

# Dietary niche and population dynamic feedbacks in a novel habitat

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Population dynamics and resource use are often intricately connected via density-dependent intraspecific competition. However, experimental studies of concurrent change in population and resource use dynamics are scarce. In particular, the impact of factors such as genetic diversity, which can affect both population dynamics and competition, remains unexplored. Using stable isotope analysis and periodic population censuses, we quantified both diet and population dynamics in wheat-adapted *Tribolium castaneum* (flour beetle) populations provided with an additional novel resource (corn). Populations were initiated with different levels of genetic variation for traits relevant to population growth and resource use (e.g. fecundity and survival). We found that high population size decreased subsequent corn use, and high corn use in turn lowered population size. Surprisingly, we did not detect a significant effect of founding genetic variation on resource niche expansion, although genetic variation increased overall population size and stability. In contrast, dietary niche expansion decreased both population size and stability. Finally, larval and adult niche dynamics were uncorrelated, suggesting that various life stages perceive or respond differentially to intraspecific competition and resource availability. Our experiments indicate that population performance in a novel habitat depends on stage-specific interactions between resource use, standing genetic variation, and population size.

It is increasingly recognized that feedbacks between evolutionary and demographic change within populations may be more common than previously understood (Bull et al. 2006, Kokko and Lopez-Sepulcre 2007, Pelletier et al. 2007). In particular, change in individual resource preferences due to interactions between population density and resource availability can dramatically affect population dynamic trajectories. For instance, the inclusion of alternative prey in the lynx diet can decouple lynx–hare population cycles (Roth et al. 2007); cannibalism and resultant resource polymorphism stabilize populations of multiple fish species (Andersson et al. 2007); and phenotypic change alters the dynamics of various ungulate populations (Ezard et al. 2009). Additionally, behavioral responses to changing resource densities can also affect population dynamics and stability (Ma et al. 2003). Therefore, adaptation to a novel habitat or resource may depend greatly on the interactions between individual resource or habitat use and density dependent population growth.

Theory and experiments both show that individuals should adopt a newly available resource if the increased resource niche breadth confers higher fitness than the narrower ancestral niche; e.g. if it is energetically more profitable than the ancestral diet. Alternatively, the ancestral resource may be energetically more valuable, but may impose density-dependent fitness costs arising from ecological interactions such as inter- or intra-specific competition (Schluter

1994, Bolnick 2001, Svanbäck and Bolnick 2007). The rate at which an adaptive niche change occurs thus depends on the strength of selection for niche expansion, individual ability to respond to it via phenotypic or behavioral plasticity, or the population's ability to respond to selection via genetic changes (including heritable components of plasticity).

The strength of selection for dietary niche expansion itself depends on competition, mediated by population density and among-individual interactions (e.g. interference vs exploitative competition). Therefore, intrinsically fluctuating population size can cause temporal changes in selection pressure for dietary niche expansion, such as that observed in perch fish (Svanbäck and Persson 2004). At the same time, without niche expansion a population may be unable to persist in a novel habitat; hence, population density is itself contingent on resource use. However, experimental demonstration of such feedbacks between resource use dynamics and population dynamics remain scarce (reviewed by Kokko and Lopez-Sepulcre 2007). Furthermore, the impact of genetic diversity (which is necessary for evolutionary adaptation) on the nature of these associations also remains unexplored. Understanding this effect is important because genetic diversity can not only decrease intraspecific competition but also increase population growth (López-Suárez et al. 1993, Tagg et al. 2005, Hanski and Saccheri 2006, Agashe 2009, Agashe and Bolnick 2010). Therefore, genetically diverse populations may experience weaker

density-dependence and also respond faster to selection for resource use, altering the strength of interaction between resource and population dynamics.

In a previous paper, Agashe (2009) showed that founding genetic variation for ecologically significant traits (such as fecundity and survival) in flour beetle *Tribolium castaneum*-populations increased population size and stability across nine months, in a habitat containing two resource patches (ancestral wheat flour and novel corn flour). Here we analyze the temporal change in dietary niche width in the same populations, to test for interactions between resource use and population dynamics, and to determine the impact of genetic variation on these dynamics. For historically wheat-adapted *T. castaneum* laboratory populations, larval development and survival are both negatively correlated with the proportion of corn in flour mixtures (Focken 2007, Agashe 2009), suggesting that corn use is typically maladaptive and should be ignored by an optimally foraging beetle. However, *T. castaneum* populations are regulated via negative density dependence (Sokoloff 1977); therefore increasing population size can alter both population growth and selection for niche expansion (Bolnick 2001). Finally, existing genetic variation for fitness on alternative resources (Dawson and Riddle 1983) means that the strength of the association between resource use and population size (i.e. density dependence) may also vary across populations. A large body of literature documents extensive analysis of *Tribolium* population dynamics in a standard homogeneous habitat, such as the larvae–pupae–adult ‘LPA’ model (Dennis et al. 1995). However, the dynamics of various life stages in novel habitats, particularly in conjunction with niche use, remain poorly understood. Here we describe joint population size and niche dynamics in populations exposed to a new habitat, and document mutual feedbacks between change in population size and resource use.

