University of Nevada, Reno

Using *Callosobruchus maculatus* as a model system for studying ecological responses to genetic diversity and evolutionary responses to selection

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Ecology, Evolution, and Conservation Biology

By

Kevin J. Burls

Guy Hoelzer/Dissertation Co-Advisor
Matthew Forister/Dissertation Co-Advisor

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The Graduate School

We recommend that the dissertation prepared under our supervision by

Kevin J. Burls

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be accepted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Guy Hoelzer, Ph.D., Advisor

Matthew Forister, Ph.D., Committee Member

Marjorie Matocq, Ph.D., Committee Member

Elizabeth Leger, Ph.D., Committee Member

Thomas Nickles, Ph.D., Graduate School Representative

Marsha H. Read, Ph.D., Dean, Graduate School

May, 2014
Abstract

Genetic variation for fitness-related and ecological traits is crucial for the ecological and evolutionary responses of populations. Studying the processes by which genetic variation influences ecological and evolutionary processes has greatly benefitted from experimental studies. The seed beetle *Callosobruchus maculatus* has become a model system for studying quantitative trait variation and evolution in experiments that either manipulate genetic variation or artificially select on one more traits. In my dissertation I use *C. maculatus* as a model system for studying the ecological and evolutionary responses of a population via two different experimental approaches.

Chapter II details an experiment to measure the ecological consequences of genetic diversity. We used replicate populations that varied in their genotypic diversity, and also their individual host preference, and measured how different combinations of genotypes and genotypic diversity influenced group resource use and productivity. We found a nonlinear increase in the benefits of diversity; that is, intermediately diverse groups had the highest productivity.

In Chapter III we used an artificial selection experiment to measure if traits involved in the evolution of dispersal are related to important life history traits in this system, and if these correlations between traits interact to influence the ability of the population dispersal distributions to shift over time. We selected on replicate *C. maculatus* populations for 20 generations. While we found an initial increase in offspring dispersal, this was not followed by continuous evolution in dispersal distance over time. However, following selection we found clear differences between dispersal and life
history traits that suggest energetic constraints involved in life history traits may constrain the ability of dispersal distributions to increase.

In Chapter IV we used beetles from the selection experiment to measure the presence and strength of correlations between different traits that are involved in organismal movement. We found that high dispersal individuals moved faster, rested less, and moved further overall than their control counterparts. These results suggest that these traits may be developmentally linked, which may make it easier for a complex trait (organismal movement over a lifetime) to evolve multiple components simultaneously.
Dedication

To the community of people who helped me complete this project.
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CHAPTER I: Introduction

Genetic variation in a population is a vital part of both the evolutionary and ecological responses of populations to a given environment. Genetic variation has long been known to be a necessary component for adaptation, without which there can be no response to selection (Fisher 1937, Ford 1964). In addition to variation for single traits, genetic correlations between traits can cause correlated evolution of traits other than those that are being selected, and these correlations have the potential to increase or decrease the magnitude of trait evolution seen in a given population (Roff 2007, Conner 2012).

One topic where the concept of correlated evolution may be especially important is the evolution of dispersal, or movement of organisms that results in gene flow across space and time (Ronce 2007). Dispersal traits— including morphological, physiological, and behavioral traits— have been shown to be responsive to selection (Palmer and Dingle 1986). In addition, life history traits have also been shown to co-evolve in a correlated manner with dispersal traits (Roff and Bradford 1996). These correlations may be positive or negative, depending on the genetic architecture of the traits and the nature of selection on life history traits in contrast with dispersal traits. However, the consequences of correlated selection of life history and dispersal traits for the realized dispersal ability of individuals and of populations has rarely been studied due to the difficulty of isolating genetic and environmental causes of dispersal variation (Perkins et al. 2013).

In addition to evolutionary responses, genetic variation has the potential to influence the ecological characteristics of populations. For example, genetically diverse populations may exhibit increased population productivity or resource use, increased
community diversity, increased or decreased pressure from natural enemies, and increased ecosystem-level functionality (Hughes et al. 2008). When these traits depend on the genetic diversity of a population, they may be able to influence the amount of standing genetic variation, as well as the selective pressures on different genotypes in the population (Donohue et al. 2005).

The ecological and evolutionary consequences of genetic diversity are topics that have received a large amount of theoretical and empirical research, and both are topics that have benefitted from an experimental approach. For example, understanding the effects of genetic diversity on ecological traits like population productivity depends on maintaining other characteristics, like population size and density, genotype frequencies, and resource quality, constant. Similarly, a population’s response and correlated responses to selection are contingent on the amount of genetic variation present at the start of selection, along with the direction and magnitude of covariance between traits, the strength and direction of selection, and effective population size (Fuller et al. 2005).

In my dissertation, I use the seed beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) as a model system for answering questions about the ecological and evolutionary consequences of genetic variation and co-variation in various traits. This species of beetle is a cosmopolitan pest of legume seeds and has become a model system for studying many aspects of quantitative trait evolution, including maternal effects, trait liability, correlated trait evolution, cross-environment genetic correlations, and inbreeding depression (Messina 1991, Fox 1993, Fox et al. 2004, Fox et al. 2007). Many studies have used *C. maculatus* in experimental evolution studies, and populations typically show strong, consistent responses to selection (Messina et al. 2009). This body
of past research and the ability to efficiently select particular phenotypes makes *C. maculatus* an excellent system to study both ecological and evolutionary processes that involve genetic variation.

In Chapter II, we experimentally manipulate the genetic diversity of replicate populations that consist of individuals that vary in their host preference, and measure the population-level resource use and productivity of the populations. This experiment tests the hypothesis that genetically diverse populations will exhibit a wider array of individual host preferences, leading to more varied resource use and increased productivity of populations relative to genetic monocultures. In Chapters III and IV, we use replicate *C. maculatus* populations in an artificial selection experiment to investigate the evolution of dispersal traits and life history traits. In Chapter III we specifically examine if evolution in dispersal traits is constrained by correlated evolution in life history traits that experience antagonistic trade-offs with dispersal. In Chapter IV, we more closely examine multiple short-term movement traits associated with dispersal and ask if the traits are different between high dispersing individuals and control individuals, and also if different parts of the movement process co-vary with each other and how this might influence complex trait evolution.

REFERENCES


CHAPTER II: A nonlinear relationship between genetic diversity and productivity in a polyphagous seed beetle

AUTHORS: Burls, K.J., J. Shapiro, M.L. Forister, and G.A. Hoelzer

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INTRODUCTION

While the importance of genetic variation as a basis for adaptation has been clearly described by evolutionary biologists (e.g., Fisher 1937, Ford 1964, Roff 2007), the effects of genetic diversity on ecological interactions and processes are less well studied. There has recently been an increased interest in the ecological literature to understand the consequences of genetic diversity for population productivity (Hughes et al. 2008, Agashe 2009, Wilkinson et al. 2010), as well as community and ecosystem processes (Weltzin et al. 2003, Crutsinger et al. 2006, Crutsinger et al. 2010, Fridley and Grime 2010). These studies largely reveal positive relationships between intraspecific diversity and population viability, similar to the consequences of species diversity on community stability (Tilman and Downing 1994, McCann 2000).

The effects of genetic diversity may either be additive, where the effect on a population is proportional to each genotype’s performance and frequency in the population; or non-additive, where it depends on interactions among genotypes and thus is not proportional to the frequencies of individual genotypes (Hughes et al. 2008). One example of an additive effect is the ‘sampling effect,’ in which more diverse populations are more likely to contain productive genotypes, thus increasing population productivity (Huston 1997, Tack and Roslin 2011). An important feature of additive effects is that
there is no predicted change in the average value of a trait for replicates at a given diversity (Hughes et al. 2008), though certain genotype combinations may be more or less productive. This leads to the prediction that the variance in productivity should decrease as diversity increases.

In contrast to additive effects, interactions among genotypes leading to non-additive effects of diversity are expected to increase mean population responses. As an example of a non-additive effect, niche partitioning can occur if genotypes are functionally distinct; con specific competition is reduced if genotypes use different resources when in combination. Niche partitioning has been invoked for most studies displaying non-additive effects of diversity, including increased aboveground plant productivity (Fridley and Grime 2010), organismal mass and growth rate (Benard and Maher 2011), increased fungal biomass and CO₂ production (Wilkinson et al. 2010), increased plant biomass and associated herbivore biomass and diversity (Crutsinger et al. 2006, Crawford et al. 2007, Kotowska et al. 2010, Cook-Patten et al. 2011), and bacterial community growth rate and carrying capacity (Jousset et al. 2011). In contrast to niche partitioning, genotypes that are more similar to each other may compete strongly, thus lowering the overall productivity of a homogeneous system (Benard and Maher 2011, Jousset et al. 2011). Thus while functional diversity is important to population ecology, interactions may not always be positive; certain sets of genotypes may be more productive than others and this may vary in environments that either contain different resources or are heterogeneous and contain multiple resources of varying quality.

