly amongst sexual and asexual species, we specifically need long-term studies on the community and ecosystem effects of intraspecific diversity in sexual species. Such studies would also benefit from explicitly considering environmental impacts on the measured response variables. Although there are understandable limits to the experimental design of such studies, it is also important to include all possible genotype or strain combinations for a mechanistic understanding of the observed impacts of genetic diversity. Changes in intraspecific genetic diversity are common in natural populations and are associated with various events such as colonization, range expansion and extinction of species. Hence, measuring the impact of changes in intraspecific genetic variation for population dynamics has far reaching implications for our understanding of the generation and maintenance of biodiversity.

CHAPTER 2

THE RELATIVE ROLES OF BEHAVIORAL AND EVOLUTIONARY CHANGE DURING RESOURCE NICHE EXPANSION

ABSTRACT

Adaptive niche expansion to include novel resources in the diet can arise via behavioral plasticity or evolutionary change. Theory suggests that the rate of evolutionary adaptation increases as a function of additive genetic variation in fitness, but can be inhibited by phenotypic plasticity. However, there have been no empirical tests of the impact of genetic variation on niche evolution and the relative roles of behavioral versus evolutionary change in niche use. I initiated flour beetle (*Tribolium castaneum*) populations with varying degrees of founding genetic variation in a heterogeneous habitat containing patches of an ancestral (wheat) and a novel (corn) resource. Stable carbon isotope analysis of sampled beetles shows that significant resource niche expansion occurred during ~6 generations, with the novel resource comprising ~ 40% of the diet at the end of the experiment. Most (87%) of this dietary change was explained by rapid behavioral niche expansion within the first generation. The magnitude of behavioral niche expansion was negatively correlated with the magnitude of subsequent evolutionary niche expansion and with mean population size. Therefore, behavioral niche expansion led to rapid utilization of the novel resource but decreased long-term population performance. Contrary to expectation, genetic variation did not affect the rate of behavioral or evolutionary niche expansion in these populations. On the other hand, under strong directional selection in a habitat containing only the novel corn resource, genetic variation significantly increased population persistence and adaptation. These results show that the impact of genetic variation on adaptation may be constrained by behavioral plasticity and by the strength of selection acting on resource use.

INTRODUCTION

Niche evolution is a central concept for our understanding of how biological diversity is generated and maintained. An organism's niche greatly determines its geographic range and distribution (Grinnell 1917; Pearman et al. 2008), and changes in niche use may lead to subsequent diversification and speciation (Pfenninger et al. 2007; Rundle and Nosil 2005; Schluter 2000). A number of experimental studies have identified ecological conditions that promote niche diversification, reflecting both ecological opportunity and selective pressure for niche change. These include biotic interactions such as interspecific competition (Pfenning et al. 2007; Schluter 1994; Tyerman et al. 2008), intraspecific competition (Bolnick 2001; Svanback and Bolnick 2007), and predation (Eklov and Svanback 2006; Meyer and Kassen 2007; Nosil and Crespi 2006; Rundle et al. 2003); as well as abiotic factors such as resource supply rates (Hall and Colegrave 2007; Kassen et al. 2004) and spatial or temporal heterogeneity in available niches (Barrett et al. 2005; Kassen et al. 2004; Rainey and Travisano 1998). These ecological factors determine the strength of selection favoring or constraining niche evolution, and thus govern whether and under what conditions niche shifts are adaptive.

The rate of adaptive niche shifts depends both on the magnitude of selection imposed by ecological factors, and the population's ability to respond to such selection. Theory posits that response to selection increases with the amount of additive genetic variation for fitness in the population (Fisher 1930). Therefore, if a niche shift is adaptive, we expect that populations with greater heritable variation for resource use should exhibit faster adaptation when exposed to a novel resource or habitat. While the positive impact of genetic variation on the rate of adaptation is supported by a few experimental tests (e.g. Ayala 1968; Crenshaw 1965; Pujol and Pannell 2008), the impact of genetic variation on niche evolution per se has not been explicitly tested. Genetic diversity correlates positively with greater niche breadth in many species (reviewed in Steiner 1977) (also see Hallett 1980), lending support to the hypothesis that genetic diversity is associated with wider niches. However, correlations alone do not establish a cause-effect relationship. For instance, niche heterogeneity may itself facilitate the maintenance of genetic variation (see Hedrick 1986; Hedrick 2006 for reviews), and the presence of competitors or predators can alter ecological opportunity for niche evolution; hence, genetic variation may be only indirectly related to niche breadth. Furthermore, the selective environment can affect expression of heritable variation (reviewed in Charmantier and Garant 2005; Hoffmann and

Merila 1999), so the impact of genetic variation on niche evolution may depend on the environment. In addition, the mechanism of population niche expansion also plays a role in determining the degree of genetic polymorphism, so that correlations between genetic diversity and niche diversity may be a byproduct of the mechanism of niche diversification. When niche expansion involves increased among-individual niche variation rather than increased homogeneity in individual resource use, more genetic variation in resource use can be maintained (Bolnick et al. 2003; Roughgarden 1972). Thus, it remains unclear whether and under what conditions intraspecific genetic variation can facilitate niche evolution. Here, I address this question by directly manipulating intraspecific genetic variation in different habitats, and quantifying the rate of niche evolution and individual specialization.

Apart from genetic variation, theory shows that behavioral plasticity can also alter the rate of evolutionary response to selection, although the magnitude and direction of the effect are debated (Abrams 2006; Ancel 2000; Baldwin 1896; Behera and Nanjundiah 2004; Hinton and Nowlan 1987; Paenke et al. 2007; Pigliucci and Murren 2003; Price et al. 2003; Whitlock 1996). Behavioral responses to novel environments are seen commonly across taxa and often precede evolutionary change (Ghalambor et al. 2007). Plasticity has been proposed to be beneficial in novel environments for multiple reasons, which may be grouped into two classes (Ghalambor et al. 2007). First, plasticity can allow populations to invade and persist in new habitats. During this period, phenotypic plasticity can shield genotypes from selection, and therefore aid in the maintenance of genetic variation upon which subsequent selection can act (Pigliucci 2001; West-Eberhard 2003). Second, plastic responses may be fixed in populations via genetic assimilation, if selection increasingly favors heritable rather than plastic responses to the environment (Pigliucci and Murren 2003; Waddington 1952; Waddington 1953; Waddington 1959). However, classical quantitative genetic models indicate that plasticity slows the evolutionary response to selection, because lower heritability implies that additive genetic means change more slowly. Hence, it has been suggested that plasticity can promote evolution only under very restrictive conditions: depending, e.g., on the shape of the adaptive landscape, the cost to plasticity, and whether there are persistent fitness tradeoffs in the different environments (Ancel 2000; Ghalambor et al. 2007; Paenke et al. 2007). While a number of comparative studies indicate that phenotypic plasticity can affect adaptive divergence between populations (reviewed in Ghalambor et al. 2007; Robinson and Dukas 1999) the theoretical prediction that plasticity can alter the rate of evolution has only rarely been experimentally tested (see Mery and Kawecki 2004). Studies show that

phenotypic plasticity can be responsible for most of the total resource polymorphism observed within natural populations (Robinson and Wilson 1996; Svanback and Eklov 2006) (but see Proulx and Magnan 2004), suggesting that rapid niche shifts in response to novel habitats can be facilitated by plasticity in resource use (also see Ghalambor et al. 2007). To directly test the importance of plastic responses for the rate and dynamics of niche evolution, however, it is necessary to conduct long-term experiments where the roles of evolutionary and behavioral niche expansion may be contrasted.

Here I describe such an experiment using populations of the red flour beetle *Tribolium castaneum*, a global pest of grain stocks reared on wheat flour in the laboratory. I manipulated founding genetic variation of replicate populations and maintained them for ~6 generations on habitats containing varying proportions (0, 0.5, or 1) of a novel corn resource. Previous studies show that *T. castaneum* has heritable variation and gene x environment interactions for fitness on different flours (Via 1991; Via et al. 1995). *T. castaneum* populations are regulated via negative density dependence (Sokoloff 1977), and experience high resource competition that should lead to selection for niche expansion in the presence of the novel resource (Bolnick 2001). By directly assessing diets of beetles sampled periodically from each population (using stable carbon isotope analysis), I tested whether genetic variation in resource use increased the rate of resource niche expansion. Furthermore, I quantified the relative roles of behavioral and evolutionary niche expansion during adaptation to the experimental habitats, and tested whether behavioral niche expansion affected the magnitude of evolutionary niche expansion.

METHODS

Data presented here were obtained from the same experimental populations described in Chapter 1. To facilitate understanding of the results described here, relevant methods (stocks, maintenance and experimental populations) from Chapter 1 are reiterated below, along with methods specific to the analysis presented here.

Beetle stocks and maintenance

The Beeman lab (Biological Research Unit, Grain Marketing and Production Research Center, Kansas) has maintained numerous stock populations of *T. castaneum* on wheat flour under laboratory conditions for over 20 years (~250 generations). I obtained four of these strains,

originally collected from geographically different source populations: Col-2, Pak-3, Z-7 and Tiw-5, for use in my experiments. I maintained my own stock populations in laboratory incubators, in conditions similar to the Beeman lab stock conditions: 95% wheat flour + 5% yeast at 33°C ($\pm 1^{\circ}\text{C}$) and 70% relative humidity. Experimental populations were also maintained under identical conditions. Organically produced flour was obtained from a single supplier throughout the experiment. I maintained my stock populations for six months before starting my experiments, to allow them to fully adjust to local lab conditions.

Experimental populations

I initiated experimental populations in a staggered manner over three months; each population starting with 120 randomly picked adult beetles from stocks (Table 1.2). All stocks had an ~1:1 sex ratio (based on sexing 50 adults per stock), so all experimental populations likely received approximately the same number of females. I created four different levels of genetic variation using varying proportions of each of the four strains. Replicate populations with the lowest level genetic variation (single-strain populations) were founded using 120 adults of a single strain (Col-2, Pak-3, Z-7 or Tiw-5). Populations with the next level of genetic variation (two-strain populations) were founded using 60 adults per strain for each of two strains. I established populations of all six possible two-strain combinations. Populations with the third highest level of variation (three-strain populations) were founded using 40 individuals from each of three strains, for each of the possible three-strain combinations. Populations with the highest level of genetic variation were founded by mixing 30 individuals of each of the four strains. I chose to maximize accuracy at the two extreme levels of genetic variation using five replicates per strain combination, and two replicates for the intermediate levels (Table 1.2). The experimental populations shown in Table 1.2 was replicated in three different resource/habitat treatments: one containing 50g wheat flour, one with 25g each of wheat and corn flour in adjacent patches (allowing free movement between patches), and the third with 50g corn flour. Two single-strain populations in the wheat treatment, one in the wheat + corn treatment, and two from the corn treatment were lost during the study due to incubator failure, decreasing the number of replicates of the relevant strains to 3 or 4 instead of five.

I censused each population every two weeks for a total of 16 census points, or ~8 overlapping generations. During each census, I counted larvae, pupae and adults in each flour patch within a container and removed dead beetles. I replaced the used flour with fresh flour and

returned larvae and pupae to the same flour type in which they were found (wheat or corn). I froze dead beetles and used flour at -80°C for 24 hours before discarding to prevent accidental spread of the pest species.

Stable carbon isotope analysis

The two naturally occurring stable isotopes of carbon (^{13}C and ^{12}C) are differentially assimilated by C3 and C4 plants, and result in distinct $^{13}\text{C}:^{12}\text{C}$ ratios specific to the two plant groups. These ratios are also reflected in the isotope ratios of herbivores that consume the plants. Thus it is possible to detect diet differences between individuals by comparing their carbon isotope ratios (Tieszen 1978), measured as the difference between the $^{13}\text{C}:^{12}\text{C}$ ratio of a tissue sample and that of a laboratory standard, denoted $\delta^{13}\text{C}$:

$$\delta^{13}\text{C} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] * 1000$$

The wheat + yeast resource used in this experiment had a $\delta^{13}\text{C}$ value of -23.82, while corn flour (without yeast) had a $\delta^{13}\text{C}$ value of -11.96. Individual beetles that consumed the novel resource (corn flour) in addition to the wheat flour should thus have a ^{13}C isotope ratio intermediate to control individuals raised on only wheat or only corn. Thus, the carbon isotope ratio of individuals indicates their relative use of corn and wheat flour. The mean isotope ratio of beetles in a population indicates average resource use within the population, and the variance in isotope ratios indicates the amount of among-individual variation in resource use (Bolnick et al. 2003; Matthews and Mazumder 2004). Therefore, temporal change in the mean isotope signature of a population indicates a change in the resource niche, and temporal change in the variance of isotope ratios indicates a change in the degree of niche variation among individuals.

During censuses 1, 6, and 12 (corresponding to weeks 2, 12, and 24 of the experiment), I randomly picked ten adult beetles from each population in the wheat + corn treatment (after mixing adults from both corn and wheat patches) and stored them at -80°C. All single-strain Z-7 populations had less than 50 adults at weeks 12 and 24, and hence beetles were not removed from these populations for isotope analysis at those times. At the end of the experiment, frozen beetles from two replicate populations per strain combination (three for populations with the highest diversity) were dried in an oven at 60°C for 48 hours, weighed individually, packed in tin capsules, and shipped to the UC Davis Stable Isotope Facility. The facility analyzes ^{13}C isotopes using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

Calculating the proportion of corn flour in individual diets

To estimate the proportion of corn in the diets of individual beetles, one would need to quantify the functional relationship between the proportion of corn consumed by individuals and their resulting isotope ratio, using beetles known to have consumed different proportions of corn flour. However, beetle flour consumption can be accurately predicted only for the two extreme corn flour proportions (0 and 1). I measured isotope ratios of beetles reared either on corn ($n = 13$) or wheat flour ($n = 155$), and used the least-squares regression equation for these data ($\text{Proportion corn} = 1.911 + 0.08 \times \delta^{13}\text{C}$) to calculate the proportion of corn consumed by each beetle sampled from experimental populations in the wheat + corn habitat (right y-axis, Figure 2.2). I also measured carbon isotope ratios of flour samples with varying, known proportions of corn and wheat flour (6 samples, corn proportion ranging from 0 to 1), fitting a least squares regression line to the data ($\text{proportion corn} = 1.992 + 0.084 \times \delta^{13}\text{C}$; $R^2 = 0.99$). The two lines are parallel, confirming the linear relationship between measured isotope ratios of individual beetles and the relative proportion of corn in beetle diets.

Assays of relative female fecundity in corn

Fecundity assays were carried out at week two for each population in the wheat and wheat + corn habitats, and two years after the start of the experiment for corn-only populations (these suffered large mortality during the experiment and therefore females were not removed from them for assays at intermediate time points). I randomly chose six females from each population and isolated them in vials containing either wheat or corn flour. After being allowed to oviposit for a day, I moved them to another vial containing the alternative resource and counted the number of eggs laid in each vial. For each female, I repeated the process to get two counts of fecundity in each flour type. I calculated the proportion of total eggs laid in corn per female. Females were not returned to their source population after the assay.