## Methods

We used four strains of *Tribolium castaneum* (Col-2, Pak-3, Tiw-5 and Z-7; denoted C, P, Z and T) for our experiments. The strains had different fecundity and survival on wheat and corn and larval behavioral preference for corn, but similar within-strain variation for fecundity (Appendix 1 Table A1). Thus, we expected among-strain variation rather than within-strain variation to play a major role during the experiment. We manipulated founding genetic variation (GV) in our experimental populations by varying the number of strains used to initiate each population (GV = number of founding strains = 1, 2, 3 or 4); due to the combinatorics, the four levels were represented by unequal numbers of distinct strain combinations. There are four combinations with GV = 1 (C/P/Z/T), six for GV = 2 (CP/PZ/ZT/CZ/PT/PZ), four for GV = 3 (CPZ/PZT/CZT/CPT), and one for GV = 4 (CPZT). We replicated each combination twice (GV = 2 and 3) or five times (GV = 1 and 4). We founded each population with 120 adults chosen randomly from stock populations (stocks had a 1:1 sex ratio). Multi-strain populations were founded with equal proportions of component strains; note, however, that strain identity would be quickly lost during the experiment as adults interbred. Our experimental design of mixing strains resulted in an

immediate increase in among-individual variance in fecundity on both wheat and corn (Appendix 1 Fig. A1), which is expected to increase further with sexual recombination.

Experimental populations were reared at 33°C and ~70% humidity, duplicating rearing conditions used for > 20 years since their collection from the wild. Although stock populations originated from diverse geographic regions, this long history of adaptation to the lab should minimize differences in temperature or humidity tolerance. Populations were reared in identical 200 ml round ziploc containers with adjacent patches (25 g each) of wheat (plus 5% yeast) and corn flour (‘WC’ habitat) allowing free across-patch movement. In wheat flour, egg to adult development is completed in 3–4 weeks, and adults can live for a few months. However, development typically requires more than five weeks when eggs are provided only with corn (Agashe et al. 2011). Every two weeks (up to 24 weeks from the start of the experiment), we counted all stages (larvae, pupae and adults) separately for the two habitat patches in each population. We discarded dead individuals and old flour and transferred all live individuals from each flour patch to corresponding patches of fresh flour. Examples of time series data for adults, pupae and larvae are shown in Fig. 1. (For population census data for all populations and more detailed methods, see Agashe 2009).

To determine temporal change in adult corn use, we analyzed stable carbon isotope ratios of individual beetles (described below) at three different time points during the study: after two weeks (within-generation, immediate change in resource use which we term ‘behavioral niche expansion’), and after 12 and 24 weeks (longer-term change in resource use: behavioral and/or evolutionary niche expansion), chosen to maximize information on different stages of niche expansion. Because of the relatively long beetle lifespan and overlapping generations, higher sampling resolution for adult diet would be unlikely to add critical information on niche dynamics (below). We refer to increased corn use as ‘niche expansion’ because it represents an increase in the diversity of resources being used (i.e. the realized niche). We are thus not necessarily implying a change in the beetle’s fundamental niche, which may theoretically include corn flour even when corn is not present in the environment.

We also estimated the change in larval corn consumption using the proportion of larvae occupying the corn patch in each population as a proxy. We first confirmed that larval flour patch choice reliably indicates resource use. We added nontoxic neutral red vital stain to flour in one half of a 3.5 cm petri-dish and placed four larvae from stock populations in the center of the plate. Due to the vital stain, larvae that ate flour from the dyed patch appeared blue. After 24 h, we recorded larval color and position. Larval patch occupancy significantly matched resource consumption ( $n = 12$  plates; one-sample t-test for % larvae per test whose patch occupancy matched resource consumption:  $\mu_{\text{expected}} = 50\%$ ;  $\mu_{\text{observed}} = 72\%$ ;  $p = 0.002$ ). Therefore, we could use larval patch occupation as a proxy for larval resource consumption.

## Stable isotope analysis

The stable carbon isotope ratio of a sample is measured against a standard as  $\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ ,

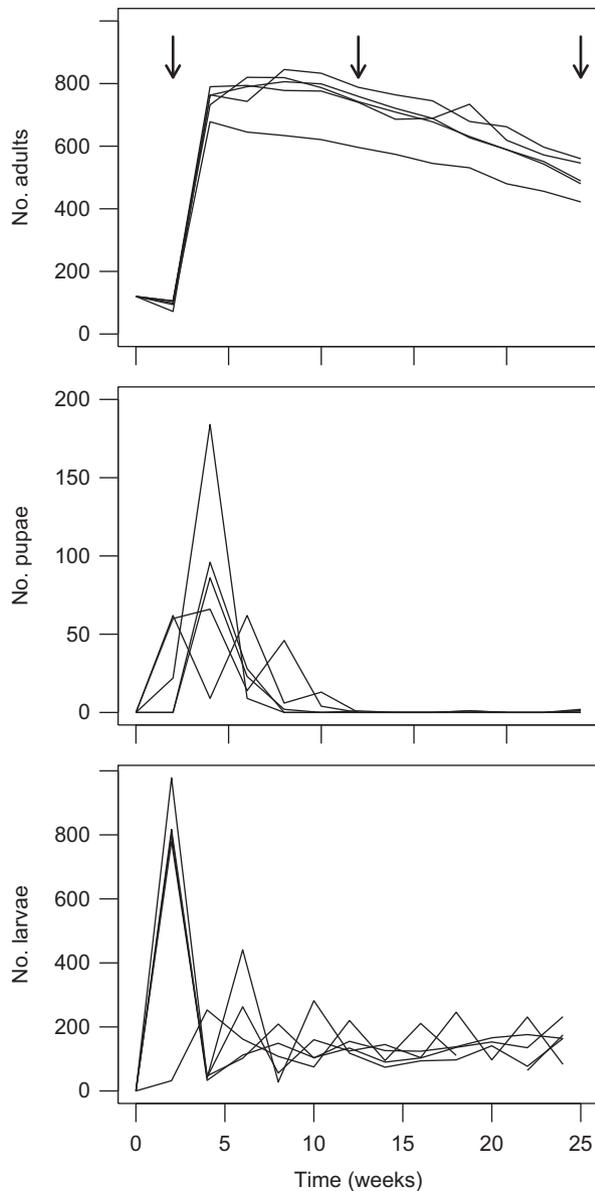


Figure 1. Example of population census data, with number of individuals of three life stages found in each of five replicate populations with the highest founding genetic variation ( $GV = 4$ ). Arrows indicate time points at which beetles were sampled for stable carbon isotope analysis.