The studies above have rarely examined the ecological processes by which genetic diversity manifests increased population productivity. For example, genetic
variation in resource use and phenotypic plasticity in resource use could both contribute to higher productivity for diverse populations in a heterogeneous environment. In order to begin testing these alternative hypotheses, Agashe (2009) and Agashe and Bolnick (2010) investigated the role of genetic diversity on population stability using the flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae). These studies found that genetic diversity increased both population stability and population size on an ancestral host and in a mixed-host environment containing a novel host. However, variation in host use over multiple generations was largely driven by behavioral shifts, rather than by genetic variation in host use. Our study was designed to add to this line of research by more closely investigating the role of genetic diversity and plasticity in host preference in determining population productivity in a different model system.

We used inbred lines of the seed beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) to assemble genotypic diversity in different combinations. We focus on two important ecological parameters in insect populations: total productivity and host plant preference. Host plant preference is an important ecological and evolutionary trait in insects, and there is a large literature investigating the maintenance of genetic variation for such preferences within and between populations (Futuyma and Peterson 1985, Thompson 1988, Jaenike 1990, Forister et al. 2007). Genetic variation in host preference has been shown to be important in altering ecological interactions such as mate choice, predation risk, and mutualisms (Forister et al. 2011, Forister and Scholl 2012, Wilson and Leather 2012); and in influencing population dynamics (Singer and Hanski 2001, Wallin and Raffa 2004, Hanski 2011).
In addition to genetic variation, phenotypic plasticity in host preference is common in insects (Agrawal 2001). High levels of plasticity may initially mask genetic variation for a trait and thus slow down the effects of selection, but moderate levels of plasticity are expected to speed up the process of genetic evolution (Price et al. 2003), and may facilitate the colonization of novel hosts (Nylin and Janz 2009). However, plasticity can also have fitness costs (de Witt et al. 1998, Relyea 2002, Lind and Johansson 2009). Despite interest in the effects of both genetic variation and plasticity (Wennersten and Forsman 2012), expectations for the effects of genetic diversity typically assume traits are genetically controlled and are not phenotypically plastic.

We isolated 10 genetically distinct lineages of C. maculatus that varied in their fecundity and host preference for three different species of host seeds. We then created groups that contained 1, 3, 5, or all 10 lineages, and measured total productivity (fecundity) and resource use of these groups. We hypothesized that if variation in fecundity and host preference among lineages facilitate the beneficial effects of genetic diversity, genetically diverse groups will have higher productivity and lower resource competition, as there will be more individuals utilizing a wider range of resources. We can also distinguish between additive and non-additive effects of diversity. Additive effects of diversity could occur if diverse groups contain lineages that are more productive than average. We tested this by comparing the additive expectations of productivity and resource use of intermediately-diverse groups to that of the most diverse group. In addition, additive effects are indicated by a reduction in the variance of productivity as diversity increases. Non-additive effects of diversity could occur if genotypes use a less preferred resource when in the presence of other genotypes, and
would be indicated by increases in both productivity mean and variance, and an increase in the use of less preferred resources at higher diversity.

MATERIALS AND METHODS

Organism and experimental design

*Callosobruchus maculatus* is a cosmopolitan crop pest of seeds from plants in the Fabaceae family. Adult outbred females typically lay between 70-80 eggs during their lifetime. After developing as eggs for approximately 5 days, individuals live as larvae inside a seed for 11-12 days and emerge as adults for 3-6 days if given free access to seeds. The populations used in this study were originally captured on mung beans (*Vigna radiata*) and have been maintained on this host for hundreds of generations (Messina 1991). This species has both high preference for and high performance on mung beans relative to other potential hosts. Variation in host preference for various leguminous hosts has been shown to have both a genetic and a maternal component (Fox 1993, Messina and Slade 1997, Messina 2004, Messina et al. 2009).

To manipulate genetic diversity we created 10 genetically distinct lineages of beetles through isolation of founding mating pairs and 5 successive rounds of full-sib inbreeding. The founding full-sib pairs were haphazardly selected from stocks originating from three different populations in South India (Messina 1991; labeled as SI-7, SI-8, and SI-9). Our 10 lineages consisted of 5 SI-7 pairs, 2 from SI-8, and 3 from SI-9. After inbreeding using a single full-sib pair from each lineage for five generations, all sibs within a lineage were allowed to breed to increase population sizes. Following this generation, we measured individual fecundity and host preference of each lineage by
creating 18-20 replicates of single male-female pairs of beetles from each lineage in 100 mm petri dishes with 40 mung beans, cowpeas, and adzuki beans each, for a total of 120 beans, such that no host was limiting (n = 188 replicates). Individuals were placed into dishes within 18 hours of emergence and were allowed to mate and lay eggs. The number of eggs on each host was counted after the pair of beetles had died as a measure of preference and the total number of eggs across hosts was used as a measure of fecundity.

Testing for the effect of genetic diversity on total productivity and resource use was done by creating a series of experimental groups, each containing 10 males and 10 females, that contained either 1, 3, 5, or all 10 genetic lineages; the lineages used in the 3- and 5-lineage groups were chosen randomly. In the 3-lineage mixtures, two lineages were represented by three males and three females, and one lineage was randomly chosen to have four males and four females; in 5-lineage mixtures each lineage was represented by two males and two females. The proportion that each lineage contributed to a given group was accounted for in all analyses. Each replicate contained 400 mung, cowpea, and adzuki beans each, for a total of 1200 beans, such that no host was limiting. Assemblages of genotypic diversity were as follows: the single-lineage groups (3-5 replicates per lineage; n = 41); three different 3-lineage mixtures (n = 5-6 per mixture; n = 16); three different 5-lineage mixtures (n = 5-6 per mixture; n = 17); and replicates containing all ten lineages (n = 6). Variation in sample size between levels of diversity was due to limitations in population size of the lineages, with many individuals from each lineage needed for single-genotype replicates. Individuals were placed into dishes within 18 hours of emergence and were allowed to mate freely. As with individual fecundity and preference experiments described above, the eggs laid on each host in these diversity
experiments were counted as the measure of resource use after all individuals in the replicate had died, and the total number of eggs across hosts was used as the index of total productivity. Note that productivity in this sense includes all eggs laid, and not those that hatch into larvae or adults. We believe this is an appropriate measure of productivity, as resources were not limiting in our design so there should be little intra-bean competition for larval survival.

Statistical analyses

Differences in individual fecundity and group productivity between lineages were measured using a linear mixed-effects model with lineage as the predictor variable and stock as a random factor. Testing for differences in total productivity with increasing diversity was done using a linear mixed-effects model with number of lineages as the predictor variable and lineage or mixture as a random factor. Mixed-effects models were run using the lmer function in the package lme4 using R version 2.14.1 (Bates et al. 2011, R Core Development Team 2012). Pairwise comparisons for all models were done using the Tukey all-comparisons argument of the glht function in the package multcomp (Hothorn et al. 2008). Testing for a decrease in the variance of productivity with increasing genetic diversity was done using a modified robust Brown-Forsythe Levene-type test for homogeneity of variances using the treatment.levene function in the lawstat package (Noguchi et al. 2009).

We also distinguished whether greater productivity of more diverse groups might be due to the ‘sampling effect,’ when diverse groups contain more productive genotypes (an additive effect of diversity); or niche partitioning, when genotypes facilitate a higher
overall performance (a non-additive effect). To do this, we used the productivity of lineages in monoculture to make additive estimates of productivity of the 3-, 5-, and 10-lineage groups, accounting for the proportion of each lineage in a mixture. These estimates were compared using a mixed-effects model with number of lineages as the predictor variable and mixture as a random effect in R. We also completed post-hoc correlations between the proportion of each lineage for a group and group productivity to test for sampling effects of individual lineages on population productivity.