DATA ANALYSIS

Quantifying population performance in the novel corn-only habitat

Populations in the novel corn-only habitat either declined quickly and went extinct, declined slowly and went extinct, or declined for a period but subsequently increased in number and thus avoided extinction (Figure 2.1). Therefore, population performance could be quantified in

multiple ways; for instance, by estimating the rate of initial population growth (or decline). All populations in the corn-only habitat declined during the first 10-15 weeks of the experiment (except one replicate of each of the strains Z-7 and Pak-3; Figure 2.1), although reproduction added new adults to most populations during this period (population census data, not shown). Therefore, I estimated $t_{1/2}$, the time taken for replicate populations of each strain combination to reach half the initial founding population size of 120 adults. I used a generalized additive model (gam) in R (R Development Core Team 2008) to fit a non-parametric smoothing curve describing the change in population size over time (Figure 2.1), and used the “predict” function to estimate $t_{1/2}$. Because $t_{1/2}$ measures the net effect of birth and death events, it is an estimate of population performance during the decline phase immediately following exposure to the novel corn flour. I then conducted an ANOVA to test for a significant effect of genetic variation on $t_{1/2}$.

A second, longer-term measure of population performance is the probability of population extinction: adaptation to the novel corn resource would allow populations to shift from negative to positive growth and thus avoid extinction. Due to the low number of replicates for strain combinations with intermediate levels of genetic variation (five, two, two, and five replicates, in increasing order of genetic variation), I could not get reliable estimates of extinction probability for each strain combination. Therefore, I calculated the extinction probability for each level of genetic variation and tested for a significant effect of genetic variation on the proportion of populations that went extinct, using Fisher’s exact test.

A third measure of population performance is the rate of population growth and recovery following initial decline in population size in the novel habitat. Populations showing greater adaptation to the novel corn resource would be expected to show a greater increase in population size following the initial population decline. Therefore, for populations that did not go extinct, I used a non-parametric smoothing procedure (gam, described above) to ascertain the minimum population size for each strain combination, and calculated the slope of the subsequent increase in population size. This slope represents the population growth rate, which indicates the rate of adaptation to the novel corn resource. I used Pearson’s product moment correlation to test whether the slope was positively correlated with the degree of founding genetic variation.

Niche expansion in the wheat + corn habitat

Beetles were first sampled for isotope ratios two weeks after starting the experiment. Since egg to adult development cannot be completed in two weeks, all adults sampled at this time were the

founding adults. Isotope ratios of beetles in the wheat-only habitat represent baseline isotope ratios of adults from each population, while isotope ratios of beetles in the wheat + corn habitat reflect the proportion of corn resource used by founding beetles in two weeks. Corn consumption within two weeks represents behavioral plasticity in resource use, defined as environment-dependent variation in behavior relevant for resource use. Such plasticity may arise either from active resource choice or from an innate response to environmental cues. Therefore, for populations of each strain combination, the difference between the mean isotope ratio in the wheat-only and wheat + corn habitats is a measure of amount of corn consumed within two weeks due to differential foraging behavior in the heterogeneous habitat. This paired comparison controls for strain-specific differences in carbon assimilation, as well as potential differences in yeast utilization (added as supplement to the wheat flour) that could lead to small differences in isotope ratios in spite of consuming the same resource. I used an ANOVA to test for the effect of strain combination on behavioral change in resource use (measured as above), using data for 2-3 replicates of each strain combination (with strain combination as a factor). Effect of genetic variation on behavioral change was measured using a permutation test (described below).

Beetles were subsequently sampled for carbon isotope analysis at weeks 12 and 24, representing change in resource use over approximately 3 and 6 generations. Using data from weeks 2, 12 and 24, I analyzed the temporal change in resource use across ~6 generations. To test for significant effects of genetic variation on evolutionary niche expansion, I calculated the mean $\delta^{13}\text{C}$ values among all replicates of each strain combination at each time point. Since the resulting dataset was unbalanced (with 4, 6, 4 and 1 data points for each level of genetic variation), I conducted non-parametric, Monte Carlo permutations of the dataset to test whether genetic variation affected resource use. To test for a significant effect of time using Monte Carlo permutations of the data, I randomly shuffled data across time points within each strain combination, removing any effect of time on the isotope ratio of each strain combination. I generated 50,000 datasets, using a linear model to calculate the slope of mean $\delta^{13}\text{C}$ vs. time for each dataset. Using this distribution of null slopes, I tested whether the observed slope from the original dataset was significantly larger than that expected by chance. Similarly, I shuffled data across strain combinations within each of the three sampling time points to calculate the probability of obtaining the observed effect (slope) of genetic variation on resource use. Finally, to test for a significant interaction effect of genetic variation and time, I randomly shuffled all data across time points and strain combinations, and calculated the slopes of $\delta^{13}\text{C}$ vs. time for

each level of genetic variation. Significant variance in these slopes would indicate a significant interaction effect of time and genetic variation. The significance of the observed variance in slopes was calculated using the distribution of variance in slopes from 50,000 permuted datasets.

Individual variation in resource use in the wheat + corn habitat

Variance in the carbon isotope ratio of individual beetles sampled from a given population at a given time represents among-individual differences in resource use. Therefore, a small variance in within-population carbon isotope ratio indicates that individuals ate very similar proportions of corn flour, while a larger variance would show that different individuals consumed different proportions of corn flour (individual specialization, Bolnick et al. 2003). To test whether individual variation in corn use changed over time, and whether genetic variation affected the rate of such change, I used a non-parametric analysis equivalent to the Monte Carlo permutations described above for analyzing mean $\delta^{13}\text{C}$ values. It should be noted that the low number of beetles sampled from each population (10) leads to imprecise estimates of between-individual variance in resource use.

Correlation between relative population size and niche expansion

To determine whether niche expansion in the wheat + corn habitat enhanced population performance I tested for a positive correlation between population size and degree of niche expansion. For replicates of each strain combination, I calculated percent mean population size in the wheat + corn habitat relative to the mean size in the wheat-only habitat. Relative population size is a measure of performance in the novel, heterogeneous wheat + corn habitat relative to the performance in the ancestral wheat habitat, and is therefore specific to each strain combination. I tested for significant correlations between relative population size and the rate of behavioral and across-generation niche expansion (slope of mean $\delta^{13}\text{C}$ versus time over the first two weeks, and across generations), using strain as the level of replication. Since all variables were normally distributed (tested using Shapiro-Wilk tests), I calculated the Pearson product-moment correlation for each pair of variables.

RESULTS

To first establish that the experimental habitat treatments were effective, I show below that the novel corn-only habitat was suboptimal for experimental populations and generated strong directional selection for increased fitness in corn flour. I describe the effects of behavioral niche expansion and founding genetic variation on population performance and adaptation to the novel corn-only resource. I then present results for the novel heterogeneous wheat + corn habitat, dissecting the roles of behavioral and evolutionary niche expansion in this habitat. Lastly, I analyze the relationship between behavioral and evolutionary niche expansion in this habitat, and their effects on population performance relative to the ancestral wheat resource. The results section therefore progresses from testing the impact of behavioral and evolutionary niche shifts under strong directional selection on resource use in a novel homogeneous habitat, to weaker selection on resource use in a heterogeneous habitat.

Population persistence and adaptation in the novel corn-only habitat

In the corn-only habitat, survival of the 120 founding adults required an immediate niche shift from the ancestral wheat resource: individuals had to utilize the novel corn flour to survive and reproduce. The egg-to-adult development time in corn flour is typically at least five weeks (Agashe, unpublished data) and egg survival in corn flour is very low (Table 1.1); therefore, population size in the first few weeks of the experiment depended on whether founding individuals carried alleles allowing them to subsist solely on corn flour. In support of this prediction, genetic variation did not affect the time required for populations to decline to half their original size ($t_{1/2}$, a measure of initial population performance; Pearson's product moment correlation = -0.44, $t = -0.69$, $df = 2$, $p = 0.56$). $t_{1/2}$ was also not correlated with the proportion of extinct populations within each level of genetic variation (Pearson's product moment correlation, $t = -0.69$, $df = 2$, $p = 0.55$), indicating that survival and performance of the founding generation did not determine the overall probability of population extinction or persistence.

Longer-term population persistence in the corn-only habitat would require adaptation to the novel corn resource, and should therefore increase as a function of genetic variation for fitness on corn. Following the initial decline in population size (weeks 2-10), some populations went extinct while others exhibited a partial recovery in the adult population size (Figure 2.1). The latter represent populations that successfully adapted to the novel corn resource to permit a

positive growth rate, and the rate of this increase in population size is therefore a measure of the degree of adaptation. I found that this rate of population recovery increased significantly as a function of founding genetic variation (Pearson's product moment correlation = 0.99, $t = 64.49$, $df = 1$, $p = 0.0099$). Genetic variation also significantly decreased the overall probability of population extinction in the novel corn-only habitat (Fisher's exact test, $p = 5.2 \times 10^{-6}$; Chapter 1, Figure 1.1 and Results), indicating that both adaptation and persistence in the novel corn-only habitat was facilitated by genetic variation for resource use.

Approximately two years after the beginning of the experiment, surviving populations in the corn-only habitat were assayed for relative fecundity in corn flour, and for larval flour preference in two-flour choice assays (measured as described in "Among-strain variation", Methods, Chapter 1). At the beginning of the experiment, relative fecundity in corn was 31% ($n = 15$ data points, each being the mean of replicate populations of each strain combination; 6 females were assayed per population), which increased significantly to 44% in less than two years ($n = 5$ populations, 6 females per population; Welch two-sample t test: $t = -4.31$, $df = 6.87$, $p = 0.003$). Furthermore, 72% of tested larvae ($n = 18$) preferred corn over wheat flour, a significant increase compared to the maximum larval corn preference measured before starting the experiment (43.48%, $n = 46$, Table 1.1; two-sample test for equality of proportions, $\text{Chi-sq} = 4.28$, $df = 1$, $p = 0.038$). These results indicate that adaptation to the corn-only habitat during two years led to increased egg allocation to corn flour and greater larval preference for corn flour, compared to ancestral populations that had never been exposed to corn flour.

Behavioral niche expansion in the novel heterogeneous habitat

Within two weeks of exposure to the heterogeneous wheat + corn habitat, sampled beetles from all populations had consumed significant amounts of the novel corn flour (Figure 2.2A), with corn constituting up to 42% of individual diets in some cases. Mean $\delta^{13}\text{C}$ of all populations decreased significantly during this period (mean change = 2.36; one-sample t test for change in $\delta^{13}\text{C}$: $t = 12.02$, $df = 14$, $p = 9.1 \times 10^{-9}$) indicating that beetles had begun consuming corn flour (for beetles reared to adulthood on wheat flour, $\delta^{13}\text{C} = -22.74 \pm 0.08$; for those raised only on corn flour, $\delta^{13}\text{C} = -11.84 \pm 0.16$). However, genetic variation or the specific strain combination had no effect on the behavioral change in resource use (one-way ANOVA for genetic variation: $F_{1,13} = 1.75$, $p = 0.21$; for strain combination: $F_{14,16} = 1.96$, $p = 0.098$). Therefore, beetles in all

populations began to use corn flour within two weeks of starting the experiment, and the degree of use of corn flour was not affected by genetic variation or strain composition.

I suggest that rapid utilization of corn within two weeks was due to behavioral plasticity in resource choice: within two weeks of exposure to the wheat + corn habitat, individuals altered their foraging behavior so that their former wheat-only diet included between 10-42% corn flour. However, a number of alternative mechanisms could also lead to such apparent behavioral niche expansion. First, if beetles that did not consume corn flour within this period suffered greater mortality, isotope ratios after two weeks would be biased toward indicating high corn consumption. If so, we would expect a negative correlation between mortality and corn use within two weeks. However, adult mortality within two weeks was not correlated with the degree of corn use in each population (Pearson's correlation = 0.06, $t = 0.33$, $df = 28$, $p = 0.74$), showing that the observed use of corn flour was not confounded by differential mortality within the founding generation. Second, the observed use of corn flour within two weeks may simply be a result of the availability of a novel resource and may not reflect behavioral resource choice. In the absence of such choice, relative resource consumption should reflect resource availability. In this experiment, corn and wheat flour were available in a 1:1 ratio; yet average corn flour consumption was only 28%, significantly lower than the expected value of 50% (one-sample t test: $t = -10.72$, $df = 14$, $p < 0.0001$). Hence, the use of corn flour within two weeks likely represents behavioral choice by founding-generation adult beetles. Lastly, the observed use of corn flour may reflect innate or heritable resource preferences rather than resource choice. E.g. beetles may use a fixed proportion "X" of a novel resource irrespective of its relative abundance, and the value of X may be determined genetically rather than via a plastic behavioral response in the presence of a novel resource. Strain-specific flour preferences exhibited by isolated larvae (Table 1.1) serve as a measure of innate resource preference of each strain. I used these data to predict the innate resource preference of each of the experimental strain combinations (calculated as the mean preference of component strains). If resource use within two weeks were determined solely by innate resource preferences, we would expect the predicted corn use of each strain combination to match the observed corn use (estimated using isotope ratios). However, I failed to find such a correlation between predicted and observed corn use (Pearson's correlation = 0.44, $t = 1.78$, $df = 13$, $p = 0.098$), indicating that variation in corn use within two weeks was not a function of innate resource preferences. Therefore, the most plausible explanation for rapid

utilization of corn flour within two weeks is that beetles responded to the presence of conspecifics and the availability of the novel corn resource via plastic resource choice.

Evolutionary niche expansion in the novel heterogeneous habitat

Experimental populations significantly increased their use of the novel corn flour over ~6 generations (excluding behavioral niche expansion; time effect, Table 2.1; Figure 2.2B). However, genetic variation did not affect niche use during this period. Analyzing temporal change in mean $\delta^{13}\text{C}$ separately within each level of genetic variation, it is apparent that significant niche expansion occurred in populations with the highest degrees of genetic variation, while niche use did not change significantly for other levels of diversity (MC permutation results: for genetic variation (GV) = 1, $p = 0.0597$; for GV = 2 and 3, $p > 0.17$; for GV = 4, $p = 0.016$). However, this difference in the degree of niche expansion across levels of genetic variation is not significant (interaction term, Table 2.1). Therefore, contrary to expectation, genetic variation affected neither overall niche use (mean $\delta^{13}\text{C}$) nor the speed of niche expansion (change in mean $\delta^{13}\text{C}$). Testing whether niche expansion was a function of strain composition rather than genetic variation, I found that overall niche use (mean $\delta^{13}\text{C}$) but not the rate of niche expansion was affected by strain composition (ANOVA, Strain combination effect: $df = 14$, $F_{14,56} = 3.32$, $p = 0.0007$; Time effect: $df = 1$, $F_{1,56} = 8.63$, $p = 0.005$; Strain combination x Time effect: $df = 14$, $F_{14,56} = 1.46$, $p = 0.16$). Therefore, neither strain composition nor genetic variation significantly affected the evolutionary rate of niche expansion during six generations.