where  $R = {}^{13}C: {}^{12}C$ . Wheat and corn have different  $\delta^{13}C$  values ( $-23.82$  and  $-11.96$  respectively). A beetle's  $\delta^{13}C$  ratio is effectively a linear function of the percentage of corn in its diet (Focken 2007), and hence can be used to infer individual and population resource use. We reared beetles solely on wheat ( $n = 15$ ) or corn ( $n = 13$ ) to obtain the relationship between beetle  $\delta^{13}C$  and diet ( $\% \text{ corn} = 1.911 + 0.08 \times \delta^{13}C$ ). At each temporal sampling point (described above), we measured  $\delta^{13}C$  of ten adults from two replicate populations per strain combination (three for  $GV = 4$ ). For each population, mean  $\delta^{13}C$  of beetles indicates average resource use. Since single-strain Z-7 populations had  $< 50$  adults after week 12 they were not sampled for isotope analysis, and these populations were not included in the final dataset. Sampled beetles were oven-dried at  $60^\circ C$

for 48 h, individually packed in tin capsules, and shipped for analysis.

Mean  $\delta^{13}C$  of beetles in the W habitat ( $\delta^{13}C_W$ ) represents baseline isotope ratios of adults in the absence of the novel corn flour. Therefore, for populations of each strain combination, the difference between mean  $\delta^{13}C$  in W and WC habitats after two weeks is a measure of the degree to which founding-generation adults added newly available corn flour to their diet. The  $\delta^{13}C$  value of beetles sampled at week 2 is an underestimate of the actual fraction of dietary corn consumed in two weeks, because some beetle tissues may still retain the signature of their larval wheat resource. However, the fraction of such tissue with slow isotopic turnover should be similar for beetles in all populations, and therefore we could compare the relative degree of corn use across populations and treatments. Similarly, the difference in mean  $\delta^{13}C$  between weeks 2 and 12, or weeks 12 and 24 measures any additional subsequent increase in corn use. Because adults can live for a month or more, these subsequent niche shifts may represent a mix of founding adults (whose behavioral niche shift was already measured) and new adults, comprising new generations. Since resource use can change via individual specialization or generalization, the degree of individual variation in resource use is also useful to understand niche dynamics (Bolnick et al. 2003). We used the within-population standard deviation in  $\delta^{13}C$  (or  $\% \text{ corn}$  used) as a measure of the degree of individual variation in resource use, analyzing it as described for mean  $\delta^{13}C$ .

*Tribolium castaneum* adults and larvae are cannibalistic (Sokoloff 1977), feeding on eggs and pupae. To test whether cannibalism played a role in resource use or population dynamics, we also quantified the stable nitrogen isotope ratio ( $\delta^{15}N$ ) of the 10 beetles sampled at week 12. If lower corn use were correlated with increased cannibalism as an alternative response to niche expansion, we would expect to find a significant negative correlation between the two isotope ratios. A positive correlation between  $\delta^{15}N$  and  $GV$  would indicate that genetically variable populations increased cannibalism, potentially altering population dynamics and feedbacks with niche dynamics.

## Data analysis

Population density data were available at two-week intervals for each experimental population, but diet was only analyzed thrice during the experiment (Fig. 1), each representing an aggregate diet measure over the intervening time period (0–2 weeks, 2–12 weeks and 12–24 weeks; corresponding to  $t = 1, 2$  and  $3$ ). We converted population size data into mean log-transformed adult population size for each interval for each population, so that both population size and corn use were now time-aggregate measures for a given time interval, rather than for a specific point in time. While 'lumping' data into slightly different-sized bins (2, 10 and 12 weeks) is not ideal, note that we are interested in quantifying both short-term change in resource use (measured after two weeks) and long-term niche expansion (measured after 12 and 24 weeks). Since the stable isotope ratios represent an aggregate diet measure, for long-term niche expansion the mean population size is the most relevant way to determine competitive and other interactions between individuals for each time period (Fig. 1).

To test for an effect of corn use on subsequent population size (i.e. population growth), we tested for a correlation between corn use for interval  $t$  and population size for interval  $t + 1$ . Likewise, to test for an effect of population size on subsequent corn use, we could test for a correlation between population size for interval  $t$  and corn use for  $t + 1$ . However, beetles perceive changes in population size immediately, and can rapidly change their resource use (Agashe and Bolnick 2010). Hence, this measure may miss concurrent changes in diet in response to population size, particularly since the longer time intervals each spanned a number of overlapping generations. Therefore, we measured both short-term and longer-term effects of population size on corn use, by measuring correlations of size at time  $t$  with corn use at time  $t$  and time  $t + 1$ , respectively. Data for larval corn use were analyzed similarly by averaging patch-specific census data for each time interval.