Host preference was measured using the package bayespref in R (Gompert and Fordyce 2011). This package utilizes a hierarchical Bayesian framework to analyze ecological count data (i.e., the number of eggs on a given host; Fordyce et al. 2011, Forister and Scholl 2012, Forister et al. 2012). Briefly, the hierarchical model uses the count data to estimate individual female preference for different hosts as parameters of a multinomial distribution. Preference at the population level is measured simultaneously (as a Dirichlet distribution from which individual female preferences are drawn), given the estimated preferences at the individual level (models are implemented using Markov chain Monte Carlo (MCMC)). This Bayesian framework is advantageous in that preference levels are estimated along with 95% credible intervals, which is not accomplished when simply testing the null expectation of no preference, as with analyses commonly used for preference data (e.g., the Kruskal-Wallis nonparametric ANOVA).

In the context of the hierarchical model, the hypothesis that individuals in a given lineage express host preference can be tested by comparing the deviance information criterion (DIC) between the constrained and unconstrained models for a single lineage. In the constrained model, females are constrained to lay approximately equal numbers of
eggs across hosts; a better fit of the unconstrained model thus indicates preference (i.e., non-uniform distribution of eggs across hosts). Differences in DIC are interpreted in a manner similar to differences in AIC, with lower scores indicating a better model fit and differences of >8 DIC units suggesting more support for a given hypothesis (Fordyce et al. 2011). In addition, strength of preference for each host can be directly compared using the 95% credible intervals around each parameter estimate, with non-overlapping preference estimates indicating significantly different preference between hosts.

For analyses comparing resource use of our experimental groups, the hypothesis that resource use differs within a lineage is similar to the individual analysis, comparing DIC values between unconstrained and constrained models for each lineage. However, in this case the replicate experimental dish of 10 males and 10 females serves as the “individual,” and the summed replicates of each lineage serves as the group. Differences in resource use within and between lineages were compared using the comparative DIC approach and by comparing 95% credible intervals.

Finally, the hypothesis that increasing genetic diversity will affect resource use is tested by estimating preference where each group is the set of experimental dishes containing 1, 3, 5, or all 10 genetic lineages. DIC values were compared for each model assuming preference does or does not differ among the groups (levels of diversity), and 95% credible intervals of preference were compared between each group.

Because preference estimates in bayespref are comparable between levels of study (Fordyce et al. 2011), we were able to measure differences in preference between the individual and group level analyses for each lineage. Differences in host preference across levels of analysis would be evidence for phenotypic plasticity. If some lineages are
more plastic in their host preference when in the presence of conspecifics, this could reduce competition in diverse groups that contain plastic genotypes. To test this hypothesis, we began by measuring plasticity for each lineage. We measured the plasticity for all three kinds of host beans in each lineage as the absolute difference between preference estimates at the individual and group levels. These estimates were then summed across hosts to create a single estimate of plasticity for each lineage. Lineage measures of host preference plasticity were then used to estimate plasticity of the 3-, 5-, and 10-lineage mixtures. We used a correlation test to ask if the plasticity of lineages or mixtures was related to their productivity.

As with productivity, we wished to determine if resource use of the more diverse groups was complementary due to an additive sampling effect or due to an interaction between lineages when in combination. Additive estimates of preference for 3-, 5-, and 10-lineage mixtures were created using preference estimates of each lineage in monoculture; we compared these estimates to observed preference estimates of mixtures using multiple analysis of variance (MANOVA). We then estimated the range of preferences for each mixture by taking the difference of the strongest and weakest preference for each host and summing across hosts. We tested for a correlation to determine the relationship between range in preference and group productivity.

RESULTS

The five consecutive rounds of inbreeding eliminated sufficient genetic diversity to create lineages that varied in both their fecundity and host preference. The inbred lineages showed a significant reduction in fecundity during inbreeding (Fig. 1), which is
consistent with the observation that populations of *C. maculatus* have previously been shown to harbor a significant genetic load (Fox et al. 2007). However, inbreeding should remove deleterious alleles after several generations, and the relatively high fecundity of many lineages (Fig. 2A) and variation in host preference despite equal inbreeding between lineages suggests at least some quantitative variation in fecundity and preference was retained between lineages.

The 10 lineages showed considerable variation in fecundity (Fig. 2A, $\mu = 35.5, \sigma = 11.6; F_{9,187} = 12.29$). Also, as expected, individuals displayed a hierarchy of preference for the three different hosts; $\Delta$DIC values for unconstrained models (i.e., those allowing individuals to display varying preference for hosts) were greater than 8 units for all lineages (Table 1). However, beetles from different lineages varied in the strength of their preference, with most lineages strongly preferring mung beans, but with some lineages showing significantly less preference than others (Fig. 3).

Increasing genetic diversity resulted in a non-linear, non-additive effect on group productivity, with 3-lineage mixtures being the most productive, 5-lineage mixtures being intermediate, and single-lineage and ten-lineage mixtures being the least productive ($F_{3,79} = 4.77; \text{Fig. } 4$). There was no decrease in the variance of productivity across levels of diversity (Brown-Forsythe $F_{3} = 0.9221, p = 0.43$). Additive estimates of productivity for lineages used in diverse groups did not differ across levels of diversity ($F_{2,64} = 0.035, p > 0.05; \text{Fig. } 4$), and since additive effects predict no change in fecundity across diversity, we did not find any evidence of an additive sampling effect for our intermediate diversity groups. In addition, none of the post-hoc correlations between lineage contribution and
productivity were significant (not shown), suggesting high performing lineages were not controlling the productivity of intermediate diversity groups.

Regarding group resource use, ΔDIC values favored models with varying resource use for all treatment levels (i.e. different numbers of eggs were laid on the different hosts, and this was true across levels of diversity; Table 2). There was no change in preference strength (i.e relative resource use) at higher levels of diversity as measured by 95% credible intervals (Fig. 5), though the 10-lineage treatment did tend to have a slightly stronger preference for mung beans and against adzuki beans. Observed estimates of host use did not differ from additive expectations within levels of diversity as measured by MANOVA (Pillai $F_{6, 158} = 0.64, p = 0.70$; Fig. 5), so there is no evidence of a sampling effect on group resource use. Preference range, or the span of preferences contained by a diverse mixture, was negatively correlated with group productivity ($r = -0.92, t_5 = -5.09, p < 0.01$).

We found considerable evidence for phenotypic plasticity in host preference. First, group productivity was not significantly different between lineages, despite differences in individual fecundity (Fig. 2 $B, \mu = 174.9, \sigma = 33.4; F_{9, 40} = 1.27$). In addition, the rank-order fecundity of lineages changed and, in some cases, the relative variance of a lineage differs between the individual and group experiments (e.g., lineage 9.3; Fig. 2). Second, almost all lineages showed significantly lower preference for mung beans as measured by 95% credible intervals and ΔDIC values when in a group as compared to individual analyses of host preference (Fig. 3). Preference for mung beans largely traded off for the lowest preferred host, adzuki beans. This effect was most evident in lineages 9.4 and 9.6, as ΔDIC values were less than 8 units apart (Table 3),
indicating individuals from these lineages displayed no preference for different hosts when conspecifics are present. Plasticity in host preference, measured as the difference in preference between the group and individual level analyses, was negatively related with group productivity ($r = -0.496$, $t = -2.22$, $p_{15} = 0.042$; Fig. 6).

**DISCUSSION**

We found partial support for the hypothesis that increasing genetic diversity would increase population productivity. Specifically, 3-lineage mixtures were more productive than monocultures and the most diverse mixtures. Five-lineage mixtures were intermediate in productivity (Fig. 4). Our lineages of *C. maculatus* varied in fecundity (Fig. 2) and host preference (Fig. 3). However, diverse mixtures did not have different resource use as compared to the monocultures (Fig. 5).

We tested for both additive and non-additive effects of diversity in this study. The sampling effect, an additive response where populations that are more diverse are more likely to include productive genotypes, would not be expected to change the average productivity at a given level of diversity. In addition, variance between replicates should decrease as diversity increases if genotypes are contributing equally to population performance. Our results show an increase in fecundity, and no decrease in variance, suggesting additive responses were not controlling dynamics of diverse groups. We also analyzed the role of component lineages in determining productivity of diverse mixtures and found no correlations between the proportion of a productive lineage and group productivity. These results suggest that the group responses to diversity are driven by non-additive mechanisms.
There are several non-exclusive, non-additive mechanisms that might explain the nonlinear response of our populations to increasing genetic diversity. First, diverse mixtures may have exhibited niche complementarity, when different genotypes facilitate co-existence by shifting niches when in the presence of other genotypes. In many systems, the ability of different genotypes to interact in a positive manner appears to be driven by two variables: the functional diversity of the different genotypes and variation in the environment that facilitates niche partitioning. Conspecifics of a similar phenotype have been shown to be more likely to compete and inhibit each other’s overall fitness, while conspecifics that are phenotypically disparate typically compete less for resources, allowing for increased population growth (Benard and Mahler 2011). In addition, the ability to use different resources is often constrained by the complexity of the environment; it has been shown at both the species and genotype level that more heterogeneous environments can often support more species by creating a wider range of niche space (Tilman 2004, Wacker et al. 2008, Jousset et al. 2011).