Among-individual variation in resource use

The within-population variance in carbon isotope ratio of individuals did not change temporally or as a function of genetic variation (permutation test: time effect, $p = 0.49$; genetic variation effect, $p = 0.57$; genetic variation x time effect, $p = 0.61$). Within populations, the variance in the proportion of corn consumed was very small (mean within-population variance in proportion corn used = 0.0120 ± 0.0007), indicating that individuals within each population ate similar proportions of corn flour at a given time. Thus, individuals in successive generations simultaneously expanded their individual niche width, and the observed niche expansion occurred via individual generalization rather than increased among-individual variation (contrary to the results presented in Bolnick et al. 2007).

Relationship between behavioral and evolutionary niche expansion

Populations exhibiting greater behavioral niche expansion showed slower evolutionary niche expansion, as evidenced by the significant negative correlation between the respective slopes of $\delta^{13}\text{C}$ vs. time for each strain combination (Figure 2.3; Pearson's product-moment correlation = -0.56; $t = -2.42$; $df = 13$; $p = 0.03$). Slopes for both two-week and subsequent change were normally distributed (Shapiro-Wilk normality test, $p > 0.2$ in both cases). Populations with the highest level of genetic diversity showed the smallest two-week behavioral niche expansion but the greatest across-generation rate of niche expansion (Figure 2.3, strain combination 15). Therefore, initial behavioral niche expansion hindered subsequent evolutionary change in resource use.

Relationship between population size and niche expansion

As expected, most populations in the study had a lower mean size in the wheat + corn habitat compared to their size in the wheat-only habitat, which contained twice as much ancestral resource (Figure 1.2B). As populations expanded their resource niche, however, we would expect this size difference between habitats to get smaller. Hence, the degree and rate of niche expansion should be positively correlated with relative population size (wheat + corn relative to wheat). Instead, I find that relative population size was negatively correlated with behavioral niche expansion (Figure 2.4A; Pearson's product-moment correlation = -0.59, $t = -2.63$, $df = 13$, $p = 0.021$). Hence, populations with greater behavioral niche expansion eventually showed weaker overall performance in the wheat + corn habitat, implying a cost to plasticity.

On the other hand, I find a non-significant positive relationship between the rate of across-generation niche expansion and relative population size (Figure 2.4B; Pearson's product-moment correlation = 0.26, $t = 0.98$, $df = 13$, $p = 0.34$; all variables were normally distributed: Shapiro-Wilk test for normality, $p > 0.2$ in all cases). However, one strain combination (combination 3, Col-2 single-strain populations) had disproportionate influence over the estimated slope and fit (influence analysis: $\text{hat} = 0.19$, $\text{DFbeta}_{\text{slope}} = -1.36$, $\text{DFfit} = -1.69$). I therefore re-tested the correlation after removing data for strain combination 3. I found a significant positive correlation between relative population size and evolutionary rate of niche expansion (Pearson's product-moment correlation = 0.59, $t = 2.5$, $df = 12$, $p = 0.027$). Omitting this data point retained the significant negative correlation between relative population size and rate of behavioral niche expansion (Pearson's product-moment correlation = -0.69, $t = -3.32$, $df =$

12, $p = 0.006$; no single data point had a large leverage on or influenced this correlation). Thus, barring strain combination 3, populations with a higher rate of across-generation niche expansion maintained a significantly greater population size in the wheat + corn habitat relative to the wheat-only habitat. The slopes of the relationship between relative population size and behavioral or evolutionary niche expansion were significantly different, regardless of whether the influential data point was omitted (t tests for difference in slopes; including all data: $t = -2.61$, $df = 24$, $p = 0.015$; excluding combination 3: $t = -4.37$, $df = 22$, $p < 0.001$). The opposite effects of within- and across- generation niche expansion on mean population size are perhaps not surprising given that the two rates of the niche expansion were negatively correlated (Figure 2.3). At the mechanistic level, it is also intuitive that across-generation niche expansion should be a better predictor of long-term population size in a heterogeneous habitat, compared to within-generation behavioral niche expansion. The question of why that is not the case for populations of strain combination 3 is addressed in the discussion.

DISCUSSION

Niche evolution and adaptation under strong directional selection

Fisher's fundamental theorem states that "*the rate of increase in fitness of any organism at any time is equal to its genetic variance in fitness at that time*" (Fisher 1930). In the experiment presented here, populations in the corn-only habitat experienced strong directional selection for increased fitness on the novel corn flour. In support of Fisher's prediction, I found that population persistence, a direct measure of fitness in the novel corn habitat, increased as a function of genetic variation for resource use (Figure 1.1). For populations that did not go extinct during the experiment, the rate of population recovery following an initial decline was also positively correlated with the amount of founding genetic variation (Figure 2.1). Subsequent assays of these recovered populations show greater fecundity and lower mortality in corn flour, as well as increased larval preference for corn, relative to initial trait values. Thus, greater genetic variation allowed populations to persist and adapt faster under strong directional selection.

Previous theoretical work has generated various predictions about conditions in which closed populations could evolve sufficiently fast to avoid extinction in a novel environment (Gomulkiewicz and Holt 1995; Holt and Gomulkiewicz 1997; Orr and Unckless 2008). Population persistence via adaptation is predicted to occur when populations start out at relatively

large sizes compared to the critical size under which they face greater extinction risk from demographic stochasticity (generally thought to be between 10-100). Population persistence is also increasingly likely when heritability for fitness in the selective environment is higher, and the degree of initial maladaptation is lower. Thus, the models concluded that only weakly maladapted populations at high initial densities could persist in novel environments. In the experiment presented here, all populations were equally and strongly maladapted to the novel corn-only habitat: time to loss of half the initial population size, $t_{1/2}$, was not significantly different between populations, and mean $t_{1/2} = 7.5$ weeks (less than two generations). Populations also had equally low starting densities (120 adults, close to the critical size mentioned above). The only difference between populations was the amount of heritable variation for fitness on corn flour, and all high-diversity populations evolved rapidly and sufficiently fast to avoid extinction in the novel environment. Although these results do not constitute a direct test of the above models, they show that contrary to model predictions, closed populations at low densities can avoid extinction via rapid adaptation given sufficient genetic variation for resource use.

Niche expansion in the heterogeneous habitat

The experiment presented here shows that in a novel heterogeneous habitat, populations rapidly expanded their resource niche via an immediate within-generation behavioral change in resource use (Figure 2.2). Subsequent evolutionary niche expansion also occurred in the populations, but was responsible for a smaller proportion (13%) of the total increase in corn use (Figure 2.2). Contrary to expectation, genetic variation did not increase the rate of evolutionary niche expansion in this experiment. Below I discuss the implications of these results and consider alternative mechanisms that could potentially explain the observed changes in niche use.

First, what was the nature of selection acting in the heterogeneous wheat + corn habitat? Beetles in this habitat were supplied with 25g each of the ancestral wheat and the novel corn resources. Individuals that consumed only the ancestral wheat resource would face greater competition, while individuals consuming largely corn flour would suffer from longer egg-to-adult development times (Agashe, unpublished data) and greater mortality (Table 1.1). Therefore, selection would act to increase fitness on both resources (rather than only on the novel corn resource). This conjecture is supported by the observation that initial survival and performance of different strain combinations in the corn-only habitat (measured as $t_{1/2}$) was not correlated with niche expansion in the wheat + corn habitat (Pearson's product-moment correlation: within-

generation slope: $t = -0.49$, $df = 13$, $p = 0.63$; across-generation slope: $t = 1.19$, $df = 13$, $p = 0.25$), indicating that the ability to use only corn flour alone was not sufficient to predict niche expansion in the heterogeneous habitat.

In principle, the beetles' response to the presence of 25g of wheat flour and 25g of the novel, nutritionally suboptimal corn flour could be classified into three broad categories (a) increased efficiency on wheat flour without using any corn flour, resulting in a population size greater than half the size on 50g wheat flour alone (b) continue to use only wheat flour with the same efficiency, resulting in half the population size on 50g wheat flour (c) adapt to use corn flour in addition to wheat (niche expansion), resulting in population sizes lower than on 50g wheat. The first two possibilities can be ruled out as stated here, because carbon isotope analysis indicates that all populations adapted to utilize corn in addition to wheat flour (Figure 2.2), and the size of all populations in wheat + corn was $> 50\%$ that in wheat (Figure 2.4). However, some strain combinations (e.g. combinations 15, 9 and 7, Figure 2.4) maintained a population size greater than or equivalent to their size in wheat-only habitats; these high relative population sizes cannot be explained solely by the incorporation of $\sim 40\%$ corn flour in the diet. Instead, in response to simultaneous selection for increased fitness on both resources, efficiency on wheat flour may have also increased in these populations, although I am unable to explicitly test this hypothesis. Strains probably faced selection for increased efficiency on wheat flour even prior to the experiment. However, stocks were maintained at lower densities and propagated using only a few adults every 1-2 generation (Beeman Lab, personal communication), which would generate relatively weak selection for efficiency on wheat flour compared to that imposed by high population density during the experiment. Thus, while all populations expanded their niche to include corn flour in their diets, it is plausible that populations that also increased their efficiency on wheat flour maintained equivalent or greater population sizes compared to the ancestral wheat flour. Future experiments that quantify temporal change in resource use efficiency along with niche expansion are required to test this hypothesis.

In the heterogeneous habitat (wheat + corn), it is possible that the measured behavioral (two-week) increase in corn use was a result of sampling bias due to selective death of beetles that did not expand their resource niche. However, all populations had similar numbers of live adults when beetles were sampled for isotope analysis (mean = 105.46 adults, $se = 0.85$, range = 83, 118), and there was no correlation between the mean isotope ratio and adult population size at week two (Pearson's product-moment correlation = -0.17 , $t = -0.62$, $df = 13$, $p = 0.55$).

Therefore, the observed change in resource use within the first two weeks of the experiment represents rapid behavioral niche expansion. Significant among-strain differences in survival in corn flour indicate that corn use is not adaptively neutral, but entails costs associated with delayed development and increased mortality (Agashe, unpublished data, and Table 1.1). Furthermore, isolated individuals showed significant differences in behavioral preferences for oviposition and resource use (Table 1.1), and such variation in plastic behavior could not lead to the uniform increase in corn use observed in experimental populations (Figure 2.2). Therefore, behavioral plasticity for resource use is likely to have been a response to increased density of conspecifics.

What was the mechanism responsible for the observed across-generation change in resource use? I discuss three (mutually non-exclusive) possibilities. First, a consistent plastic (behavioral) response to intraspecific competition could lead to a temporal increase in niche expansion: every generation, as population density increased, utilization of the novel resource would increase without a change in the strength of the response itself. Second, selection could act to increase the strength of the plastic response to intraspecific competition: again, as population density increased, each generation would show a stronger plastic response to density. Third, selection could act on heritable components of resource use to increase utilization of the novel resource, apart from a baseline plastic response. If behavioral plasticity in response to resource competition were responsible for the observed increase in corn use, we would expect a high rate of across-generation niche expansion, since population density increased in all wheat + corn populations during generations 1-6 (population census data, not shown). However, the rate of niche expansion across six generations was much lower than the initial rate of niche expansion within the founding generation (Figure 2.2). Therefore, it is unlikely that the observed across-generation niche expansion was due to a linear plastic response to intraspecific competition or due to selection on the strength of the plastic response. On the other hand, selection could have acted on heritable components of resource use, typically leading to slower rates of niche expansion compared to that observed due to behavioral plasticity. In the corn-only habitat, behavioral plasticity in resource use (which largely determined initial performance in corn flour, measured by $t_{1/2}$) did not decrease the probability of population extinction. Hence, the slower rate of niche expansion observed across generations was likely due to selection acting on heritable fitness components, representing evolutionary niche expansion that was also observed in corn-only populations. This could be tested by comparing heritable fitness components of individuals from each population at the end of the experiment to measurements made before the experiment.

However, most populations were lost prematurely due to incubator failure at generation 8 (the experimental was intended to continue for nine generations), precluding such an assay.

Relative roles of behavioral and evolutionary niche expansion and the Baldwin effect

The relative contribution of behavioral and evolutionary change during niche expansion have not been previously been quantified and contrasted in a single experimental study. Here, I show that behavioral niche expansion by founding adults resulted in rapid increase in utilization of the novel corn resource in individual diets (from the initial 0% up to ~35% corn in 2 weeks), while across-generation, evolutionary niche expansion contributed to a smaller increase in corn use (~35% at week 2, to ~40% at week 24). Initial behavioral shifts thus accounted for approximately 87% of the niche expansion observed over the entire six generations. The negative correlation between behavioral and evolutionary niche expansion (Figure 2.3) shows that rapid behavioral niche expansion may in fact hinder subsequent evolutionary niche expansion. It is unlikely that the observed negative correlation between behavioral and evolutionary niche expansion is a spurious result of evolutionary constraints placed on some populations by genetic variation, for two reasons. First, the degree of niche expansion (and the behavioral component) was not explained by the presence of individual strains or overall genetic diversity. Second, replicate strain combinations spanned the spectrum of rates of niche change, as did different strain combinations within a given level of diversity (e.g. combinations 5-10 with GV = 3, Figure 2.3).

Numerous studies have compared the degree of adaptive plasticity and evolutionary diversification in native and invading species following habitat invasion (Dybdahl and Kane 2005; Latta et al. 2007; Strauss et al. 2006). These studies show that adaptive plasticity can play a major role in the spread of invasive species. In spite of such important applications, direct experimental studies of the relative impacts of plasticity and evolution during multi-generation adaptation are extremely rare. In a previous test of the hypothesis that plasticity can facilitate evolution (the “Baldwin effect” (Baldwin 1896)), flies were allowed to learn to avoid one of two resources and were artificially selected based on their oviposition preferences (Mery and Kawecki 2004). The study showed that depending on the direction of selection (which resource was to be avoided), learning either hindered or facilitated the evolution of an innate oviposition preference. The study presented here does not support the Baldwin effect, because behavioral niche expansion hindered evolutionary niche expansion. Mathematical models predict that plasticity is more likely to hinder evolutionary change in populations when the plastic response is not

heritable but is extremely rapid, or when behavioral plasticity is highly heritable (Papaj 1994); or when there exists a single fitness optimum rather than multiple adaptive peaks (Ancel 2000; Anderson 1995; Borenstein et al. 2006). In *T. castaneum*, behavioral niche expansion can occur as rapidly as within a week of introduction to a wheat + corn habitat (Chapter 3), but currently there is no data on the shape of the fitness landscape in this habitat and the heritability of the plastic response. Therefore, the most plausible explanation for the observed negative correlation between the degree of evolutionary and behavioral niche expansion is that rapid behavioral niche expansion buffered populations from strong selection arising from resource competition, mitigating subsequent evolutionary response.