We could not test the effect of genetic variation on corn use at each time interval with an ANOVA, because the experimental design was unbalanced with respect to genetic variation (with 3, 6, 4 and 1 strain combinations for each GV level). Therefore, for each set of paired differences in isotope ratios calculated above (weeks 0–2, 2–12 and 12–24), we first fit a linear model to estimate the effect of each strain combination on the change in isotope signature using population replicates ( $\Delta\delta^{13}\text{C} \sim -1 + \text{strain combination}$ ). We extracted the average effect size for each GV level and associated variance in the effect size estimate from this model. Using these, we fit a weighted linear regression to test whether  $\Delta\delta^{13}\text{C}$  varied as a function of genetic variation (average  $\Delta\delta^{13}\text{C} \sim \text{GV}$ , weights =  $1/\text{estimated variances}$ ). The weighting ensured that any differences in variance due to different numbers of strain combinations within each GV level (3, 6, 4 and 1), or heteroscedasticity, did not influence the regression. We followed this procedure for each set of  $\Delta\delta^{13}\text{C}$  values (corresponding to change in isotope ratios between two time points), to test whether genetic variation altered the degree of niche expansion. To test whether there was significant niche expansion during each time period, we used a paired t test in each case (i.e. comparing  $\delta^{13}\text{C}$  at time 0 and 12, and 12 and 24 for each strain combination).

To determine whether corn use in the WC habitat enhanced net population performance, we tested for a positive correlation between average population size and degree of behavioral or total niche expansion (calculated as the difference between mean  $\delta^{13}\text{C}$  at week 24 and the initial  $\delta^{13}\text{C}_w$ ). These two correlations capture the effect of immediate niche expansion (which was large in our populations), as well as overall niche expansion. Unless mentioned otherwise, we report Pearson's product-moment correlation coefficients ( $r$ ), with  $n = 14$  for all tests. We confirmed that data were normally distributed when using t tests. Statistical analysis was conducted in R (R Development Core Team 2008).

## Results

### Population dynamics

Adult population size at week 2 was equivalent across populations, since very few adults died and none were recruited in the first two weeks (egg to adult development requires at least three weeks). Despite the presence of the novel corn

resource patch (in which development is slower), population dynamics progressed largely as predicted by the LPA model and previous experiments (Dennis et al. 2001). For *Tribolium castaneum* populations maintained under overlapping generations, eggs laid within the first few days in a new habitat have high survival, whereas subsequent eggs and pupae are largely cannibalized by growing larvae and adults. Thereafter, adult recruitment remains low until the previous larval cohort matures and older adults die, allowing a new cohort to survive to adulthood (Sokoloff 1977, Costantino et al. 1995). Accordingly, a peak in larval numbers at week 2 was followed by a sharp rise in pupae and adults (Fig. 1). After the first wave of adult recruitment (paired t-test for weeks 2 and 12: mean increase = 1.41,  $p < 0.001$ ), adult numbers declined in weeks 12–24 (Fig. 1, 2; paired t-test: mean decrease = 0.64,  $p < 0.001$ ). Overall, adult population size and stability (the coefficient of variation in population size through time, within a replicate) were both significantly higher for genetically diverse populations (Agashe 2009).

### Niche expansion

Within the first generation, all populations significantly expanded their resource niche to include the novel corn resource (Table 1, Fig. 2), which constituted up to 42% of the diet of an individual beetle. Average corn consumption was 28% (significantly lower than the null expectation of 50%; one-sample t-test:  $t = -10.72$ ,  $DF = 14$ ,  $p < 0.0001$ ), indicating active choice by founding-generation adults rather than resource use based solely upon resource availability. Therefore, resource quality rather than quantity determined resource use within this period. The diet change was not a result of differential mortality: adult survival was not correlated with the degree of corn use ( $r = 0.06$ ,  $p > 0.1$ ). The initial, rapid behavioral niche expansion (from 0% to 28% corn use in two weeks) was not followed by niche expansion in subsequent time intervals (mean corn use increased from 28% to 31% by week 12 and 33% by week 24; Table 1, Fig. 2). Therefore, maximum niche expansion during the experiment occurred within the first two weeks, via adult behavioral resource choice when beetles were initially exposed to the novel corn resource. Corn use was not affected by founding genetic variation during any time interval (Table 1). Additionally, the degree of cannibalism was not correlated with corn use at week 12 ( $r = 0.08$ ,  $p = 0.31$ ; Fig. S2) or genetic variation ( $r = 0.05$ ,  $p = 0.86$ ; Appendix 1 Fig. A2), indicating that cannibalism did not alter niche expansion in our experiment.

Table 1. Analysis of temporal change in adult resource use (see Data analysis in Methods for details of model and analyses).

Change in resource use $\Delta\text{mean } \delta^{13}\text{C}$	Effect						
	Time (DF = 13)			Genetic variation			
	$\Delta\delta^{13}\text{C}$	t	$p^a$	Est.	SE	t	p
Weeks 0–2 (behavioral)	2.395	11.5	<0.001	-0.41	0.22	-1.91	0.20
Weeks 2–12	0.34	1.1	0.28	-0.22	0.44	-0.49	0.67
Weeks 12–24	0.25	1.7	0.11	0.29	0.14	2.18	0.16
Weeks 0–24 (total)	2.961	12.4	<0.001	-0.30	0.32	-0.94	0.45

<sup>a</sup>With a Bonferroni correction for multiple tests,  $p$  adjusted = 0.009.