Our experiment tested for niche complementarity by measuring individual host preference and group resource use in monoculture and in the more diverse groups. We expected variation in host preference along with a heterogeneous environment would facilitate niche complementarity; however, we found no evidence that diverse groups had lower strength of preference (Fig. 5), and in fact a large range in preference between lineages was associated with lower productivity in mixtures. Some studies have found that genetic diversity is most beneficial, or that inbreeding depression is most detrimental, in stressful environments (Wise et al. 2002, Kristensen et al. 2008). As we wanted to focus specifically on host preference rather than competition in this study, resources were
non-limiting. Thus, it is possible that there was not sufficient competition for high quality hosts for genotypes to act in a facilitative manner in the most diverse groups, though this does not explain the nonlinear increase in productivity seen in our study.

A second non-additive mechanism is selection of productive genotypes via hard selection, where selective mortality of poorer lineages occurring on top of some level of background mortality could reduce the population size and thus productivity during the early stages of selection. This effect might be expected to be weakest when poor genotypes are absent in certain mixtures, and strongest when poor genotypes are at a higher relative proportion. The feedback between selection and population ecology has been a subject of renewed interest under the theme of eco-evolutionary dynamics (Hairston et al. 2005, Carroll et al. 2007, Schoener 2011). In our system, it is possible that selection in the intermediate diversity groups allowed high-productivity lineages to more fully exploit the most preferred resource, though we are unable to test this hypothesis as we do not have data on individual fecundity within group replicates. Multi-generational studies are needed to investigate the feedback between changes in the amount of trait variation due to selection and the process of natural selection on those traits (Schoener 2011).

A third mechanism to explain the nonlinear response in productivity is that individuals in different lineages responded plastically in their host preference and fecundity to the presence of other females, regardless of genotype. Plasticity in oviposition preference in response to increased density has been shown in numerous arthropod species (Krasnov et al. 2003, Pienaar and Greeff 2003, Wallin and Raffa 2004, Davis et al. 2011). In our study, many lineages showed substantial phenotypic plasticity
in host preference in response to the presence of conspecifics (Fig. 3), though not all lineages responded equally. In addition, total group productivity of single lineages was well below the additive expectation from the individual fecundity analyses. Finally, both the rank-order fecundity of lineages and the amount of variation in fecundity within lineages differed between individual and group analyses (Fig. 2). While differences in productivity were not significant in the group analysis, major changes in rank order (e.g., lineage 7.5 going from third ranked to seventh ranked) are suggestive of variation in the lineage’s response to conspecifics. These results are consistent with previous studies that have shown *C. maculatus* will avoid beans with eggs already present on them, depending on resource availability and selective background (Messina 1990, Messina and Karren 2003, Messina 2004). In addition, other chrysomelid and bruchid beetles display plastic responses in traits like egg size and number to changes in host availability and quality (Fox et al. 1997, Teixeira et al. 2009).

Plasticity may be adaptive or maladaptive depending on environmental conditions. Both genetic polymorphisms and plasticity are expected to result in broader niches and subsequently lower intraspecific competition (Van Valen 1965, Bolnick et al. 2003, Forsman et al. 2008, Wennersten and Forsman 2012). However, plasticity is expected to be costly relative to fixed traits in certain environments, especially for highly plastic genotypes (Relyea 2002, Lind and Johannson 2009). We found a significant negative relationship between plasticity and group productivity (Fig. 6). Given the change in preference for most lineages when in the presence of conspecifics and the low variation in host preference between levels of diversity, it is possible that plasticity in host preference masked potential niche complementarity provided by genetic variation.
while allowing more fecund and less plastic lineages to increase productivity of intermediate diversity groups.

In addition to the mechanisms listed above, our lineages experienced a significant decrease in fecundity during inbreeding (Fig. 1), and it is possible that inbreeding depression in some lineages may be obscuring the effects of diversity on productivity. However, many of the lineages retained relatively high fecundity during inbreeding, suggesting quantitative genetic variation was retained between lineages. In general, separating the effects of inbreeding from the effects of ecological trait variation is not a trivial issue. First, inbreeding depression can be contingent on environmental conditions, which may confound typical genotype-by-environment interactions (Fox and Reed 2011). In contrast to inbreeding, experiments using multiple populations of sexual organisms to create new populations with genetic diversity may confound outbreeding benefits or depression with ecological trait variation (Agashe 2009, Aguirre and Marshall 2012). Replicate populations that vary in their quantitative genetic diversity and environment, but not population size, may more accurately reflect the effects of genetic diversity in natural populations (Forsman et al. 2012).

This study has implications for understanding genetic diversity in both ecological and evolutionary contexts. Hughes (2008) pointed out three issues confronting research for understanding the ecological consequences of genetic diversity: knowing the relevant measures of genetic diversity; understanding the extent and magnitude of effects across taxa and traits; and identifying ecological mechanisms. The results of this study indicate the effects of diversity may be non-additive and nonlinear. An increasing body of empirical work suggests that positive consequences of genetic diversity depend not only
on adequate trait variation in the population, but also environmental conditions that
capitalize on genetic variation in the population, such as high competition or novel
resources (Weltzin et al. 2003, Wacker et al. 2008, Agashe 2009, Agashe and Bolnick
2010, Jousset et al. 2011). While our study focused on host preference variation, we did
not directly control the amount of variation in host preference at a given level of
diversity. Future experiments should focus on manipulating trait variance and means in
multiple environments; these factorial designs will lead to a broader understanding of the
conditions and traits that control the consequences of genetic diversity.

Second, the phenotypic plasticity in host preference observed in this study
suggests plasticity may have important consequences for the net ecological effect of
genetic diversity in populations. When genotypes vary for both a trait and for phenotypic
plasticity in that trait, as in our study, this can directly confound attempts to estimate the
effects of genetic diversity. Studies wishing to address this issue should experimentally
manipulate not only the amount of genetic diversity present in a population, but also the
phenotypic plasticity of different mixtures and of the population as a whole. Controlling
plasticity and genetic diversity in a factorial manner can then elucidate when plasticity
may be enhancing or constraining niche partitioning between genotypes.

Finally, the interaction between plasticity and diversity can be particularly
intricate when the plasticity is controlled by conspecific density, as density dependence
may regulate any positive effect of diversity on population productivity. If trait plasticity
reduces the strength of selection on different genotypes at high density, then trait
evolution could be constrained or augmented over time by changes in population
dynamics (Donohue et al. 2005, Wender et al. 2005, Bassar et al. 2010). Studies wishing
to investigate this interaction will need to measure quantitative trait variation, population demography, and selection gradients over multiple generations. This type of density-dependent plasticity may be an important mechanism in maintaining genetic variation in ecologically important traits in populations, even under strong directional selection, and this topic deserves further research.

In summary, we investigated the role of genetic diversity in fecundity and host preference in determining population productivity and resource use in a polyphagous seed beetle. We found increased productivity at intermediate levels of diversity but no change in relative use of different resources. While a mechanism for the rise in productivity associated with intermediate diversity remains unknown, we suggest substantial density-dependent plasticity in host preference may play a large role in regulating population productivity. Furthermore, we suggest that ecological studies of genetic diversity may need to include this interaction in order to more accurately predict the response of genetically diverse populations.

ACKNOWLEDGEMENTS

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Table 1. DIC values for individual analyses of host preference for genetic lineages. Constrained models hypothesize no difference in preference between hosts, while unconstrained models assume preferences differ and allow preference values to vary. Lower ΔDIC values within a lineage between levels of analysis indicate lower preference at that level.

<table>
<thead>
<tr>
<th>Lineage</th>
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<th>Unconstrained DIC</th>
<th>Δ DIC</th>
</tr>
</thead>
<tbody>
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<td>44.74</td>
<td>-21.97</td>
<td>66.71</td>
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<tr>
<td>7.2</td>
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</table>
Table 2. DIC values for models of group resource use for different levels of diversity. Constrained models hypothesize no preference between hosts, whereas unconstrained models allow preference to vary.