Niche expansion and population size

Does niche expansion actually improve population performance in the novel habitat? To understand this, it is important to dissect the opposite effects of behavioral and evolutionary niche expansion on population size in the wheat + corn habitat relative to size in ancestral wheat-only habitat. As shown in Figure 2.4, the rate of behavioral niche expansion had a significantly negative impact on relative population size, while the evolutionary rate of niche expansion tended to increase relative population size. The latter trend is significant if the single, highly influential data point for Col-2 single-strain populations (combination 3) is omitted. Populations composed of this strain had high rates of evolutionary niche expansion coupled with unusually low growth rates and population sizes (Figure 2.4); but is this trait combination biologically significantly different from other populations? In other words, why did populations composed of Col-2 individuals perform so poorly in wheat + corn in spite of exhibiting niche expansion equivalent to other populations, and an adaptation rate exceeding most other populations? A plausible explanation lies in the nature of selection acting on resource use in the heterogeneous habitat (see section above): population size in the wheat + corn habitat depended on fitness in both wheat and corn flour. Of the four experimental strains, Col-2 had the highest fitness in corn flour, but had very low fecundity overall (Table 1.1). Therefore, Col-2 populations could use corn to survive and reproduce, and could respond to selection for corn use, but could not maintain a large population size relative to wheat flour because their low fecundity in both resources. Thus, the uniquely low fitness of Col-2 individuals in both wheat and corn flour likely resulted in high rates of evolutionary niche expansion but not in increased population size in the wheat + corn habitat.

Hence, the experiment shows that the mechanism of niche expansion may determine its long-term impacts on population ecological parameters. Whereas evolutionary niche expansion can increase population size in novel habitats, behavioral niche expansion can significantly decrease population performance.

Niche specialization versus generalization

In this experiment, population niche width largely expanded via resource generalization rather than increased among-individual variation, such that all individuals consumed on average equivalent amounts of corn flour at a given time. This result supports theoretical predictions that evolution of individual specialists is generally more difficult (Ackermann and Doebeli 2004; Roughgarden 1972; Wilson and Turelli 1986). On the other hand, there is overwhelming evidence for individual niche specialization in natural populations (Bolnick et al. 2003), and population niche width is positively correlated with individual variation in niche use in diverse taxa (Bolnick et al. 2007). However, the evidence presented in these studies is largely comparative or observational, and may therefore be representative of stable end points rather than the dynamics of niche evolution tested in this experiment. The experimental results shown here may also be an artifact of laboratory settings; however, this is presently difficult to evaluate due to the paucity of other laboratory experiments. Mathematical models (cited above) predict that strong tradeoffs associated with resource use, or habitat selection or assortative mating between resource specialists can promote niche specialization. The experimental setup of this study would be expected to hinder the evolution of niche specialists to some extent, because there was no barrier between alternative resource patches and no evidence for habitat selection or assortative mating by adult beetles (Agashe and Patel, unpublished data). Lastly, the lack of individual niche specialization may simply be due to a lack of inherent tradeoffs for fitness on the alternative resources in *T. castaneum*. While there is little data to quantify the strength of tradeoffs, fitness on the two resources in this experiment was asymmetric- even under strong competition for the ancestral wheat flour, fitness on corn flour was too low (Table 1.1; see discussion above) to allow a selective advantage to corn-flour specialists. Therefore, under strong competition for wheat, fitness was maximized not by consuming corn exclusively, but by consuming some corn in addition to wheat: i.e. resource generalists had greater fitness than either resource specialist under strong competition. It is possible that longer-term evolution could substantially increase fitness on

corn flour, in which case generalists may have lower fitness than either niche specialists and the evolution and maintenance of niche specialists could be possible.

Conclusions

The experiment presented here shows that under strong directional selection on a novel resource, behavioral plasticity in resource use may have no effect on long-term adaptation, but genetic variation for resource use can increase population persistence and adaptation rate. On the other hand, in a heterogeneous habitat containing both ancestral and novel resources, rapid behavioral niche expansion decreased overall population performance. Surprisingly, genetic variation for resource use did not facilitate the rate of evolutionary niche expansion in the heterogeneous environment. Thus, the relative impacts of intraspecific genetic variation and plasticity for resource use depend on the selective environment to which populations are exposed. Lastly, this study represents one of the few direct experimental tests of the Baldwin effect, showing that contrary to his prediction behavioral niche expansion can significantly decrease the rate of subsequent niche evolution.

CHAPTER 3

THE INTERACTION BETWEEN GENETIC VARIATION AND COMPETITION, AND THEIR IMPACT ON NICHE EXPANSION

ABSTRACT

Theory and empirical evidence show that intraspecific competition facilitates niche expansion by increasing the strength of selection favoring the use of novel resources. Theory also suggests that genetic variation for niche use can facilitate the speed of adaptive niche shifts. Thus, in a novel habitat, intraspecific competition and genetic variation should together increase the rate of niche expansion. However, if genetically diverse individuals compete less, genetic variation could also hinder niche evolution by decreasing the strength of selection acting on niche use. In a factorial experiment, I manipulated the degree of genetic variation and population density (a proxy for intraspecific competition) in populations of *Tribolium castaneum* in a heterogeneous habitat containing a novel resource. Corroborating previous results, I found that a large fraction of the total niche expansion (~73%) was explained by behavioral niche expansion, which did not depend on the experimental treatments. Genetic variation hindered niche expansion in the first four experimental generations, but later facilitated it in interaction with competition. During the latter half of the study, populations with greater competition showed a higher increase in utilization of the novel resource, adding to the results of previous studies. Lastly, more diverse populations had more individual variance in niche use at the end of the experiment. Together, these results show that intraspecific competition and genetic variation act on different components of niche evolution; that their impacts on niche expansion can vary temporally; and that they interact positively to increase the degree of niche expansion.

INTRODUCTION

A major focus of research in evolutionary ecology is to understand conditions that promote niche evolution and diversification. Theory predicts that intraspecific competition for shared resources leads to disruptive selection acting on resource use, which promotes niche diversification (Simpson 1953). Empirical proof of this prediction comes from laboratory populations of *Drosophila* maintained at high densities (high intraspecific competition) that evolved to tolerate growth medium containing toxic cadmium chloride more rapidly than populations maintained at a lower level of intraspecific competition (Bolnick 2001). Experimentally elevated intraspecific competition also led to niche expansion in stickleback fish kept in enclosures in natural habitat, though this was caused by behavioral rather than genetic shifts (Svanback and Bolnick 2007). Other studies with spadefoot toads and sea otters also show that intraspecific competition facilitates variation in resource use (Pfennig et al. 2007; Tinker et al. 2008). Thus, it is clear that intraspecific competition generates selection for niche expansion within populations.

The response to selection is predicted to increase as a function of additive genetic variation for fitness (Fisher 1930). Various laboratory experiments with *Drosophila* and *Tribolium* provide support for this prediction: populations with greater genetic variation (either derived from hybrid lines or from radiation-induced mutations) significantly increased population size, productivity and adaptation rate in new environments (e.g. Ayala 1965; Ayala 1968; Crenshaw 1965). Thus, intraspecific genetic variation for niche use should increase the speed of adaptive niche expansion. A previous experiment with *Tribolium castaneum* beetle populations revealed high levels of phenotypic plasticity in resource use. Perhaps as a result, genetic variation had no detectable effect on the rate of niche expansion (Chapter 2). However, population densities in this experiment were allowed to fluctuate naturally and therefore intraspecific competition generating selection for niche expansion was not directly manipulated. Previous work shows that the impact of genetic variation on population parameters depends on the degree of intraspecific competition: for instance, heterogeneous groups of *Drosophila melanogaster* larvae exhibit higher productivity and develop faster than homogeneous groups, but only under high competition (Martin et al. 1988). Hence, it is plausible that genetic variation could have a significant impact on niche evolution only under stronger selection for niche expansion, such as that generated by very high population densities and limited resource availability. If so, genetically more diverse populations facing high competition should show the highest rates of

evolutionary niche expansion, and populations facing low competition, when limited by low genetic variation, should have minimum rates of niche evolution.

As an alternative to the positive interaction between genetic variation and competition discussed above, genetic variation may also hinder the rate of niche evolution if it alters the degree of competition between individuals. For instance, genetically dissimilar individuals may compete less than genetically similar individuals, a hypothesis proposed to explain the evolutionary advantage of sexual over asexual reproduction (Barton and Post 1986; Bulmer 1980; Maynard Smith 1978; Young 1981). Although evidence in favor of this hypothesis is equivocal, experiments with plants (Allard and Adams 1969; Boyden et al. 2008; Cheplick and Kane 2004; Reusch et al. 2005), *Drosophila* (Fowler and Partridge 1986; Lopezsuarez et al. 1993; Martin et al. 1988; Pérez-Tomé and Toro 1982), territorial salmon (Griffiths and Armstrong 2001), and fire-bellied toads (Jasienski 1988) show that genetically heterogeneous groups of individuals have greater productivity than genetically similar groups due to weaker competition or more efficient resource partitioning between individuals. Therefore at a given population density, more diverse populations may be under weaker selection for niche diversification compared to less diverse populations. The outcome of the interaction between genetic variation and competition thus depends on the relative strength of the long-term positive evolutionary impact of genetic variation and its immediate and negative impact on intraspecific competition. If the immediate negative impact of lowering intraspecific competition is greater, genetic variation in niche use could potentially hinder the rate of niche evolution. Because there have been no experimental tests of this hypothesis, the impact of the interaction between genetic variation and competition for niche evolution remains unclear.

I tested whether genetic variation and intraspecific competition interact to facilitate the rate of niche expansion, using laboratory populations of *T. castaneum*. Previous work shows that relatedness between *T. castaneum* larvae determines between-individual interactions such as cannibalism and competition (Garcia and Toro 1992; Jasienski et al. 1988), indicating that genetic variation within populations alters the strength of intraspecific competition in this species. *T. castaneum* also exhibits additive genetic variation for resource use (Via and Conner 1995), so that populations can respond to selection generated by intraspecific resource competition at high densities. Therefore, both positive and negative interactions between genetic variation and competition discussed above could operate in this species to alter the rate of niche evolution. In a factorial experimental design, I manipulated the level of competition and genetic variation in

replicate *T. castaneum* populations and quantified the rates of behavioral and evolutionary niche expansion in a heterogeneous habitat containing a novel resource.

METHODS

Experimental populations

Experimental populations were initiated at two population densities (200 and 400 adults, for low and high competition) crossed with two levels of genetic variation (two or four strains of *Tribolium castaneum*; Table 3.1). Populations were maintained in identical plastic containers with 20g wheat flour and 20g corn flour in adjacent patches that allowed free movement between patches. Wheat flour (supplemented with 5% yeast) is the ancestral resource for *T. castaneum* (for > 20 years in the laboratory), while corn flour represents a novel resource. The four experimental strains had significant variation for traits relevant to growth and fitness in the two resources (Table 1.1). Populations were maintained in incubators at 33°C ($\pm 1^\circ$ C) and 70% humidity.

I initiated the first generation with adults chosen randomly from stock populations of each of the four strains, with all possible 2- and 4- strain combinations as shown in Table 3.1. The optimal density for *T. castaneum* is ~15 beetles per g flour (Sokoloff 1977); hence, high-competition populations were started with 400 adults (20 beetles per g wheat flour) and low-competition populations were initiated with 200 adults (10 beetles per g wheat flour). I allowed adults to mate and oviposit for one week, after which I removed all adults and stored them at -80°C for carbon isotope analysis. The number of eggs laid in each population during this period depends on the number of ovipositing adults; hence, the number of founding adults determined the degree of competition experienced by larvae as they developed. After allowing larvae to develop for four weeks, I counted the number of eclosed adults in each population. A sample of either 200 or 400 adults (corresponding to the competition treatment) was added to fresh flour to start the next generation, and excess adults were frozen. This cycle of one oviposition week followed by four larval development weeks was repeated for eight generations.

Most populations in the high competition treatment produced an average of 200-450 adults each generation, which was often lower than the required number of adults to start the next generation (400) in this treatment. In such cases, all live adults were used to initiate the next generation and none were frozen. Because the experiment aimed to look at the impact of genetic variation on niche evolution within closed populations, I did not supplement these founding

adults with adults from stock populations. Therefore, high-competition populations were sometimes founded with less than the initially planned 400 adults each generation, and the number of adults each generation varied between populations according to their productivity. Regardless, the number of founding adults used for high-competition populations was larger (>300) than that used for low-competition populations (which generally produced an excess of adults, only 200 of which were used to start the next generation). Hence populations in the high competition treatment group were effectively maintained at higher competition, although the difference between high and low competition densities was less than intended due to the unplanned within- and between-population variation in productivity.

Population productivity

It was necessary to test whether genetic variation decreased intraspecific competition and increased productivity in the experimental populations. From the census data (see above) I calculated productivity of each population, measured as the number of eclosed adults per generation averaged over the entire 8 generations. To also test the impact of genetic diversity on productivity under benign conditions in a homogeneous habitat, I initiated a set of control populations in 50g wheat flour only (ancestral resource). I used the same design shown in Table 3.1 (with equivalent numbers of founding adults), but with two replicates per strain combination instead of three. Because the control habitat contained 50g wheat flour compared to the experimental populations' 25 g wheat flour, the "low" and "high" density treatments in the two habitats resulted in different degrees of competition for the ancestral wheat resource. The number of adults (rather than competition for resources) is critical for determining fecundity in *T. castaneum* (Sokoloff 1977). Hence, to facilitate comparison between control and experimental populations, and to still allow us to test whether diversity increased productivity at high competition, the above design was necessary. Control populations were maintained and processed in the same manner as experimental populations in the wheat + corn habitat, except that beetles from these populations were not analyzed for their stable carbon isotope ratios (described below).

Stable carbon isotope analysis

The stable carbon isotope ratio of 10 beetles frozen (as above) at generations 0 (founding adults with one week of exposure to the experimental wheat + corn habitat), 4 and 8 were used to quantify temporal change in resource use in the populations. Previous results (Chapter 2) show

that the carbon isotope ratio of beetles reared only on the ancestral wheat resource is ~ -24 , while the carbon ratio of beetles reared on only corn flour is ~ -12 (see Chapter 2, Methods). Prior to the experiment, all beetles were maintained on the wheat resource and would therefore have the same isotope ratio ($\delta^{13}\text{C}$) of -24 . Therefore, the mean $\delta^{13}\text{C}$ of 10 beetles one week after exposure to the experimental wheat + corn habitat (“generation 0”) is a direct measure of the degree of behavioral niche expansion by founding adults; a ratio closer to -12 indicates greater consumption of corn flour during the first week. Similarly, for each population, the isotope ratio of beetles at generation 4 (or 8) minus the ratio at generation 0 represents the degree of evolutionary niche expansion that occurred in 4 (or 8) generations (over and above behavioral niche expansion). The rates of evolutionary niche expansion during generations 0-4 and generations 4-8 can thus be calculated and compared as successive differences between isotope ratios at generations 0, 4 and 8. By the same logic, for each population, variance in $\delta^{13}\text{C}$ (with 10 beetles) was used to measure the degree of and rate of change in among-individual variance in resource use.

DATA ANALYSIS

Populations of two strain combinations (CP and CZ) went extinct between generations 4 and 8. Therefore, data from these two combinations were not included in the analysis described below, reducing the number of data points to 4 low-diversity combinations and one high-diversity combination with two replicates for each strain combination (Table 3.1). Because the experimental design was unbalanced it could not be analyzed with standard parametric tests such as regression or ANOVA.