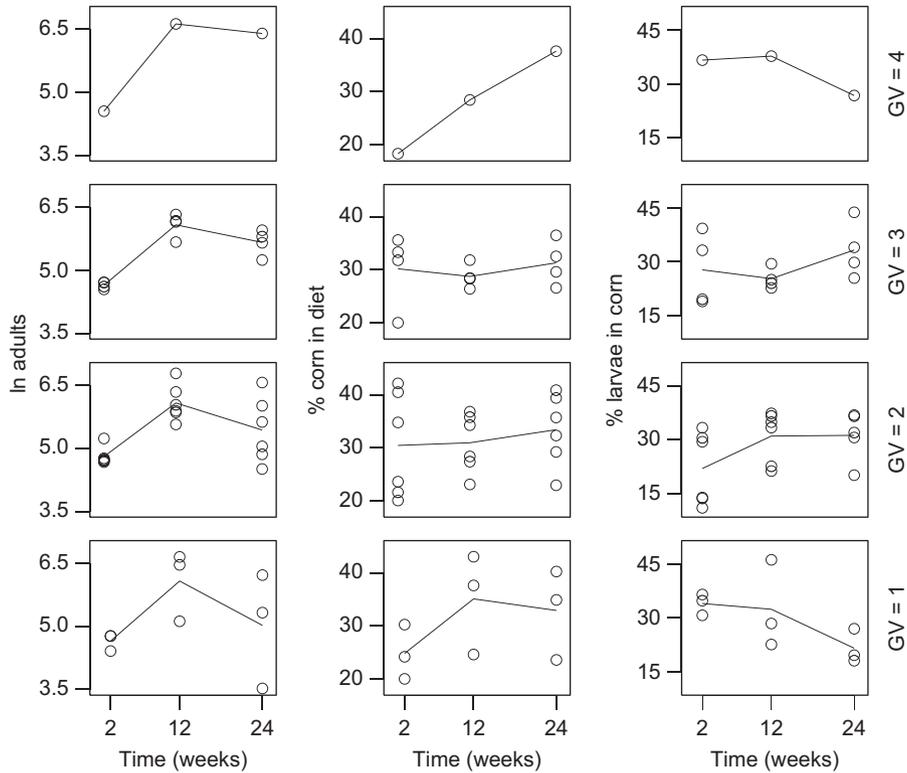


Figure 2. Panels at left show temporal change in adult population size of replicate populations of each strain combination, for each level of founding genetic variation (GV). Middle panels show corresponding change in mean resource use (% corn in diet). Panels to the right show the proportion of larvae occupying the corn patch, averaged over each time period. Lines connect means for each time point.

The degree of among-individual variation in corn use within populations was small (on average, the standard deviation in the proportion of corn used by beetles within a population was 0.098). Thus, beetles within a population mostly consumed similar amounts of corn and population niche expansion occurred via niche generalization rather than specialization (Bolnick et al. 2003), contrary to the niche variation hypothesis (Van Valen 1965, Bolnick et al. 2007). Among-individual variation in corn use did not change during the experiment, and was unaffected by genetic variation or average corn use (GLM, model:  $\sigma[\delta^{13}\text{C}] \sim \text{GV} \times \text{time} \times \text{mean } \delta^{13}\text{C}$ ,  $p > 0.25$  for all effects).

Larval corn consumption was largely constant and independent of founding genetic variation. On average, 27% of larvae in each population were found in corn patches at week 2 – significantly lower than expected from random spatial distribution (one sample t-test,  $\mu_{\text{expected}} = 50\%$ ;  $p < 0.001$ ). This proportion did not change significantly during the experiment (paired t-tests for various time intervals,  $p > 0.44$  in each case). In addition, genetic variation had no effect on larval occupancy of corn patches during the experiment ( $R^2 = 0.03$ ,  $p > 0.1$ ), or on the coefficient of variation of proportion of larvae in corn ( $R^2 = 0.68$ ,  $p > 0.1$ ).

### Concurrent diet and population dynamics

In the short term (within a time interval), adult population size was negatively associated with dietary consumption of corn within weeks 12–24 but not weeks 2–12 (Fig. 3A; weeks 12–24:  $r = -0.62$ ,  $p = 0.018$ ; weeks 2–12:  $r = -0.49$ ,  $p = 0.077$ ). However, the negative relationship between corn

use and population size was not significantly different for the two time intervals (GLM, full model:  $\text{corn use} \sim \text{size} \times \text{time}$ ,  $\text{AIC} = 175.5$ ; reduced model:  $\text{corn use} \sim \text{size}$ ,  $\text{AIC} = 172$ ,  $p_{\text{size}} = 0.002$ ). Thus, contrary to expectation, concurrent niche expansion appeared to be negatively rather than positively density dependent. In the longer term, however, population size did not have a significant effect on corn use (Fig. 3B; corn use at time  $t + 1$  is not correlated with population size at time  $t$ ;  $p > 0.13$  for both time intervals). Interestingly, the proportion of larvae occupying corn patches at week 2 was negatively correlated with the degree of adult behavioral niche expansion ( $r = -0.76$ ,  $p < 0.01$ ). Thus, in populations where adults initially consumed large amounts of corn, fewer larvae ate corn. In contrast, larval and adult corn use was uncorrelated in other time periods (Spearman correlation,  $p > 0.2$  in both cases). Therefore, resource use in the larval and adult life stages (i.e. the feeding stages) was disconnected in the experimental populations.

Adult population size at a given time is expected to be a function of previous population size (via density-dependent reproduction and cannibalism (Costantino et al. 1995) as well as previous resource use (via resource-dependent fitness). Accordingly, population size at  $t + 1$  increased as a function of previous population size, decreased with previous adult corn use, and was also affected by the interaction of population size and corn use at time  $t$  (Fig. 3C–D, Table 2). As expected, adult population size increased over time and as a function of founding genetic variation (Table 2, Fig. 2). However, genetic variation did not alter the effect of corn use on subsequent population size ( $p_{[\text{corn} \times \text{GV}]} > 0.5$ ) although it altered the positive density-dependence in population