<table>
<thead>
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<th>Number of lineages</th>
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<th>Unconstrained DIC</th>
<th>ΔDIC</th>
</tr>
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<td>10</td>
<td>71.83</td>
<td>47.25</td>
<td>24.58</td>
</tr>
</tbody>
</table>
Table 3. DIC values for analyses of group resource use for lineages. Bold numbers indicate no significant difference between constrained and unconstrained models, i.e., no preference for different hosts.

<table>
<thead>
<tr>
<th>Lineage</th>
<th>Constrained DIC</th>
<th>Unconstrained DIC</th>
<th>Δ DIC</th>
</tr>
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<tbody>
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<td>9.6</td>
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Figure 1. Changes in fecundity over 5 generations of inbreeding from a sample of *C. maculatus* lineages used in this study. Error bars are ± 1 standard deviation.

Figure 2. Rank order A) individual fecundity and B) group productivity for lineages used in this study. Exclusive letters between lineages represent significant pairwise differences (differences were only significant at the individual level). Error bars are ± standard error. Squares represent lineages from the SI-7 stock, circles are from SI-8, and triangles from SI-9. Lettering schemes represent comparisons based on a mixed-effects model (lineages are not independent), and significant differences may not be obvious based on comparison of the raw means.

Figure 3. Individual host preference (closed symbols) and group resource use (open symbols) of mung, cowpea, and adzuki beans for lineages. Error bars are 95% credible intervals. Squares represent lineages from SI-7 stock; circles represent SI-8, and triangles represent SI-9.

Figure 4. Differences in group productivity between levels of genetic diversity. Error bars are ± standard error. Closed symbols are actual data; open symbols represent additive estimates of productivity for groups at each level of diversity, where each open data point is the estimate for one combination of lineages (three combinations for the 3- and 5-lineage treatments).

Figure 5. Differences in group resource use between levels of genetic diversity. Error bars are 95% credible intervals. Squares represent preference for mung beans; circles represent preference for cowpeas and triangles are preference for adzuki beans. Closed symbols are actual data; open symbols are additive estimates of preference for groups at each level of diversity.

Figure 6. Correlation between group productivity and plasticity of component lineages. Black dots are single lineage groups, dark grey dots are 3-lineage groups, light grey dots are 5-lineage groups, and white is the 10-lineage group. Pearson $r = -0.492$, $p = 0.496$. 
Figure 1

![Average fecundity by generation](image)
Figure 2
Figure 4

A scatter plot showing the relationship between Diversity (number of lineages) and Fecundity. The plot includes data points labeled with different letters, indicating significant differences in the data.
Figure 5

The figure shows a scatter plot with preference on the y-axis and diversity (number of lineages) on the x-axis. The plot is divided into four panels labeled One, Three, Five, and Ten, each representing different levels of diversity. The data points are marked with different symbols and error bars, indicating variability or confidence intervals.
CHAPTER III: Dispersal and life history evolution via artificial selection using the seed beetle *Callosobruchus maculatus*

INTRODUCTION

The dispersal of organisms across landscapes is a dynamic process, with the decision to disperse and the distance moved dependent on a variety of factors (Hamilton and May 1977, Holt and McPeek 1996, Léna et al. 1998, Matthysen 2012). These movements have multifareous consequences for both micro- and macro-evolutionary processes, including demography, population ecology, genetic drift, natural selection, and co-evolutionary dynamics (Nathan and Muller-Landau 2000, Rousset 2003, Bowler and Benton 2005, Zheng et al. 2009, Léotard et al. 2009). Long distance dispersal, the furthest movements of individuals in a given population, has in particular been shown to be important in many evolutionary processes including metapopulation dynamics, range expansions and invasions, and range margin dynamics (Van Valen 1971, Thomas et al. 2001, Nathan 2005, Kokko et al. 2006, Ronce 2007). Thus, understanding the evolution of dispersal within a population can help inform many aspects of population biology.

There is a growing appreciation that dispersal rates and distances of individuals in a population are not static quantities, but rather can change over time, affecting spread rates of organisms over time and space (Ezoe 1998, Poethke et al. 2003, Dytham 2009). Variation in dispersal distances is quantified using a dispersal distance kernel (*sensu* Nathan et al. 2012), which measures the frequency of individuals dispersing a given distance from a point. Dispersal kernels are thought to typically exhibit two somewhat contrasting characteristics: they are leptokurtic, with most individuals moving short
distances; and they are ‘fat-tailed,’ meaning there are more far-dispersing individuals than predicted by Gaussian or exponential distributions (Kot et al. 1996, Bullock and Clarke 2000). Theoretical studies have shown the potential for dispersal kernels to evolve in response to habitat variation and competition, and these studies highlight the importance of long distance dispersal in kernel evolution (Hovestadt 2001, Rousset and Gandon 2002).

For many terrestrial animals, long distance dispersal requires expending substantial time and energy, so life history theory predicts that important fitness traits should be negatively correlated with dispersal ability due to antagonistic pleiotropy or resource allocation trade-offs (Roff 1995, Bonte et al. 2012). These trade-offs may constrain dispersal evolution in some populations. Evidence of trade-offs (i.e., negative correlations) between dispersal and life history traits is currently equivocal, with some studies showing strong signs of negative correlations (Roff and Bradford 1996, Hughes et al. 2003, Simmons and Thomas 2004, Gibbs and Van Dyck 2010), and others showing little to no cost of dispersal in relation to fitness (Hanski et al. 2006). A recent meta-analysis by Stevens et al. (2012) found that, across butterfly species, dispersal ability was typically associated with higher fecundity and shorter development time, contrary to expectations based on life history trade-offs. It is likely that, as with other trade-offs, the cost of dispersal experienced by an organism is dependent on both the environment and the nature of selection being imposed on the population (Conner 2012). These features have made it difficult to predict the ability of populations to shift their ranges in response to climate change, or to predict the magnitude of correlated trait evolution in general (Stevens et al. 2010, Delph et al. 2011).
An alternative prediction regarding dispersal and life history evolution comes from literature on range expansions (Phillips et al. 2008, Phillips et al. 2010a). If individuals of a population are dispersing in a manner such that far dispersing individuals cluster together and spatially segregate themselves from more sedentary individuals (as at an invasion front), individuals will tend to mate in a positive assortative fashion. This can lead to a ‘spatial sorting’ of differing dispersal phenotypes between the population core and range edge (Shine et al. 2011). If these individuals have higher reproductive success, dispersal distances will evolve to increase at the range edge, a feature found in several empirical studies (Cwyner and MacDonald 1987, Léotard et al. 2009, Phillips et al. 2010b, Lindström et al. 2013, Lombaert et al. 2014). In addition to differing in dispersal traits, spatial sorting predicts that individuals at the range edge will be at a lower conspecific density. This ecological shift may select for increased population growth rate, i.e. fecundity, with the potential for decreasing competitiveness at equilibrium densities (Burton et al. 2010, Phillips et al. 2010a). This prediction complements research on the Glanville fritillary butterfly (Melitaea cinxia), which has found evidence for a ‘colonizer syndrome’ consisting of fast developing, shorter-lived, high fecundity, high dispersing individuals in newly re-colonized habitats (Baker and Stebbins 1965, Saastamoinen 2007, Niitepõld et al. 2009, Zheng et al. 2009, Bonte and Saastamoinen 2012).

Few studies have simultaneously investigated the roles of life history evolution and dispersal evolution in determining the spread rate of an expanding population. Burton et al. (2010) conducted an individual-based simulation of a range advance that allowed proportional trade-offs in a fixed resource pool between competition, reproduction, and dispersal. Results from this study agreed with the spatial sorting
prediction, as reproduction and dispersal were increased at the range edge at the expense of competitive ability. Perkins et al. (2013) used data from the invasion front and population core of the cane toad (*Rhinella marina*) to model the relative influences of life history and dispersal evolution in determining spread rate. The authors found relatively equal effects of each process as well as a substantial positive interaction between the two processes. These studies suggest that positive correlations between life history and dispersal can positively influence spread rate under certain circumstances. However, if life history traits like fecundity or development rate are negatively correlated with dispersal, selection for increased dispersal could be expected to decrease fitness, lowering the potential spread rate and constraining the evolution of dispersal at the range edge.