I conducted Monte-Carlo permutations of the data in R (R Development Core Team 2008) to test whether genetic variation and competition interacted to affect (a) the degree of behavioral niche expansion (measured variable: mean $\delta^{13}\text{C}$ at generation 0) (b) the degree of evolutionary niche expansion at generation 4 (measured variable: difference in mean $\delta^{13}\text{C}$, generation 4 - 0) (c) the degree of evolutionary niche expansion at generation 8 (measured variable: difference in mean $\delta^{13}\text{C}$, generation 8 - 0). Thus in each case the measurement of interest is a paired, population-specific difference in isotope ratio, averaged for two replicate populations to give a single value for each strain combination (4 with low genetic variation and 1 with high genetic variation). For each test, null distributions of the following effect sizes were calculated using 50,000 shuffled datasets. For illustration, I describe below the test used for the

degree of behavioral niche expansion with the mean $\delta^{13}\text{C}$ values of populations at generation 0. The same procedure was used for each of the other three tests (see above) using the appropriate measured variable; this information is also summarized in Table 3.2 (column 3).

- (a) Effect of genetic variation: populations were separated into two groups with low and high genetic variation, with the genetic variation assignment randomized in each permutation. The average “mean $\delta^{13}\text{C}$ ” of strain combinations within each group was calculated. The difference in these averages quantifies the effect of low vs. high genetic variation on mean resource use (i.e. mean $\delta^{13}\text{C}$). The distribution of 50,000 such differences from randomized datasets represents the null distribution under no effect of genetic variation. If genetic variation increased the degree of behavioral niche expansion, we would find that the difference between groups observed in the original data would occur very rarely in this null distribution, unlikely to be explained by chance alone. The p value for effect of genetic variation on the degree of behavioral niche expansion was therefore calculated as the probability of finding a between-group difference at least as large as that observed.
- (b) Effect of competition: populations were separated into two groups with low and high competition, with the competition assignment randomized in each permutation. The effect size and null distribution for the competition effect was calculated exactly as described for genetic variation (above).
- (c) Interaction effect of genetic variation and competition: populations were separated into four groups, representing the four experimental treatments (genetic variation crossed factorially with competition, Table 1; henceforth called “interaction groups” to distinguish from the genetic variation and competition treatments). The average “mean $\delta^{13}\text{C}$ ” was calculated using strain combinations within each group, and the between-group variance in this average was used as the effect size for measuring the interaction effect. The interaction group assignment was randomized in each permutation to obtain the null distribution for between-group variance in average mean $\delta^{13}\text{C}$. If there were no effect of interaction group on mean $\delta^{13}\text{C}$, the probability of finding a variance as large as observed would be very high. This p value for the effect of interaction group was calculated using the null distribution generated from 50,000 randomizations.

The methods described above were also used to test for significant effects of genetic variation, competition and their interaction on the degree and change in among-individual variance in

resource use (i.e. using variance $\delta^{13}\text{C}$ instead of mean $\delta^{13}\text{C}$ for each population as the response variable).

RESULTS

Behavioral niche expansion

The mean proportion of the novel corn resource in beetle diets increased from 30% at generation 0 (behavioral niche expansion within one week) to 37% at generation 4 and 39% at generation 8. Thus, most niche expansion during the experiment occurred due to behavioral changes within one week of exposure to the wheat + corn habitat (Figure 3.1; one-sample t test for mean $\delta^{13}\text{C}$ at generation 0 with $\mu = -24$: $t = 28.08$, $df = 9$, $p < 0.001$). The mean carbon isotope ratio of adult beetles from each population was not affected by the genetic variation, competition or their interaction (Figure 3.1; permutation test: p values for all effects > 0.33). Among-individual variance in resource use within one week was also not affected by the experimental treatments (Figure 3.2; permutation tests, $p > 0.27$). Therefore, irrespective of the adult beetle density (competition) or standing genetic variation for resource use, beetles rapidly utilized the novel corn resource within a week.

Evolutionary niche expansion

Genetic variation significantly affected the degree of evolutionary niche expansion at generation 4 (Table 3.2, column 4). However, the direction of this effect was opposite to that expected: more diverse populations exhibited less niche expansion than less diverse populations (Figure 3.1). Competition did not affect evolutionary niche expansion at generation 4, either alone or in interaction with genetic variation (Table 3.2). Therefore, genetic variation suppressed the degree of early evolutionary niche expansion.

Between generations 4 and 8, genetic variation alone had no effect on the degree of evolutionary niche expansion, but in interaction with competition it increased the amount of corn flour used by beetles during this period (Table 3.2; Figure 3.1). Competition alone also significantly increased the degree of evolutionary niche expansion between generations 4 and 8. Together, these results show that genetic variation for resource use had an initial detrimental effect on evolutionary niche expansion, but later interacted with competition to facilitate niche expansion. This temporal variation in the effects of genetic variation and competition may explain

the lack of significant treatment effects on the overall degree of evolutionary niche expansion (generation 0 to generation 8, Table 3.2). Without data for generation 4, therefore, I would have missed detecting the dynamics of niche expansion and transient treatment effects.

Among-individual variance in niche use

At the end of the experiment, more diverse populations had significantly greater among-individual variance in resource use (variance $\delta^{13}\text{C}$) compared to less diverse populations (Figure 3.2; Welch two-sample t test, $t = 3.58$, $df = 7.87$, $p = 0.007$). Genetic variation was the sole experimental treatment that affected variance $\delta^{13}\text{C}$ at any stage of the experiment (Table 3.2). Furthermore, the effect of genetic variation was significant only between generations 4 and 8, when populations with low diversity showed a significant decline in among-individual variance in $\delta^{13}\text{C}$, in contrast to populations with high diversity in which it was maintained (Figure 3.2; Table 3.2). During behavioral niche expansion genetic variation and competition did not affect the variance in $\delta^{13}\text{C}$ within populations (Figure 3.2; permutation tests for effects of genetic variation, competition and interaction: $p > 0.1$ in all cases). The same results were found for change in variance $\delta^{13}\text{C}$ between generations 0 and 4, and for the overall change between generations 0-8 (Table 3.2). Therefore, during the last four generations in the experiment genetic variation within populations determined the degree of individual variation in resource use (Table 3.2).

Together with results for the degree of niche expansion (previous section), these results imply that genetic variation and competition affect different components of niche expansion, and therefore place different constraints on the evolution of population niches. During generations 4 – 8, competition significantly increased niche expansion, whereas genetic variation prevented the loss of among-individual variation in niche use. These dynamics ultimately affected the overall degree of evolutionary niche expansion and the maintenance of individual variance in niche use in the experimental populations.

Population productivity

I found a significant interaction effect of genetic diversity and competition on productivity in the ancestral wheat habitat (permutation test for interaction effect: $p = 0.03$), but not in the experimental wheat + corn habitat ($p = 0.42$). Results in the wheat habitat confirm that genetic diversity decreases larval competition and mortality (leading to increased productivity) at high population densities. This implies that in the present experiment, genetic diversity could

potentially hinder niche expansion because more diverse groups of larvae face weaker intraspecific competition.

The average productivity of high- and low- competition populations was statistically indistinguishable in the ancestral wheat habitat (Figure 3.3A; paired t test, $df = 4$, $p = 0.25$). However, the effect of competition on productivity was confounded by genetic diversity. As seen in Figure 3.3A, productivity of diverse populations increased at high competition (395 vs. 514; significance could not be tested due to solitary data points in each category) whereas that of less diverse populations decreased at high competition (mean difference of 80; paired t test, $df = -5.2$, $df = 3$, $p = 0.01$). This effect of diversity could occur if more diverse populations had greater fecundity at high densities; however, in a separate experiment I found that genetic diversity did not affect population fecundity at different densities (Agashe, unpublished data). Therefore, the increase in productivity of diverse populations under high competition can be attributed to low mortality arising from weaker larval competition. Thus, genetic variation decreased competition between larvae at high population density.

As noted above, genetic variation and competition did not interact to affect population productivity in the experimental wheat + corn habitat (Figure 4B; permutation test, effect of genetic variation on change in productivity: $p = 0.42$). The productivity of genetically diverse populations was similar to that of less diverse populations, and competition had no effect on productivity (mean difference in productivity = 40; paired t test for productivity of less diverse populations: $t = -2.3$, $df = 3$, $p = 0.11$). Potential causes for these results are addressed in the discussion below.

DISCUSSION

I manipulated both intraspecific competition and genetic variation for resource use in experimental populations of *Tribolium castaneum* to test whether they interact to affect the degree of niche expansion. I found that the effects of genetic variation and competition vary temporally. Genetic variation impeded early evolutionary niche expansion, but later interacted with competition to facilitate it. Competition alone also facilitated niche expansion during the latter half of the study. Ultimately, the experimental treatments did not predict the overall degree of resource niche expansion that occurred in the experimental populations. However, populations with more genetic variation maintained significantly greater among-individual variance in

resource use. These results suggest that genetic variation largely determines the maintenance of individual variance in niche use, and resource competition (generating selection for resource niche expansion) may be the major driver of evolutionary change in resource use.

As noted in the introduction, genetic variation may have both positive and negative implications for resource niche expansion. Genetic variation for resource use can increase the response to selection acting on intraspecific competition (Fisher 1930), and therefore facilitate niche expansion. On the other hand, genetic variation can lower the degree of intraspecific competition (Maynard Smith 1978), and therefore hinder niche expansion. Perhaps the most important result of this experiment is that both positive and negative effects of genetic variation occur, but at different stages of niche expansion.

Evolutionary and behavioral niche expansion

Many studies show that genetic variation has a greater impact on population parameters under strong selection (reviewed in Armbruster and Reed 2005; Charmantier and Garant 2005; Reed et al. 2003; Wise et al. 2002). The results of my experiment contribute to this literature by showing that the impact of genetic variation also depends on the degree of intraspecific resource competition. Under strong selection for niche expansion due to high population density, genetic variation aids exploitation of the novel corn resource (Figure 3.2). Similar results were observed even when resource competition was low but selection for a niche shift was strong because the ancestral resource was absent (results for corn-only habitat, Chapter 2). On the other hand, under weaker resource competition due to lower population density (Figure 3.2), or in a wheat + corn habitat (Chapter 2), genetic variation did not facilitate niche expansion. Thus, together with previous work (Chapter 2), this experiment provides empirical evidence that irrespective of the factors causing selection for exploitation of a novel niche, genetic variation can facilitate exploitation of novel resources provided selection is strong.

In this experiment, the strength of selection on niche use was determined by intraspecific competition, which could in turn be altered by genetic variation (Garcia and Toro 1992; Jasienski et al. 1988). This complex interaction between genetic variation and competition gives rise to temporal variation in genetic variation's effect on niche expansion. Between generations 0 and 4, genetic variation's effect on niche expansion was predominantly negative because it immediately decreased larval competition for the ancestral resource (Figure 3.3A) and reduced selection for niche expansion. On the other hand, its countering positive impact of increasing response to

selection could only be apparent after a few generations (here, between generations 4-8). Because the response to selection is greater under strong competition (Martin et al. 1988), genetic variation only increased niche expansion in populations with high competition (Figure 3.1; Table 3.2). In low-competition populations, genetic variation didn't matter because resource competition was too low. Thus, both positive and negative effects of genetic variation played a role in determining the niche dynamics in this experiment. Genetic variation's mitigating effect on intraspecific competition is thought to confer an ecological advantage to sexually reproducing species over asexual lineages (Bell 1982; Maynard Smith 1978). This experiment shows that the competitive advantage of genetic variation depends on the environment. While genetic variation may confer short-term competitive benefits, these benefits can only translate into a longer-term advantage under strong selection (in this case, mediated by high population density in a heterogeneous habitat). Conversely, genetic variation may impart a competitive disadvantage under a benign environment, but its effect may be reversed under strong selection. For instance, in ambient CO₂, *Arabidopsis thaliana* populations with diverse genotypes have lower fitness than monocultures; however, under elevated CO₂, genotype mixtures perform better than single-genotype stands (Andalo et al. 2001).

The results of this experiment also corroborate the previous observation that behavioral plasticity explains a large proportion of the overall niche expansion in *Tribolium castaneum* (Chapter 2). In both experiments, behavioral niche expansion within one or two weeks resulted in an average of 30% and 35% corn in beetle diets (starting from 0% at the outset of the study). In each case I observed a lack of significant effects of genetic variation on the magnitude of behavioral niche expansion. Therefore, in *T. castaneum*, behavioral niche expansion appears to have a predictably large effect on population-level responses to a heterogeneous habitat containing both an ancestral and a novel resource. Note that the competition and genetic variation treatments affected the first-generation adult beetles only for one week and only as adults. On the other hand, the treatments altered the genetic and competitive environment of subsequent generations for four weeks throughout their development from egg to mature adults. Therefore, the impact of the competition-genetic variation treatments on change in resource use would be expected to be stronger in the latter case. In agreement with this prediction, the experimental treatments facilitated evolutionary niche expansion (Table 3.2, Figure 3.1), but had no effect on the degree of behavioral niche expansion (Figure 3.1). Rapid behavioral adaptation to novel habitats via niche shifts has been frequently documented (e.g. Ghalambor et al. 2007; Jedlicka et

al. 2006; Price et al. 2003; Reznick and Bryga 1987; Svanback and Eklov 2006). My experiment adds to this body of knowledge by specifically showing that large amounts of behavioral plasticity in niche use can overcome constraints imposed by standing genetic variation or by weak selection on niche use, and facilitate rapid adaptation to novel niches.

Individual specialization

A key result from this study is that the degree of founding genetic variation facilitated the maintenance of individual variation in resource use in experimental populations (Figure 3.2; Table 3.2). An increase in population niche width can occur via an increase in either within-individual or between-individual variation in niche use (Bolnick et al. 2003; Roughgarden 1972). In the first case, all individuals increase their niche width to a similar extent, utilizing more but similar resources. In the second case, population niche width increases because different individuals mainly eat a narrow range of different resources. While it seems obvious that greater genetic variation in resource use should allow for greater individual variation in niche use, this may not always be true for the following reasons. First, sexual recombination between individuals rapidly homogenizes populations; hence, initial heritable variation in resource use cannot be maintained for very long unless other factors promote genetic polymorphism (such as habitat heterogeneity, e.g. Hedrick 1986). Second, individual resource specialization may largely be an outcome of individual differences in behavioral or physiological traits (Bolnick et al. 2003) rather than heritable variation for fitness in different resources. Lastly, mathematical models predict that individual specialization is generally difficult unless it is promoted by functional trade-offs in resource use (Ackermann and Doebeli 2004; Roughgarden 1972; Taper and Case 1985), and it is not obvious why such trade-offs should be stronger in more diverse populations.