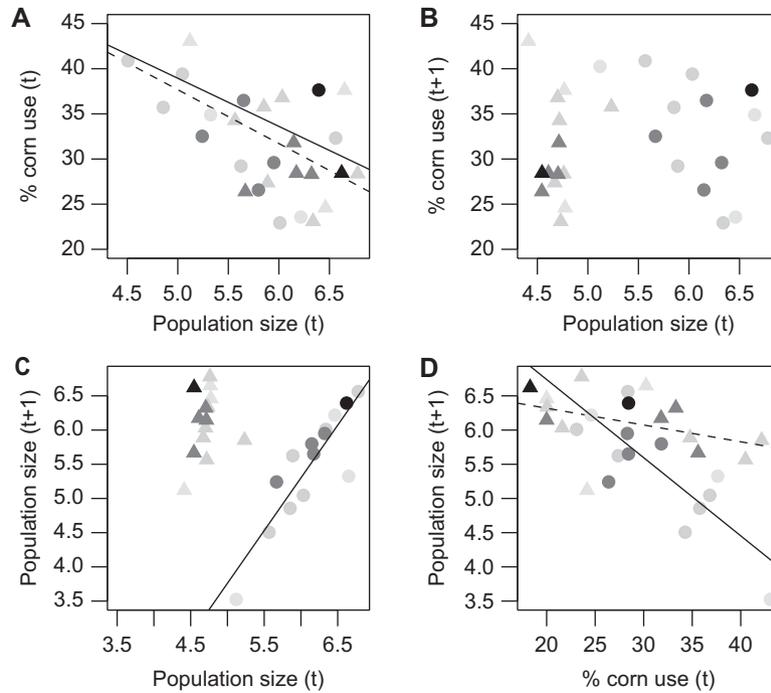


Figure 3. (A) Short-term effect of population size on corn use: proportion of adult dietary corn at time  $t$  as a function of  $\log_e$  adult population size at time  $t$ . (B) Long-term effect of population size on corn use: proportion of adult dietary corn at time  $t + 1$  as a function of  $\log_e$  adult population size at time  $t$ . (C) Average  $\log_e$  adult population size for time period  $t + 1$  as a function of population size for time period  $t$ . (D) Average  $\log_e$  adult population size for time period  $t + 1$  as a function of proportion of adult dietary corn at time  $t$ . Triangles and dashed regression lines represents data where  $t =$  weeks 0–2 (week 12 in case of panel A). Filled circles and solid regression lines represent data where  $t =$  weeks 2–12 (week 24 in case of panel A). Each data point shows the mean of replicate populations of a given strain combination. Points are shaded so that darker points show populations with greater founding genetic variation.

size ( $p_{[size \times GV]} < 0.05$ ). Finally, greater behavioral and total niche expansion was associated with low overall population size (Fig 4A;  $r = -0.57$ ,  $p < 0.05$  in both cases) and low population stability (Fig. 4B;  $r = 0.71$ ,  $314 p < 0.01$ ). Thus, niche expansion decreased overall population performance in the novel WC habitat.

## Discussion

Population density and resource use can form mutual feedback loops that are critical to understand the adaptive trajectory of populations exposed to novel habitats (Kokko and Lopez-Sepulcre 2007). Our experimental results indicate

Table 2. Analysis of adult population size.

Term	Full model <sup>a</sup> (AIC = 13.63)				Best model <sup>b</sup> (AIC = 11.66)			
	Effect	SE	t	p	Effect	SE	t	p <sup>c</sup>
Intercept	-63	38.3	-1.62	0.13	-59.0	18.8	-3.14	<b>0.008</b>
Size (t)	14.8	8.32	1.78	0.101	13.9	4.00	3.48	<b>0.004</b>
Corn (t)	1.79	1.58	1.14	0.278	1.63	0.73	2.23	<b>0.044</b>
GV	21.6	17.8	1.21	0.25	19.7	8.32	2.37	<b>0.034</b>
Time	52.2	43.7	1.19	0.256	47.8	21.5	2.22	<b>0.044</b>
Size (t) × Corn (t)	-0.38	0.34	-1.14	0.278	-0.35	0.15	-2.26	<b>0.042</b>
Size (t) × GV	-4.57	3.85	-1.19	0.258	-4.17	1.81	-2.31	<b>0.038</b>
Corn (t) × GV	-0.59	0.73	-0.81	0.432	-0.52	0.32	-1.64	0.125
Size (t) × Time	-11.5	8.93	-1.28	0.223	-10.5	3.94	-2.67	<b>0.019</b>
Corn (t) × Time	-1.32	1.67	-0.79	0.443	-1.14	0.57	-2.01	0.065
GV × Time	-6.28	22	-0.28	0.78	-3.79	5.62	-0.67	0.512
Size (t) × Corn (t) × GV	0.13	0.16	0.80	0.44	0.11	0.07	1.60	0.132
Size (t) × Corn (t) × Time	0.28	0.35	0.82	0.43	0.25	0.12	2.12	0.054
Size (t) × GV × Time	1.86	4.42	0.42	0.681	1.35	0.02	1.47	0.166
Corn (t) × GV × Time	-0.00	0.87	-0.00	0.997	-0.10	0.09	-1.16	0.266
Size (t) × Corn (t) × GV × Time	-0.02	0.18	-0.12	0.908				

<sup>a</sup>Full model: Size ( $t + 1$ ) ~ Size ( $t$ ) × Corn use ( $t$ ) × GV × Time.

<sup>b</sup>Reduced model: Full model - Size ( $t$ ) × Corn use ( $t$ ) × GV × Time interaction.

<sup>c</sup>p-values < 0.05 are highlighted in bold.