In this study we take an artificial selection approach to experimentally examine the relationship between dispersal traits and life history evolution in determining population dispersal kernels. We selected replicate populations of the seed beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) for increased spread, measured as the distance of eggs from the population’s point of origin (hereafter the dispersal kernel). We conducted selection for 20 generations by selecting the very tail of the dispersal kernel after each generation, along with corresponding control replicates. By measuring the location of all eggs in replicates between generations, we are able to measure the evolvability of the dispersal kernel over time. After selection, we measured divergence in individual morphology, life history, and dispersal characteristics to estimate trade-offs between dispersal and life history traits, and investigated how these traits interact to determine the potential spread rates of the different sets of individuals. If life history traits experience antagonistic pleiotropy or resource allocation trade-offs with
dispersal traits, dispersal evolution may be minimal despite increases in individual movement and dispersal ability. Alternatively, if spatial sorting occurs via assortative mating and selection for increased growth rate, average and maximum dispersal distances should increase in selected populations, as should life history traits that increase population growth rate, such as reduced development time and increased fecundity.

MATERIALS AND METHODS

Study system

We used the seed beetle *Callosobruchus maculatus* as a model system for studying an expanding population. This species is a cosmopolitan pest of legume seeds, meaning that its ‘natural’ habitat includes homogenous resource patches of varying size (i.e., small bags to large silos) that are locally used up within a few generations, encouraging dispersal both within and between generations. Females are extremely fecund, laying approximately 70-80 eggs each in stock populations. After eggs finish development in 4-5 days, larvae burrow into seeds and complete development in 11-12 days when at 29.5 °C. Adults are capital breeders and require no food or water after eclosion. If given access to seeds, adults typically live 3-6 days. This life history allows the investigator to create non-overlapping generations within which selection can be imposed. Males and females are sex-dimorphic, and there is some evidence of selection for sexual dimorphism as well as evidence of sexual conflict (Crudginton and Silva-Jothy 2000, Aronqvist et al. 2005, Rankin and Aronqvist 2008). Individuals in this study were originally captured on mung beans (*Vigna radiata*) and have been maintained on this host for hundreds of generations in a nonspatial environment (Messina 1991). In almost all
cases, only one larva successfully develops from each *V. radiata* seed, as larvae experience “contest competition” for resources (Messina 1991, Messina 2004). Because of this, *C. maculatus* individuals can adjust their egg laying preferences in response to conspecific density (Mitchell 1975).

**Experimental protocol**

Our artificial selection experiment took place in one-dimensional, spatially extended mesocosms consisting of discrete resource patches. Each mesocosm consisted of 31 small (70 mm diameter) jars, connected by 3.1 mm inner diameter tubing 6 cm in length. Each jar held 150 ± 10 seeds (see Fig. 1). This setup effectively creates a ‘viscous’ but homogeneous landscape: individual beetles only move across a fraction of the space in a lifetime, but movement is not restricted in any way that would limit reproduction. In addition, beans were limited near the origin but increased in availability as an individual disperses in either direction. We established 10 replicate populations consisting of 50 individuals with equal proportions from each of the three stock populations in a 50/50 sex ratio. These individuals were selected as virgins within 12 hours of emergence and placed into replicates at random in the center jar (#16). Individuals were allowed to mate, lay eggs, and move at will throughout the mesocosm. The offspring used in the next generation were selected after all adults had died. All replicates were kept at 29.5 °C ambient temperature.

For the founding generation, we examined all replicates and found the five replicates that had the 50 furthest eggs on either side of the jar of origin. These five mesocosms became the ‘high dispersal’ treatment (one of these replicates experienced an outside infestation in generation 7 and so was excluded from all analyses). The other five
replicates became the ‘control’ treatment, where eggs were randomly selected from across the mesocosm. This protocol essentially partitions the original genetic variance between treatments at the beginning of the experiment. Because the number of eggs in each jar is highly unequal, and because counting all eggs in a mesocosm is time intensive, we used a sample protocol to estimate the dispersal distribution in control replicates so that offspring could be selected at random with respect to distance. In each replicate, a sample of 20 seeds was selected from each jar and all the eggs on these seeds were counted. From this data we used frequency-weighted random selection to determine the number of eggs that were taken from each jar. These eggs were then used to found the next generation. All other seeds were taken out and saved by jar to later count all oviposited eggs, and fresh seeds were placed in each jar. Fifty eggs selected for each treatment were then placed into the center jar before eclosion, where they then emerged as adults of the next generation. After selecting control and high dispersal replicates in the founding generation, the same protocol was used within each treatment for each generation of selection. We rotated which replicate was in a given mesocosm randomly every 4 generations to account for effects of location within the room in response to selection. Each generation, all eggs from each replicate were counted by jar to measure the full dispersal kernel.

After 20 generations of selection, individuals were selected as above and then placed into a common, nonspatial environment, and allowed to reproduce. This common environment was used to eliminate maternal effects, such as effects of laying order on offspring size, from our measurements of divergence (Fox 1993). Following this generation, eggs were isolated to select individuals to measure divergence between
treatments in a number of characteristics. We selected 50 pairs of male and females from each replicate and measured divergence of life history traits in a nonspatial environment, a 90 mm petri dish (n= 450 pairs). All pairs were placed in petri dishes as virgins within 12 hours of emergence. We measured the mass of all individuals at the initiation of the experiment. We measured individual lifespan of individuals to the nearest ½ day, and measured fecundity in each replicate after both individuals had died. In addition, in each of these replicates, we selected 10 eggs at random after 24 hours of initiation to measure egg-to-adult survival and development time.

We also selected 10-15 pairs of individuals to measure divergence of dispersal distance in a spatial environment (n= 97 pairs). Spatial replicates consisted of mesocosms similar to that used for selection but were only 15 jars in length, each with 10 beans. Pairs were isolated and weighed as above, and placed in the middle jar (#7) within 12 hours of emergence as virgins and allowed to mate, lay eggs, and disperse at will. The positions of each beetle were recorded every 12 hours between initiation and death to measure movement throughout the lifespan. Afterwards, all eggs were counted by jar to record the dispersal kernel and total fecundity as an estimate of population spread.

Statistical analyses

All analyses were completed using R version 3.0.3 (R Core Team 2014). We measured changes in the dispersal kernel during selection, that is, the distance of eggs from the point of origin, using a generalized linear mixed-effect model (GLMM) using the glmer function in the lme4 package (Bates et al. 2014). We modeled the distance of an egg from the jar of origin, with treatment and generation as fixed effects and including an interaction, which tests for differing responses between treatments over time.
Replicate lineages were modeled as a random effect within generations. We included a poisson link function to account for the nonnormal distribution of distances. We also performed a similar analysis using only the furthest 50 eggs from each line in each generation to test for increases in the tail of the dispersal kernel. We also tested for changes in fecundity of lineages during selection using a GLMM, with fecundity of a replicate as the response; treatment, generation, and their interaction as fixed effects; and replicate as a random effect within generations. Finally, we measured the intensity of selection in each generation as $i = S/\sigma_p$, where $S$ is the selection differential and $\sigma_p$ is the phenotypic standard deviation.

Divergence between treatments in mass, lifespan, and fecundity was measured with a linear mixed-effects (LME) modeling approach using the `lmer` function in the `lme4` package. All responses were modeled with treatment as a fixed effect and replicate as a random effect. Tests of mass and lifespan included sex as a main effect and an interaction with treatment. Analyses for lifespan and fecundity were conducted separately for individuals in the spatial and nonspatial environments. Egg-to-adult survival and development times were modeled using survival curves in the `survival` package (Therneau 2014). Divergence in survival was measured using two methods. First, survival curves were compared using the `survdiff` function, which uses the Fleming-Harrington G-rho family of tests (Harrington and Fleming 1982). Second, survival was analyzed via a Cox proportional hazards model with mixed effects using the `coxme` function in the `coxme` package (Therneau 2012). In this model, treatment was a fixed effect and selection replicate and mother of origin were random effects. Divergence in development time between treatments was measured using an accelerated time failure
(AFT) model via the \textit{survreg} function using a log link function, with treatment as a predictor variable and replicate as a random effect.

We measured divergence in individual-level dispersal kernels and individual movement in the spatial analyses using multiple methods. We compared dispersal kernels (the distance of eggs from their point of origin) between treatments with a GLMM using the \textit{MCMCglmm} function in the \textit{MCMCglmm} package (Hadfield 2010). This model included distance of an egg as the response, treatment as a fixed effect and selection replicate as a random effect. We again used a poisson link function for these data. We also compared total lifetime movement of individuals using a GLMM with \textit{MCMCglmm} with total movement as the response, treatment and sex as fixed effects including an interaction, and selection replicate as a random effect. In addition, we compared movement between treatments using a modified Brown-Forsythe Levene-type test for homogeneity of variance with the \textit{levene.test} function in the \textit{lawstat} package (Gastwirth et al. 2013). Because this represents an analysis of point standard deviations from the median between groups, unequal variance represents far dispersing individuals moving disproportionately more in the selection treatment compared to those individuals in the control treatment. Finally, we tested for differences in short-term movement patterns between treatments by measuring the distance covered by individuals between each observation point as an estimate of movement rate. This analysis used a GLMM via \textit{MCMCglmm} with distance moved as the response, treatment and sex as fixed effects including their interaction, and replicate as a random effect.