In spite of these reasons, the impact of genetic variation on the maintenance of individual specialization had not been empirically tested. The degree of individual variation in niche use has significant implications for population ecological and evolutionary dynamics (see Bolnick et al. 2003 for a review). For instance, individual niche variation can substantially alter population dynamics (Kendall and Fox 2002; Lomnicki 1978) and species coexistence (Lichstein et al. 2007; Yoshida et al. 2003); and depending on other conditions such as niche-based assortative mating (Snowberg and Bolnick 2008), niche variation can allow populations to undergo subsequent adaptive diversification and speciation (Dieckmann and Doebeli 1999). Hence, the finding that more diverse populations maintain greater individual variance in resource use during adaptation

increases understanding of how genetic variation can maintain the within-population phenotypic diversity that is critical for generating species diversity.

Previous work suggests that niche expansion most commonly occurs via individual niche specialization rather than generalization (the Niche Variation Hypothesis, Van Valen 1965). Empirical evidence for the Niche Variation Hypothesis has been debated at length (reviewed in Bolnick et al. 2003), but recently Bolnick et al (2007) reported widespread support for it in natural populations of diverse taxa including gastropods, fish, frogs, and lizards. In my experiments, however, I found that the contribution of individual niche specialization to niche expansion was very small (average within-population variance in % corn in the diet was only ~2; Figure 3.2 and Chapter 2 Results). Potential causes for this discordance between my experimental data and evidence from natural populations were discussed in Chapter 2.

Genetic variation, competition, and their influence on productivity in different habitats

The quantification of productivity was important in this experiment because it established that genetic diversity lowered intraspecific competition (Figure 3.3A), confirming that genetic variation could have both negative (lower competition) and positive (increased response to selection) implications for niche expansion. In the control wheat-only populations, genetic variation within populations decreased larval competition and increased productivity at high density (Figure 3.3A), confirming previous results (Garcia and Toro 1992). However, in the experimental wheat + corn habitat, productivity was not affected by genetic variation, competition or their interaction (Figure 3.3B). Two mechanisms may explain this observed difference in the impact of genetic variation and competition on productivity between habitats. First, both experimental densities in the wheat + corn habitat were greater than or equal to the “high” density in the control wheat-only populations; it is possible that these densities were too high to detect density-dependent effects of genetic diversity on productivity. Second, rapid and pervasive behavioral niche expansion within populations (Figure 3.1) may have masked or mitigated the effects of genetic variation on productivity at different densities. Further experiments with a wider range of population densities in each habitat could test these alternative hypotheses and shed light on the exact mechanism through which genetic variation influenced the relationship between productivity and population density in the novel habitat.

Finally, it is important to address the fact that low productivity of high-competition populations (Figure 3.3B) lead to a discrepancy between the designed and actual difference in

population density between the competition treatments (also see Methods). High- and low-competition treatments (initiated with 200 and 400 adults at densities of 10 and 20 adults per g wheat flour) were chosen to be slightly below and above the optimal adult density in *T. castaneum*, ~15 adults per g flour (Sokoloff 1977). *T. castaneum* is regulated by negative density dependence, and thus the productivity of high-density populations was lower than what was required to initiate each generation (Figure 3.3B), and varied within populations at each generation. Consequently, the effective degree of competition in high-density treatments was lower than planned, although it was still higher than the low-density treatments (300 vs. 200). Hence, the competition effect measured by this experiment was likely underestimated due to temporal and between-population variation in the number of founding adults in high-density populations. A true two-fold difference in population density would thus have a greater impact on niche evolution than that measured in this experiment.

Conclusions

This experiment identifies a previously untested interaction between genetic variation and intraspecific competition as a factor that promotes resource niche expansion. The results further indicate that intraspecific competition may be a major factor driving the rate of niche evolution, while genetic variation in niche use may largely determine the degree to which individual niche variation is maintained in populations. Perhaps most interestingly, the experiment shows that the two factors have different impacts during various stages of niche evolution. Intraspecific competition and genetic variation for niche use are ubiquitous attributes of natural populations, and both have important implications for population ecology and evolution. Experimental evidence of their interaction and their impact during niche evolution is a step toward enhancing our understanding of how such intrinsic properties shape the future dynamics of populations.

TABLES

Table 1.1: Among-strain trait variation

Mean values (\pm standard error) are shown for each trait, measured for isolated individuals of each of the four experimental strains. n indicates the sample size for each strain (and flour type, where applicable).

Strain	Fecundity		% Egg survival		% Larvae choosing
	<i>n = 36 females</i>		<i>n = 150 eggs</i>		corn patch
	Wheat	Corn	Wheat	Corn	<i>n = 46 larvae</i>
Col-2	16.25±4.8	1.5±0.65	100±0	85.7±0.03	43.48±0.07
Pak-3	19.8±3.08	5.4±1.29	100±0	54.45±0.04	15.21±0.05
Z-7	8.33±1.67	1.0±0.58	86.65±0.03	61.65±0.04	13.04±0.05
Tiw-5	18.6±1.54	1.4±1.17	87.35±0.03	6.75±0.02	34.78±0.07

Table 1.2: Experimental design

The experimental design used for each of the three resource types (wheat only, corn only, or wheat + corn) is shown. The four *T. castaneum* strains are denoted C (Col-2), P (Pak-3), Z (Z-7), and T (Tiw-5). Each experimental population was founded with 120 adults and equal proportions of constituent strains as indicated, in 50 g flour (for the wheat + corn habitat, 25 g each of each flour type in adjacent patches).

Genetic variation (# founding strains)	# Possible strain combinations	Fraction individuals / strain	Replicates / combination	Total populations
Least (1)	4 (C/ P/ Z/ T)	1	5	20
Low (2)	6 (CP/ PZ/ ZT/ CT/ CZ/ PT)	0.5	2	12
Intermediate (3)	4 (CPZ/ PZT/ CPT/ CZT)	0.33	2	8
High (4)	1 (CPZT)	0.25	5	5

Table 1.3: Results of permutation test for mean population size and stability

Response variable	Effect	P (Permuted effect ≥ Observed effect)
CV	Habitat	< 0.0001
	Genetic variation	0.001
	Habitat x Genetic variation	0.48
Mean size	Habitat	0.001
	Genetic variation	0.026
	Habitat x Genetic variation	0.46

Table 1.4: Strain-specific response to increased genetic variation in different habitats

Results of Tukey-HSD pairwise contrasts are given for all single-strain and four-strain (“All”) populations in each habitat (W = wheat; WC = wheat + corn), along with the difference in the mean response of each single-strain and four-strain combination.

Response	Strain (combination)	W		WC	
		Difference (strain - All)	P adjusted	Difference (strain - All)	P adjusted
CV	Z-7 (1)	0.14	0.006	0.23	<0.001
	Pak-3 (2)	-0.02	0.963	0.09	0.017
	Col-2 (3)	0.15	0.008	0.22	<0.001
	Tiw-5 (4)	0.12	0.033	0.33	<0.001
Mean size	Z-7 (1)	-2.55	<0.001	-2.96	<0.001
	Pak-3 (2)	0.41	0.619	-0.47	0.262
	Col-2 (3)	-0.90	0.187	-2.44	<0.001
	Tiw-5 (4)	-0.25	0.96	-1.48	0.001

Table 2.1: Analysis of evolutionary change in resource use and individual variation in resource use

Response variable	Effect	Pr (Permuted effect size \geq Observed effect size)
Mean $\delta^{13}\text{C}$	Time	0.01
	Genetic variation	0.73
	Time x Genetic variation	0.17
Variance	Time	0.49
$\delta^{13}\text{C}$	Genetic variation	0.60
	Time x Genetic variation	0.61

Table 3.1: Experimental design

The four *T. castaneum* strains are denoted C (Col-2), P (Pak-3), Z (Z-7), and T (Tiw-5). Each population was started in 40g flour (20g each of wheat and corn in adjacent patches). Numbers in parentheses denote the number of replicate populations for each strain combination within each treatment group.

Genetic variation (Number of strains; possible strain combinations)	Intraspecific competition for wheat	
	Low (10 adults/g wheat)	High (20 adults/g wheat)
Low (2; CP/PT/TZ/CZ/PZ/CT)	6x(3) = 18	6x(3) = 18
High (4; CPTZ)	1x(3) = 3	1x(3) = 3

Table 3.2: Results of permutation tests for evolutionary change in resource use

P values from permutation tests are shown for each response variable- change in mean $\delta^{13}\text{C}$ or change in variance $\delta^{13}\text{C}$ for replicates of each strain combination, calculated between different generations (contrasts). The effect size used to calculate the effect of each explanatory variable is also shown. GV = genetic variation; Comp = competition. “Interaction group” refers to the four treatment groups created in the factorial experimental design (Table 3.1; Methods).

Response variable	Effect	Effect size	Contrasted generations		
			0 - 4	4 - 8	0 - 8
Δ (Mean $\delta^{13}\text{C}$)	Genetic variation	Δ (High GV) – Δ (Low GV)	0.02	0.261	0.19
	Competition	Δ (High Comp) – Δ (Low Comp)	0.44	0.036	0.16
	Interaction	σ^2 (Δ Interaction group)	0.53	0.028	0.70
Δ (Variance $\delta^{13}\text{C}$)	Genetic variation	Δ (High GV) – Δ (Low GV)	0.06	0.023	0.42
	Competition	Δ (High Comp) – Δ (Low Comp)	0.40	0.347	0.29
	Interaction	σ^2 (Δ Interaction group)	0.22	0.256	0.72

FIGURES

Figure 1.1: Population extinction as a function of founding genetic variation

Data for the novel resource (corn) is shown by solid circles and solid line; data for the ancestral resource (wheat) is shown using open circles and dashed line; plus signs and dotted line indicate proportion of extinct populations in the heterogeneous wheat + corn habitat.

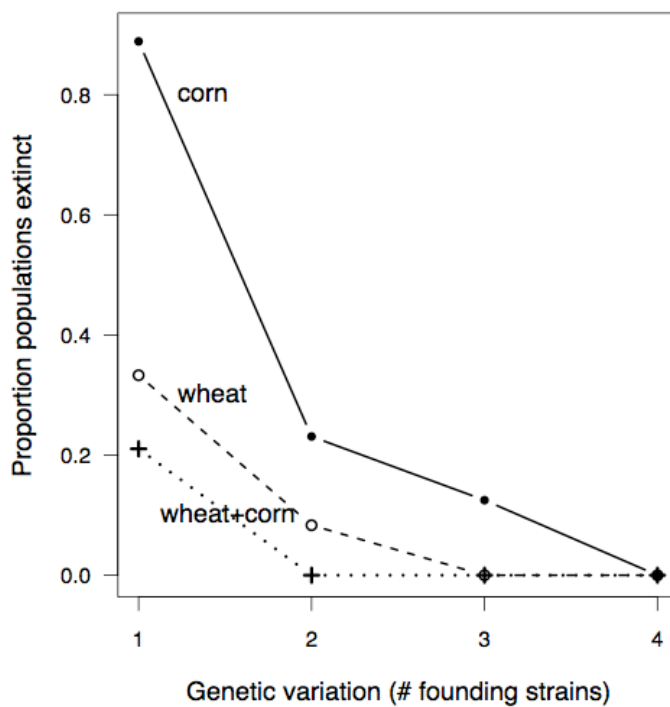


Figure 1.2: Population stability and size as a function of genetic variation

Population parameters as a function of genetic variation in two habitat treatments, wheat (filled circles, solid line) and wheat + corn (open diamonds, dashed line) are shown. (A) CV of population size, and (B) natural log of mean population size are shown as a function of founding genetic variation. Each point is the mean value for all replicate populations founded by a specific strain combination, with least squares regression lines for each habitat. Overlapping data points were slightly displaced along the x-axis while plotting so that all data points would be visible.

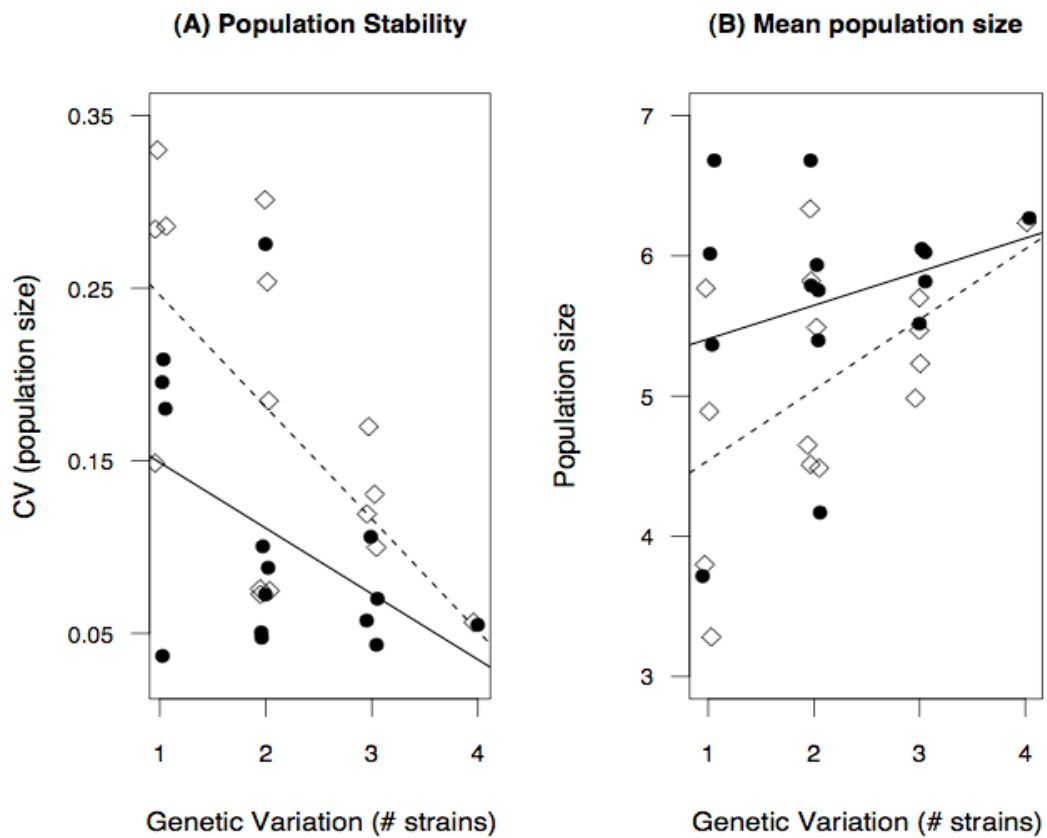


Figure 1.3: Low- and high-diversity contrasts in different habitats

Panels show boxplots for mean population size (A & B) and CV of population size (C & D) in two experimental habitats for single-strain (Col2/Pak3/Z7/Tiw5) and high-diversity populations composed of all four strains (“All”). The dashed horizontal line shows the mean value (size or CV) of the single-strain populations as indicated on the x-axis.