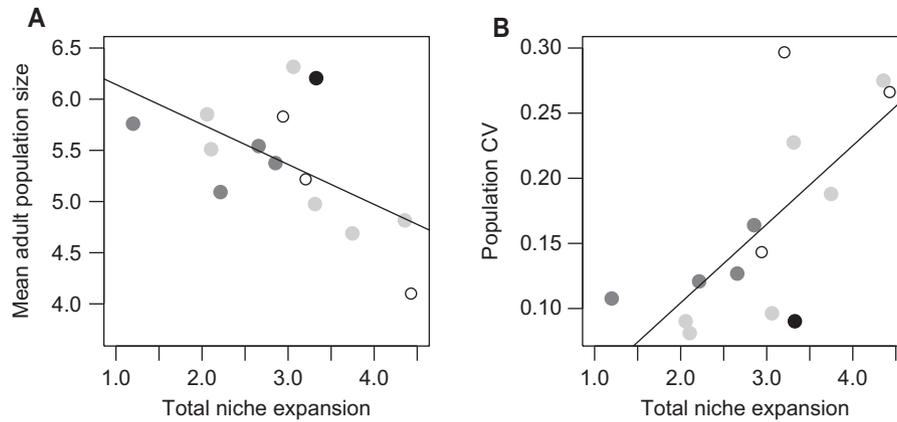


Figure 4. (A) Average  $\log_e$  adult population size and (B) population CV (inverse of population stability) as a function of total niche expansion ( $\Delta$  mean  $\Delta^{13}\text{C}$ ; Table 1), with best-fit regression lines. Each data point is the mean of 2–3 replicates of a given strain combination. Points are shaded so that darker points show populations with greater founding genetic variation.

such feedbacks between population density and resource use in a novel habitat, and show that genetic variation does not alter their strength (at least in our experimental system). Flour beetle populations where individuals quickly adopted the unfamiliar corn resource had low long-term average population size (Fig. 4). In turn, and contrary to expectation (Bolnick 2001), our results suggest that high population density can inhibit short-term niche expansion through increased use of corn (Fig. 3A). In this experiment, populations were allowed to self-regulate with overlapping generations, so that we could analyze natural population and dietary niche dynamics. Further evidence of feedbacks comes from our other experiments with *Tribolium castaneum* showing that a) forcing beetles to consume only corn decreases population size to the point of extinction (Agashe 2009, Agashe et al. 2011) and b) enforcing high population density decreases short-term corn use (Agashe et al. unpubl.) but can increase evolutionary niche expansion under discrete generations (Agashe and Bolnick 2010). Together, these results demonstrate time sensitive mutual feedbacks between population size and resource use in a novel habitat.

What was the mechanistic basis for the observed change in corn use during various time intervals? The within-generation increase in corn use represents behavioral niche expansion that could arise from a combination of pre-existing innate willingness to eat a novel resource, individual density-dependent foraging decisions, and physiological plasticity for digesting corn. Longer-term increase in corn use may represent evolutionary niche expansion (as seen in Agashe and Bolnick 2010). However, because populations were maintained under overlapping generations and some adults may live and reproduce for 12 weeks, we were unable to accurately determine the evolutionary ancestry of adult beetles sampled at weeks 12 and 24. This means that the observed longer-term change in adult corn use could be a combination of behavioral response to changing population density, evolutionary change in the degree of plasticity in corn use, and evolutionary change in non-plastic traits determining resource use. Nonetheless, it is clear that inclusion of the novel corn resource in the diet of adult beetles was associated with a subsequent decrease in population size. Compared to the ancestral wheat resource, development is

~1.5 times slower in corn, and mortality is much higher (Agashe 2009). Therefore, rapid behavioral use of corn flour is likely to be maladaptive unless it is associated with change in these heritable fitness components; i.e. unless preference was coupled with performance. We do not find such association between preference and performance: strains with particularly high or low preference for corn were not the strains with highest/lowest fitness in corn (Table 1, Agashe 2009). When preference and performance are decoupled, adoption of novel resources may thus have deleterious effects on population performance. Behavioral responses to resource limitation are widespread and are often critical for persistence in novel habitats (Ghalambor et al. 2007), e.g. during colonization by invasive species (Dybdahl and Kane 2005). Recent empirical and theoretical results also show that adaptive foraging by predators can stabilize diverse communities that are otherwise predicted to go extinct (reviewed by Valdivinos et al. 2010). Such wide-ranging effects of individual niche shifts stress the need to further understand their impact on population dynamics under various forms of selection on resource use.

Larval and adult corn use were initially negatively correlated (at week 2), and subsequently decoupled. This was counter to our expectation that larval resource use would be largely determined by female oviposition, and thus positively correlated with female resource use. However, adult resource use may not directly translate into oviposition preference if females oviposit indiscriminately (Sokoloff 1977); if higher mortality in corn alters offspring abundance (Agashe 2009); or if larval movement across patches unlinks female oviposition and larval corn use (Patel and Agashe unpubl.). Any or a combination of these factors could have resulted in the observed disconnect between larval and adult corn use. Finally, negative pleiotropic or epistatic relationships between adult and larval corn acceptance may also lead to the opposite, stage-specific behaviors. Our observations are consistent with previous experiments with a corn-oil sensitive *T. castaneum* strain demonstrating stage-specific distribution in heterogeneous habitats with corn oil patches (Jillson and Costantino 1980). Notably, in mixed species populations, *T. castaneum* consistently drove its competitor (*T. brevicornis*) to extinction despite its smaller niche breadth,

because high adult density delayed larval development in *T. brevicornis*. Thus, interactions between individuals arising from population density and niche use can also determine whether competitive coexistence is possible. It is also surprising that the amount of founding genetic variation for resource use did not alter total niche expansion (Table 1) or the relationship between corn use and population size (Fig. 3), despite its strong effects on population size and stability (Agashe 2009). However, at intermediate diversity levels (GV = 2 and 3), most niche expansion occurred initially; while at extreme levels of diversity (GV = 1 and 4), relatively greater niche expansion occurred over the longer term (Fig. 2). Overall, it is likely that the magnitude of long-term niche expansion (including both behavioral and evolutionary change) was so small compared to immediate behavioral niche expansion, that genetic variation for resource use placed a negligible constraint on niche evolution during the experiment. However, in a separate experiment where *T. castaneum* were maintained at high population density (strong competition), we found that genetic diversity significantly facilitated evolutionary niche expansion (Agashe and Bolnick 2010) and maintained greater individual variation in resource use across multiple generations. Thus, while genetic variation may not increase among-individual niche variation (as observed here), it can maintain initial variation across evolutionary time periods.