In addition to treatment-specific analyses of life history traits, we also performed \textit{post-hoc} correlations using individual data from the spatial analyses to quantify the
relationship between lifetime movement, dispersal kernels, and life history. These analyses consisted of either male or female total movement or the average egg dispersal as the response variable and mass, lifespan, and fecundity as the predictor variables.

RESULTS

Selection replicates had consistently larger dispersal kernels than control replicates throughout the experiment, but there was no significant response to selection for a shift in dispersal distances over time, which would be indicated by a treatment*generation interaction (Fig. 2, Table 1). In fact, dispersal distances represented by the 50 furthest eggs actually decreased over time in the selected lines (Table 1). Fecundity was significantly lower in the selection replicates compared with the controls, but there was no significant interaction between treatment and generation, which would have indicated a regular reduction in fecundity as a correlated response in the selection replicates (Fig. 3). Selection intensity averaged 2.15 with a standard deviation of 0.46.

Despite the lack of continuous evolution of dispersal distance, we found several significant differences between treatments in life histories in our individual-level measures of divergence. Selected individuals were less fecund than control individuals; this difference was stronger in the spatial analyses (Fig. 4, Table 2). There was no significant effect of selection on lifespan for either sex (Table 2); however, females lived longer than males in the nonspatial analyses, whereas there was no difference in lifespan between sexes in the spatial analyses (Table 2). Selected females also weighed slightly less than control females, while there was no difference in male mass between treatments (Table 2). Selected individuals had significantly lower egg-to-adult survival ($\chi^2 = 257$, df
= 1, \( p < 0.01 \); Fig. 5; Table 3). There was no difference in development time between treatments (Table 3).

We also found significant differences in movement and dispersal distances between treatments in our individual-level spatial measures of divergence. Both males and females from selection replicates moved approximately double the distance of control individuals (Fig. 6, Table 4). In addition, the variance in movement between replicates was significantly larger for females in the selection treatment \( (F=4.8125, p=0.03) \) and marginally larger for males \( (F=3.0514, p=0.08) \). Testing for differences in movement rate, that is the distance covered by an individual between time points, resulted in significant main effects of treatment and sex, with selected individuals moving further than control individuals, and males taking smaller step sizes than females. There was also a significant treatment*sex interaction, with selection males taking proportionally larger steps than selected females (Table 2). Finally, we found a small but significant increase in the dispersal kernels, or the distances of eggs laid from the female’s point of origin, of selected individuals (Fig. 7, Table 4). In addition, dispersal distance was positively correlated with movement across treatments (Fig. 8), suggesting that individuals who move further over a lifetime are indeed responsible for far-dispersed eggs.

Of the post-hoc correlations, we found significant positive relationships between lifespan and total movement in both sexes and a marginal relationship between lifespan and average egg dispersal distance in females, suggesting longer lived individuals moved further and may be responsible for dispersing offspring further in a lifetime (Table 5). Interestingly, despite the decrease in fecundity in selected individuals, there was a
significant positive relationship between total fecundity and average egg dispersal distance (Table 5).

DISCUSSION

In this experiment we artificially selected replicate *C. maculatus* populations to test the evolvability of dispersal kernels under selection for increased dispersal, and to examine how joint evolution of dispersal traits and life history traits contributes towards the rate of population spread. Due to our initial selection protocol, we saw an immediate increase in dispersal distance of selection treatment replicates, and this increase was maintained over the course of the experiment. However, despite 20 generations of intense selection, we found no additional increase over time in either average lengths of dispersal kernels, or the longest distances dispersed (Fig. 2). In addition, fecundity decreased significantly over time. Following selection, our individual-level measurements of divergence found clear evidence for life history trade-offs, with selected individuals being less fecund, smaller, and with lower egg-to-adult survival, but with increased movement and consequent egg dispersal. This evidence supports the hypothesis that life history traits, such as fecundity, can constrain dispersal evolution if they experience trade-offs with traits that contribute to dispersal, such as movement.

The lack of a continuous evolutionary response in the dispersal kernel is a surprising result given the wealth of evidence for heritability of dispersal related traits across many taxa (Roff 1990, Roff and Fairbairn 2001, Sinervo et al. 2006, Bonte and Lens 2007, Saastamoinen 2008). Typically, a lack of response to selection is attributed to low heritability in the trait. The extent of phenotypic variation is not a constraint on the
extent of heritable variation in this case, given the range of dispersal distances exhibited by individuals in both treatments. Furthermore, the increased movement and egg dispersal of individuals after selection indicates that there was heritable variation in traits related to the dispersal kernel. In addition, the strongest correlate of movement after selection was lifespan, a trait in which this species has been shown to have substantial variation and heritability (Fox et al. 2003).

Multiple lines of evidence in this experiment support the hypothesis that life history trade-offs constrained dispersal kernel evolution. First, selection replicates experienced relatively quick and dramatic decreases in fecundity, roughly 66% in 9 generations (Fig. 3), and this drop in fecundity was confirmed by spatial and nonspatial individual analyses following selection (Fig. 4). Second, selected individuals had significantly lower egg-to-adult survival than control individuals (Fig. 6), which would further reduce the effective fecundity in selection replicates. Finally, we found a positive correlation between total fecundity and egg dispersal in our individual analyses of divergence (Table 4). In light of the dramatic drop in fecundity by selected individuals, this strongly suggests a negative correlation between the ability of an organism to move and its ability to successfully generate offspring in a distant locality. In contrast, one reason increases in dispersal kernels are expected to occur is because individuals at the front of the range may exhibit increased reproductive success due to lower conspecific competition (Van Valen 1971, Phillips et al. 2008).

Inspection of individual movement and egg dispersal from the individual-level measures of divergence lend additional insight into the evolutionary dynamics of the replicate populations. Despite the lack of continuous evolution at the population level, we
found selected individuals moved almost double the distance of control individuals, and among-individual variation in movement was also greater in selection replicates. This increase in movement was also correlated with an increase in egg dispersal, though this increase between treatments is modest (approximately ½ jar; Fig. 7, Fig. 8). This difference in movement appeared to be driven by two separate processes. First, the distance moved between observation points of selected individuals was higher, though the difference was larger for males (Table 2). Second, we found that lifespan had a substantial positive relationship with both total movement and egg dispersal (Table 4). Thus, the lack of evolution in lifespan (Table 2) may demonstrate another constraint on the evolution of the dispersal kernel in this system. These data together suggest that the net outcome of dispersal in this system is controlled by differences in movement ability in both the short and long term.

It is possible that differences in environments between the selection experiment itself and our individual measures of divergence changed individual movement patterns in a way that reveal variation in movement and dispersal after selection not observed during selection. For example, individuals in our spatial analyses of divergence were in conditions with no conspecifics (as opposed to multiple individuals in selection replicates) and lower resource density (10 beans per jar as opposed to 150 beans per jar). However, dispersal is typically positively density-dependent, so finding increased dispersal under low or no competition would be opposite of the predicted pattern (Travis et al. 1999, Lambin et al. 2001). Similarly, studies of phenotypic plasticity often find increased variation under stressful conditions, with benign conditions masking extreme phenotypes (Hoffman and Merilä 1999, Donohue et al. 2005). Thus, it seems unlikely
that the differences we see in our divergence measurements are due only to plasticity, though we cannot test this with our data.

These results are consistent with other studies that have shown trade-offs between life history and dispersal. Many of these studies have used wind-dimorphic insects or heteromorphic seeds, but monomorphic species have also been examined as well (Roff and Bradford 1996, Zera and Denno 1997, Hughes et al. 2003, Simmons and Thomas 2004, Lu et al. 2013). These studies highlight the importance of genetic trade-offs between structures associated with movement and structures associated with reproduction, like wing musculature or seed dormancy. Other studies have emphasized the physiological cost of dispersal musculature; for example, Gibbs et al (2010) found that increased flight resulted in lower egg provisioning, creating smaller eggs, which then indirectly lowered egg hatching rate and increased larval development time. Our experiment demonstrates that these trade-offs are indeed capable of limiting dispersal evolution and consequently the spread of offspring.