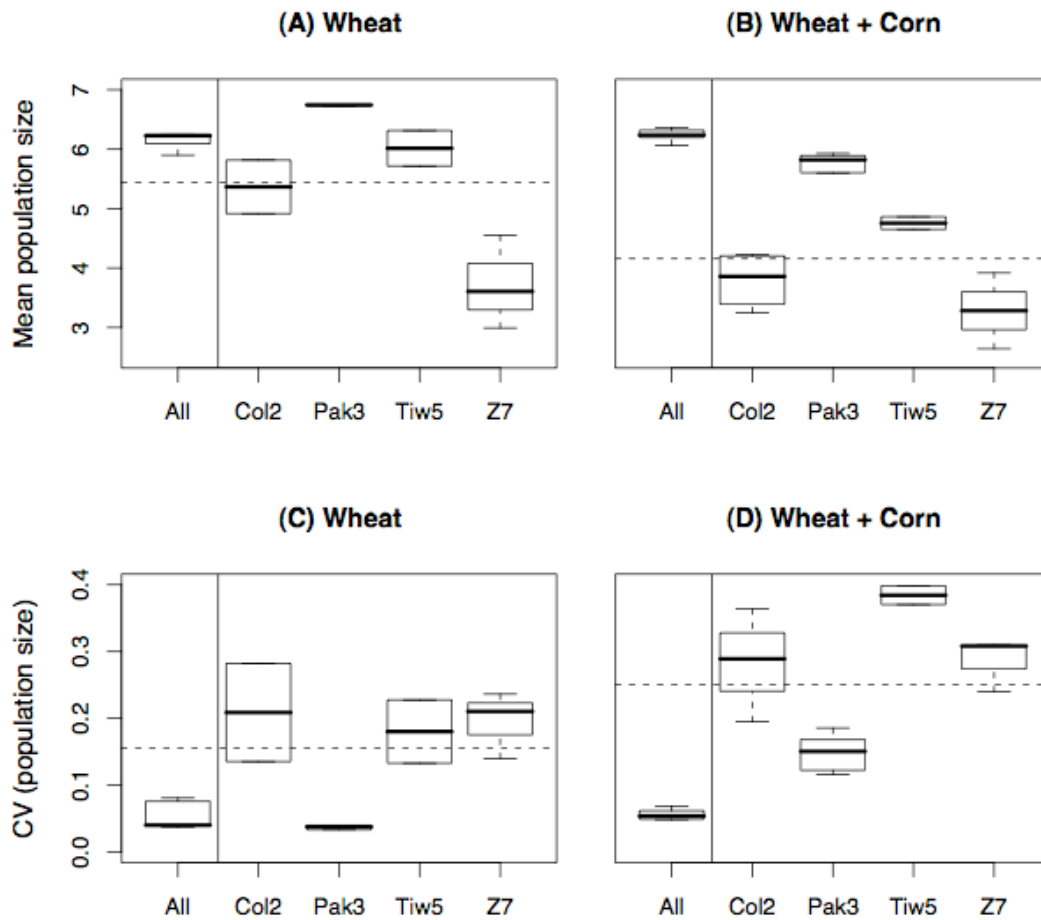


Figure 1.4: Effect of fraction of Pak-3 adults on population size and stability

Effect of fraction of Pak-3 adults on CV and mean population size is shown, where each point represents data for a single population. For values > 0 , the x-axis also represents a decreasing level of genetic variation (e.g. a Pak-3 fraction of 0.25 indicates high-diversity all-strain populations, and a Pak-3 fraction of 1 indicates single-strain Pak-3 populations). The leftmost data points in each panel represent all populations founded without any Pak-3 individuals (fraction Pak-3 = 0). After excluding these points, the least-fit regression of CV and population size on the fraction of Pak-3 adults is indicated with solid lines.

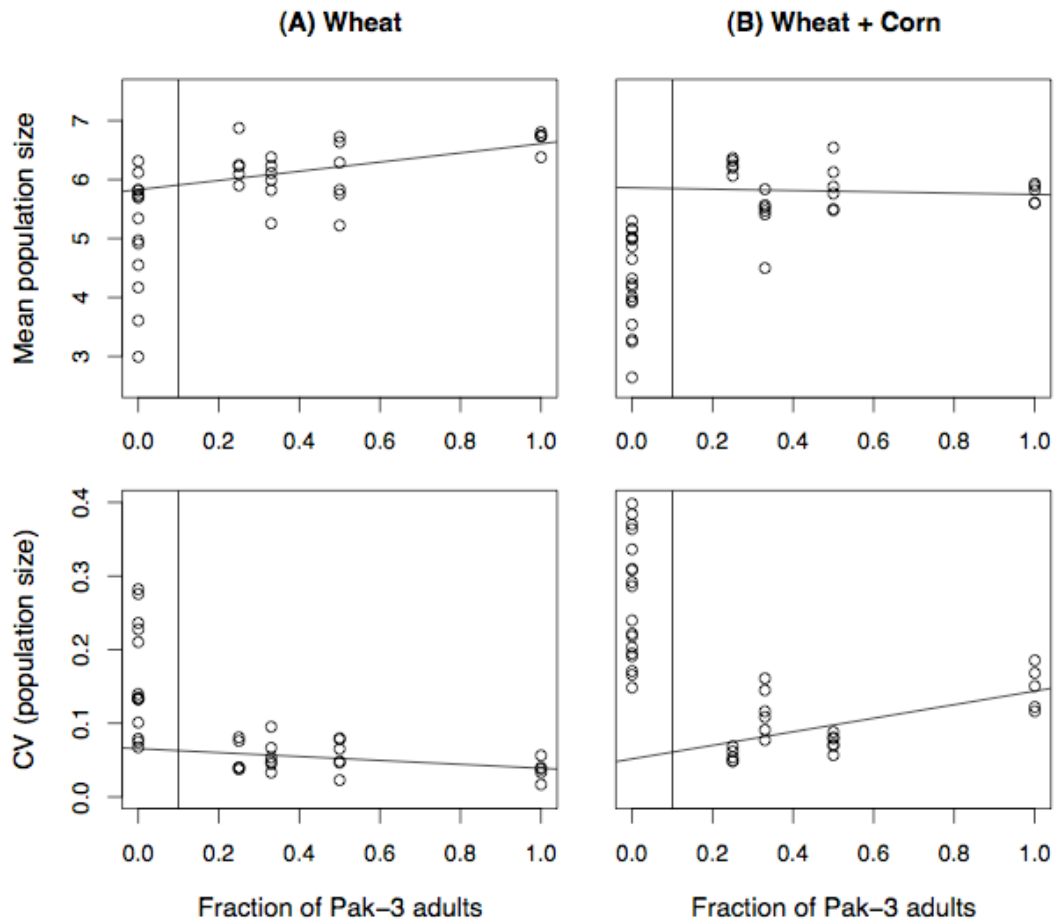


Figure 2.1: Population dynamics in the novel corn-only habitat

Each panel shows data for replicate populations of a given strain combination within each level of genetic variation: 4 combinations for single-strain (GV=1) populations; 6 combinations of two strains each (GV=2); four combinations of three-strain populations (GV=3), and a single strain combination with the highest diversity (GV=4) founded using all four strains. Smoothed lines were predicted from a generalized additive model, excluding data from one population each from strain combinations 1 and 2 (bottom left panels) that increased in size during weeks 4-6. Horizontal dashed lines indicate population sizes of 0 (extinction) and 60 ($t_{1/2}$).

Horizontal dashed lines indicate population sizes of 0 (extinction) and 60 ($t_{1/2}$).

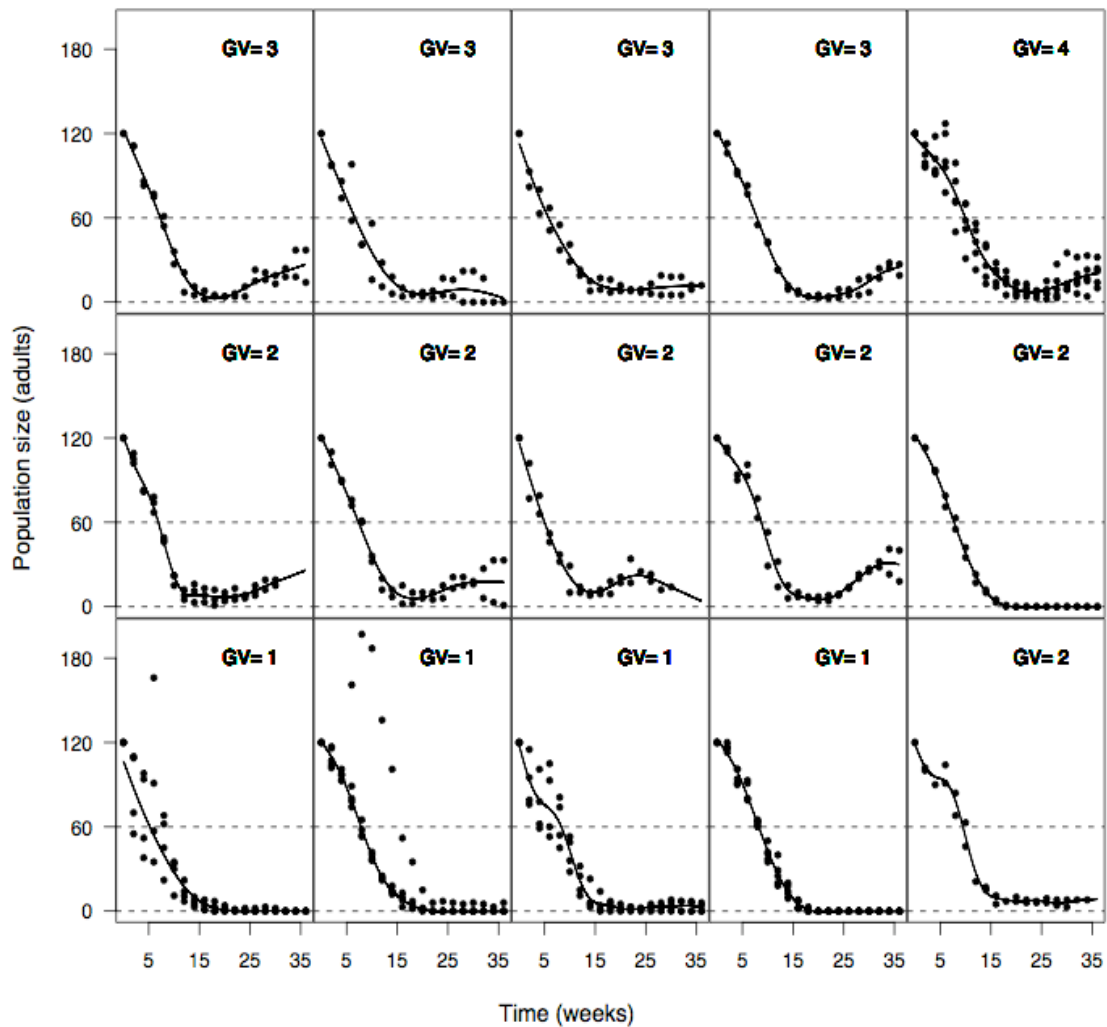


Figure 2.2: Resource niche expansion in experimental populations

Temporal change in the mean $\delta^{13}\text{C}$ of 10 beetles from 2-3 replicate populations of each strain combination is shown. Data at time 0 indicate isotope ratio in wheat + yeast, variation in which leads to a maximum error of $\pm 5\%$ in the estimated proportion of corn flour in beetle diets (see Methods), shown along the right y-axis. The degree of genetic variation is indicated by GV for each row. Left hand panels (A) show behavioral change in resource use (measured as $\delta^{13}\text{C}$) of adults within the first 2 weeks of the experiment, and right hand panels (B) show evolutionary change in resource use across ~ 6 generations. Data in the top row are labeled with the founding strain (Z = Z-7; P = Pak-3; C = Col-2; T = Tiw-5).

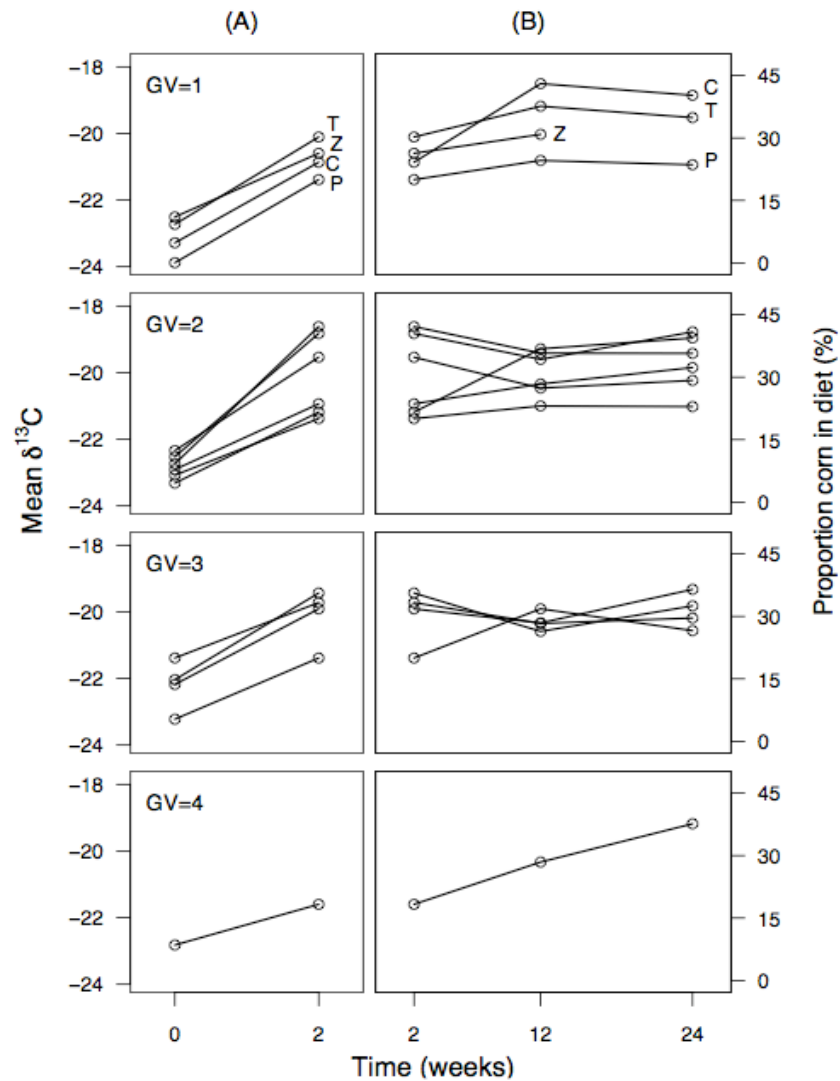


Figure 2.3: Correlation between the rate of behavioral and evolutionary niche expansion

Each data point represents the mean slope of mean $\delta^{13}\text{C}$ vs. time, calculated by fitting a linear model in turn to replicates of each strain combination. Data are labeled with the strain combination in increasing order of the degree of genetic variation: combinations 1 to 4 are single-strain populations (GV=1); 5-10 were founded by two strains each (GV=2); 11-14 are three-strain (GV=3), and combination 15 represents highest diversity populations founded using all four strains (GV=4).