In summary, stage-specific resource use dynamics, together with individual and genetic variation in behavioral preference and fitness on the two resources, determined population density and intraspecific competition in our experimental populations. These dynamics in turn affected subsequent selection acting on resource use and fitness. We have thus shown how resource use and population dynamics can affect each other in temporally invariant, novel habitats. Given the ubiquity of density-dependent resource competition and habitat heterogeneity in natural systems, we expect that such associations between resource niche and population dynamics are a widespread phenomenon. We hope that our results spur further experimental work, especially to analyze how factors such as temporal variation in resource availability, migration, spatial structure, or interspecific competition alter the relationship between resource use and population dynamics. Such investigations into the mechanics of adaptation to novel environments are especially relevant in understanding how populations may adapt to altered habitats and communities as a result of global climate change.

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## Appendix 1

Table A1. Trait variation in the four experimental strains. Mean  $\pm$  SE for three traits are shown, with n individuals measured per strain per flourtype (where applicable). Standard deviation for fecundity is shown in parentheses. Strain Col-2 has significantly higher within-strain variance for fecundity on wheat (ANOVA:  $p = 0.043$ ). Table from Agashe et al. 2011.

Strain	Fecundity n = 36 females		% egg survival n = 150 eggs		% larvae choosing corn patch n = 46 larvae
	Wheat	Corn	Wheat	Corn	
Col-2	16.25 $\pm$ 4.8 (5.36)	1.5 $\pm$ 0.65 (1.97)	100 $\pm$ 0	85.7 $\pm$ 0.03	43.48 $\pm$ 0.07
Pak-3	19.8 $\pm$ 3.08 (4.3)	5.4 $\pm$ 1.29 (2.78)	100 $\pm$ 0	54.45 $\pm$ 0.04	15.21 $\pm$ 0.05
Z-7	8.33 $\pm$ 1.67 (3.16)	1.0 $\pm$ 0.58 (1.86)	86.65 $\pm$ 0.03	61.65 $\pm$ 0.04	13.04 $\pm$ 0.05
Tiw-5	18.6 $\pm$ 1.54 (3.04)	1.4 $\pm$ 1.17 (2.65)	87.35 $\pm$ 0.03	6.75 $\pm$ 0.02	34.78 $\pm$ 0.07

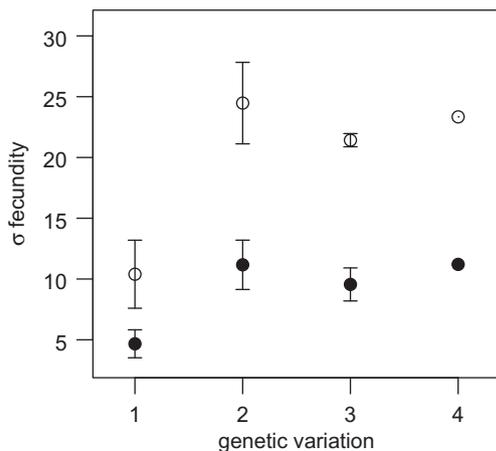


Figure A1. Initial within-population standard deviation in fecundity on wheat (open circles) and corn (filled circles) is shown for each level of genetic variation (GV). Standard error bars reflect variation between strain combinations within a given GV. Figure from Agashe et al. 2011.

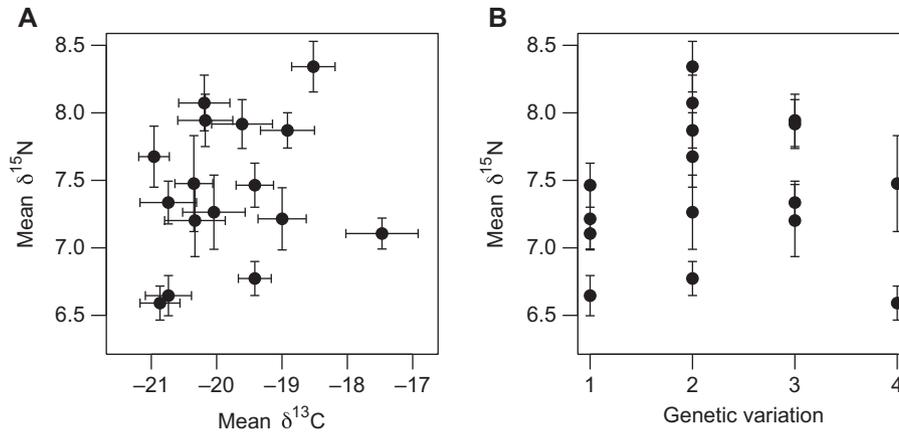


Figure A2. (A) Nitrogen versus carbon stable isotope ratios (B) Nitrogen isotope ratios versus founding genetic variation. Each data point shows the average carbon and/or nitrogen isotope ratio for ten individuals sampled from one replicate population each for strain combinations sampled at 12 weeks, with standard error bars. Two replicate populations were sampled for  $\text{GV} = 4$ .