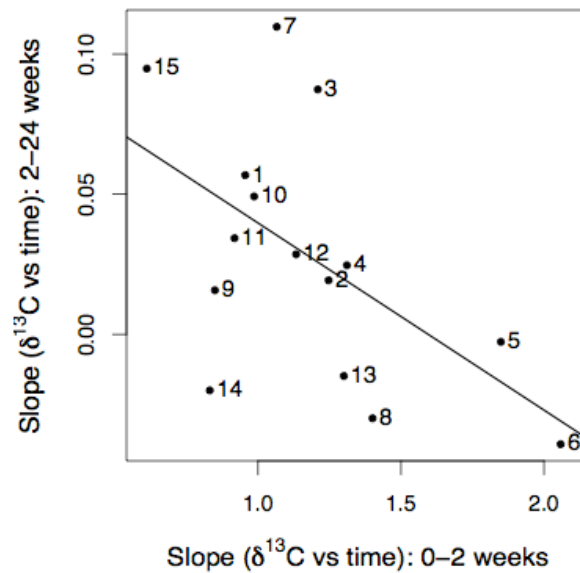


Figure 2.4: Correlation between niche expansion and relative population size

Correlation between relative population size in the wheat + corn habitat and the rate of behavioral niche expansion (A) and evolutionary niche expansion (B) is shown. Solid lines show the linear relationship between the two variables, and dashed lines show the best-fit relationship after omitting data for strain combination 3 (single-strain Col-2 population). Each data point represents the mean of all replicates of a given strain combination. Combinations 1 to 4 were single-strain populations (GV=1); populations 5-10 were founded by two strains each (GV=2); combinations 11-14 were three-strain populations (GV=3), and 15 represents highest diversity populations founded using all four strains (GV=4).

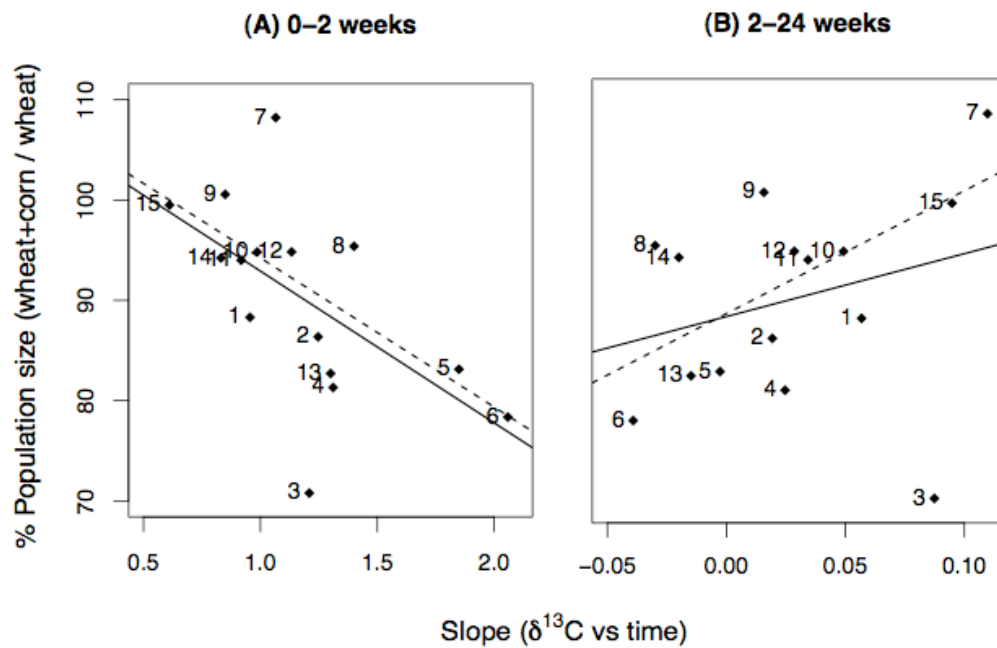


Figure 3.1: Temporal change in average resource use of populations

Each data point is the mean value of the response variable, from two replicate populations with a specific strain combination and treatment. Within-generation change represents behavioral change in resource use, calculated as the difference between mean $\delta^{13}\text{C}$ of populations after one experimental week and initial $\delta^{13}\text{C}$ in wheat flour (~ -23). Across-generation change represents evolutionary change in resource use. Lines connect means of competition treatments; asterisks denote significant differences in means. GV = genetic variation.

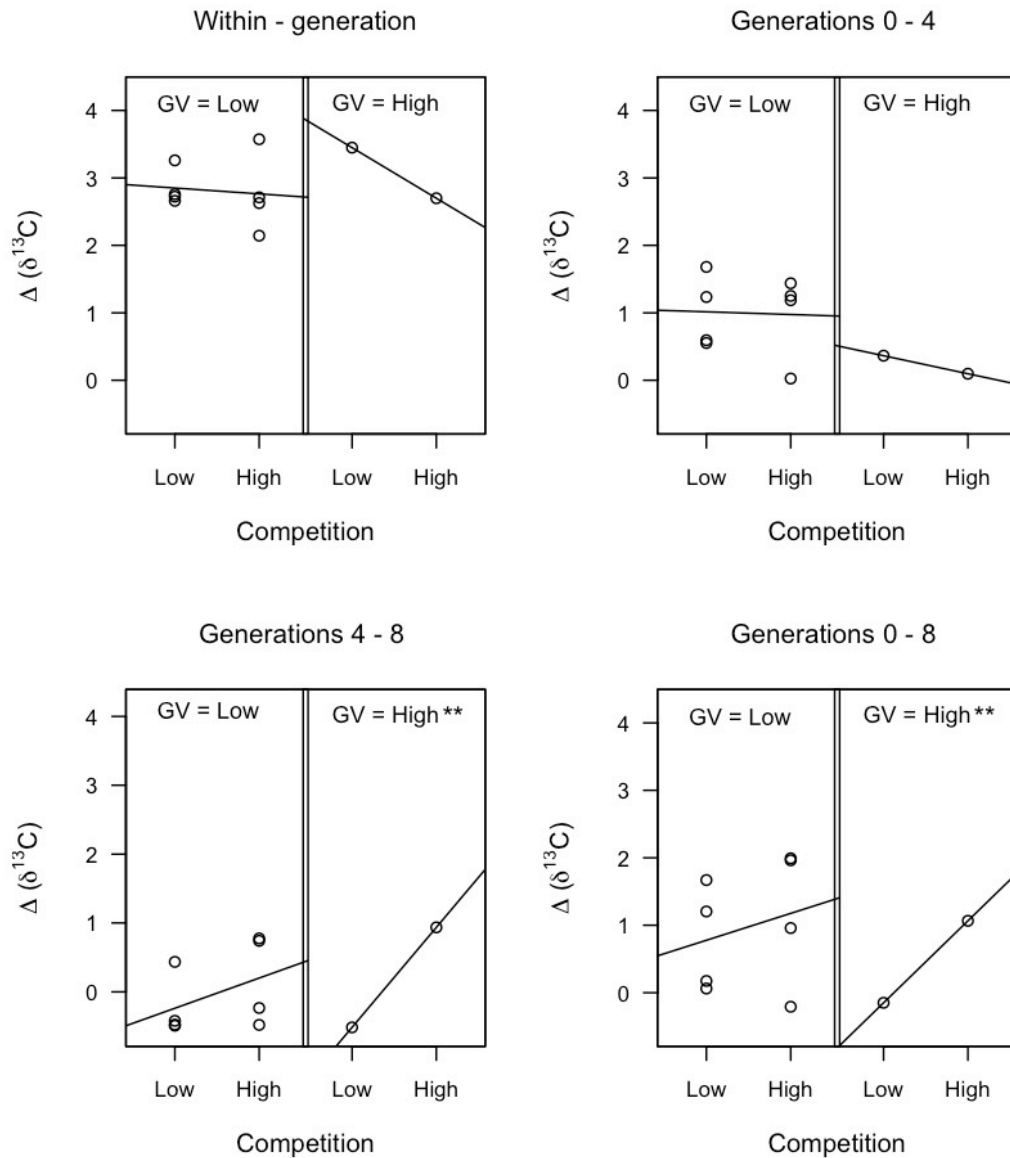


Figure 3.2: Temporal change in among-individual variance in resource use

Each data point is the mean value of the response variable, for two replicate populations with a specific strain combination and treatment. Data at generation 0 represent among-individual variance in resource use following behavioral niche expansion; data for generations 0 – 4; 4 – 8; and 0 – 8 represent change in the variance in resource use following evolutionary niche expansion. Lines connect means of competition treatments; asterisks denote a significant difference between competition treatments. GV = genetic variation.

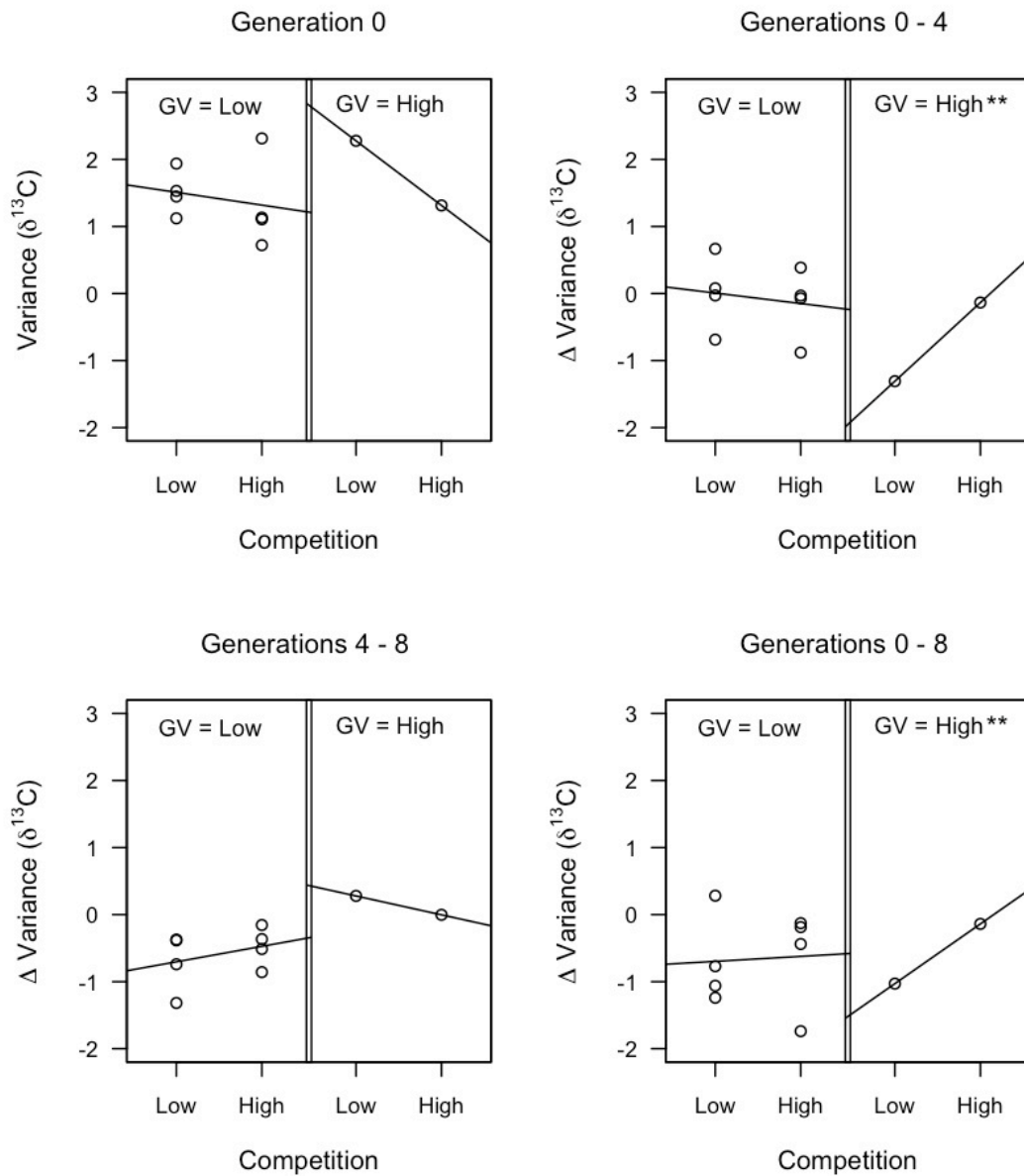
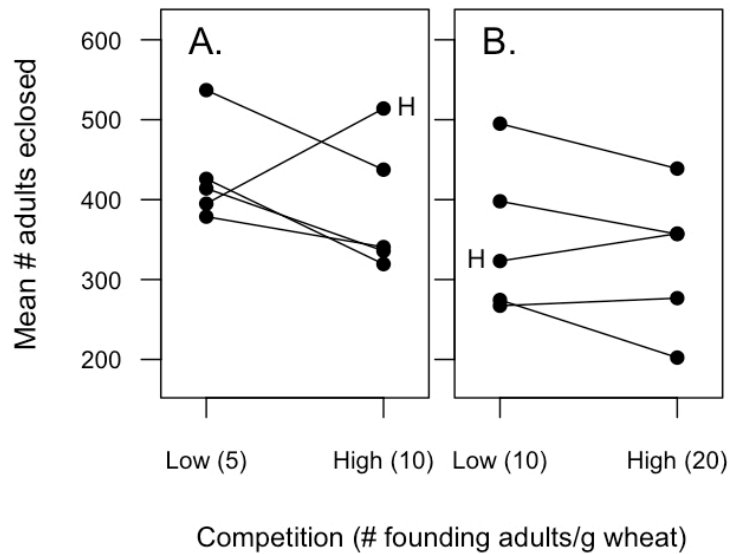


Figure 3.3: Population productivity

Mean number of eclosing adults (averaged across all 8 generations) as a function of competition, in control wheat-only (A) and experimental wheat + corn (B) habitats. Populations with high genetic variation are labeled “H”. Note that populations in both habitats were founded with equal numbers of adults (200 for low competition and 400 for high competition) in equal quantities of total resource, but the effective degree of resource competition was measured as the adult density per g of ancestral resource.



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VITA

Deepa Agashe was born in Mumbai, India on June 15, 1983, and was brought up in various Indian cities. She finished high school with the firm conviction that her career would involve “genetics research”, and that she would get started on it soon as she found out just what it meant. She enrolled in the Bachelor of Science program in Microbiology from the University of Pune and graduated in May 2003. She spent the three years during the Bachelor’s degree in the laboratory and occasionally in the forest, pottering around with a number of research projects with bacteria, invasive plants, primitive ants, and butterflies. She was duly convinced that she really enjoyed science after all- especially ecology and evolutionary biology- and that she wasn’t much good enough at anything else. In June 2003 she enrolled in a Master of Science program in Biodiversity at the University of Pune, where she saw that a career as a biologist could also entail terrifying tasks like memorizing hundreds of common and Latin names for plants, and- even worse - matching them up correctly. The following year, in September 2004, she abandoned her Master’s program to join the University of Texas at Austin as a graduate student in Ecology, Evolution and Behavior. Rumor had it that they didn’t require proficiency in Latin and had far fewer species to name than tropical India.

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