These results largely contrast with studies of spatial sorting, which have found evidence for increased dispersal distances at range edges, along with increases in traits favoring higher reproductive rate. For example, *R. marina* individuals at the range front are not only better dispersers but they also complete metamorphosis and grow more quickly than their counterparts in the population core (Phillips 2009). Similarly, *M. cinxia* individuals in newly colonized populations are often associated with a particular metabolic phenotype that includes not only increased movement but also increased fecundity and earlier age at first reproduction (Saastamoinen 2007). These traits may be favored due to correlated selection in newly colonized environments, with selection
pressures changing as the population ages (Ovaskainen et al. 2008, Bonte and Saastamoinen 2012). They are also likely the result of genetic architecture, as indicated by the association between individual genotype at the Pgi locus and dispersal in M. cinxia (Zheng et al. 2009, Hanski 2011). These studies clearly demonstrate that dispersal capability and life history traits like fecundity or development time can covary positively and jointly evolve in expanding populations or metapopulations.

Other ecologically relevant traits may also covary in expanding populations (Rogers and Siemann 2004, Simmons and Thomas 2004, Fjerdingstad et al. 2007, Léotard et al. 2009). Environmental variation during development and natal habitat quality have also been shown to influence phenotypes in such as way as to create dispersal syndromes, manifesting consistent correlations between traits in natural populations (Sinervo et al. 2006, Benard and McCauley 2008, Cote et al. 2010, Bonte et al. 2011). It is likely that these correlations are important determinants of colonization success in these and other systems (Ronce and Clobert 2012). It is possible that the environmental context and time frames associated with range expansion might yield a different outcome from this laboratory experiment.

It is also possible that our replicates showed a non-continuous response to selection due to excessive inbreeding. Inbreeding can be a confounding factor in selection experiments, especially when selection is intense, as in our experiment (Robertson et al. 1961, Fuller et al. 2005). Inbreeding has also been experimentally shown to constrain the evolutionary potential of populations and also to affect trait heritability in nonadditive ways (Wade et al. 1996, Dierks et al. 2012). In addition, inbreeding has been shown to affect fitness traits in other C. maculatus studies (Fox et al. 2007). While we attempted to
mitigate inbreeding effects by combining stock populations at the beginning of the experiment, it is possible inbreeding constrained the response of our replicate populations. However, under the hypothesized scenario for spatial sorting, local population sizes are likely to be smaller, with inbreeding and drift stronger at the range edge than at the population core (Arnaud-Haond et al. 2006, Eckert et al. 2008). This has the potential to slow the evolutionary response of populations at the range edge by limiting the genetic variance until more individuals reach a given area. Future studies could measure changes in the rate of spread over time combined with dispersal trait variation in order to understand if individuals are becoming more variable and more dispersive over time.

In conclusion, we used an artificial selection experiment to directly investigate the relationship between life history traits and dispersal traits in determining the dispersal kernels of replicate populations. We found clear evidence of trade-offs between life history traits and increasing dispersal distance, and these trade-offs likely limited the evolution of the dispersal kernels during selection, despite increasing individual movement of selected individuals. These results likely represent one end of a continuum of relationships between these types of traits, and highlight the importance of constraints in determining the evolvability of dispersal distances in populations under selection.

REFERENCES

Arnaud-Haond, S., S. Teixeira, S.I. Massa, C. Billot, P. Saenger, G. Coupland, C.M. Duarte and E.A. Serrão. 2006. Genetic structure at range edge: Low diversity and
high inbreeding in Southeast Asian mangrove (*Avicennia marina*) populations.

Molecular Ecology 15:3515-3525.


Table 1. Mixed model results for dispersal kernel and fecundity evolution during selection. Dispersal kernel measurements were fit with a poisson link GLMM; fecundity was fit using LME. Estimate is the restricted maximum likelihood parameter estimate, ±SE is the estimate standard error, $\chi^2$ is the Wald Type II Chi-square statistic, and $p$ is the associated p-value.

<table>
<thead>
<tr>
<th>Model</th>
<th>df</th>
<th>Estimate (±SE)</th>
<th>$\chi^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dispersal kernel</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.059</td>
<td>10.47</td>
<td>&lt;0.01</td>
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<tr>
<td>Generation</td>
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<td>0.003</td>
<td>2.85</td>
<td>0.09</td>
</tr>
<tr>
<td>Interaction</td>
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<td>0.005</td>
<td>1.03</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>Top offspring dispersal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
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<td>0.093</td>
<td>3.89</td>
<td>0.05</td>
</tr>
<tr>
<td>Generation</td>
<td>-0.008</td>
<td>0.007</td>
<td>5.79</td>
<td>0.02</td>
</tr>
<tr>
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<td>0.78</td>
<td>0.38</td>
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<tr>
<td><strong>Fecundity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>363.37</td>
<td>260.31</td>
<td>18.43</td>
<td>&lt;0.01</td>
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<td>Interaction</td>
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<td>26.29</td>
<td>0.27</td>
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</table>
Table 2. Mixed model results for measurements of divergence after selection. Estimate is the restricted maximum likelihood parameter estimate, ±SE is the estimate standard error, $\chi^2$ is the Wald Type II Chi-square statistic, and $p$ is the associated p-value.

<table>
<thead>
<tr>
<th>Model</th>
<th>df</th>
<th>Estimate (±SE)</th>
<th>$\chi^2$</th>
<th>$p$</th>
</tr>
</thead>
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<td><strong>Nonspatial analyses</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Fecundity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>8</td>
<td>-13.850 8.007</td>
<td>6.51</td>
<td>0.01</td>
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<td>Lifespan</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
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<td>-0.606 0.425</td>
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<td>Sex</td>
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<tr>
<td>Interaction</td>
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<td>0.21</td>
<td>0.65</td>
</tr>
<tr>
<td>Mass</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
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<td>2424.91</td>
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<td>Interaction</td>
<td>8</td>
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<td><strong>Spatial analyses</strong></td>
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<tr>
<td>Fecundity</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>8</td>
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<td>6.51</td>
<td>0.01</td>
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<tr>
<td>Lifespan</td>
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<tr>
<td>Treatment</td>
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<td>8</td>
<td>0.023 0.367</td>
<td>0.004</td>
<td>0.95</td>
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Table 3. Analysis of divergence in larval development. Egg-to-adult survival measured using a Cox mixed-effects proportional hazards model; development time was measured using regression analysis with a log-link for accelerated time failure. Estimate is the maximum likelihood parameter estimate, ±SE is the estimate standard error, $z$ is the Wald $z$-statistic, and $p$ is the associated $p$-value.

<table>
<thead>
<tr>
<th>Model</th>
<th>df</th>
<th>Estimate</th>
<th>(±SE)</th>
<th>$z$</th>
<th>$p$</th>
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<tr>
<td>Egg-to-adult survival</td>
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<td></td>
<td></td>
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<tr>
<td>Treatment</td>
<td></td>
<td>-0.772</td>
<td>0.085</td>
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<tr>
<td>Development time</td>
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<td></td>
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<td></td>
<td></td>
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<td>Treatment</td>
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<td>7.03</td>
<td>18.469</td>
<td>0.38</td>
<td>0.7037</td>
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Table 4. Divergence in individual movement and dispersal kernels. Analyses were competed with GLMM using the \textit{MCMCglmm} procedure. Posterior estimates are the parameter estimates, Lower and Upper CI are 95\% credible intervals, and \( p \) is the fraction of MCMC samples that were different from 0.

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter</th>
<th>Posterior estimate</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>( p )</th>
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<td>Treatment</td>
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<td>0.551</td>
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<td>-1.208</td>
<td>-3.700</td>
<td>1.412</td>
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<td><strong>Dispersal kernels</strong></td>
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<td>0.134</td>
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<td><strong>Movement rate</strong></td>
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<td>Interaction</td>
<td>0.401</td>
<td>0.134</td>
<td>0.709</td>
<td>0.006</td>
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</table>
Table 5. Pearson's product-moment correlations between movement and life history characteristics of individuals used in spatial analyses. Bold values are significantly different from 0, \( t \) is the \( t \)-statistic, and \( p \) is the associated \( p \)-value.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Correlation</th>
<th>( t )</th>
<th>( p )</th>
</tr>
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<tbody>
<tr>
<td><strong>Female movement</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total fecundity</td>
<td>0.176</td>
<td>1.57</td>
<td>0.12</td>
</tr>
<tr>
<td>Lifespan</td>
<td><strong>0.431</strong></td>
<td><strong>4.66</strong></td>
<td><strong>&lt;0.01</strong></td>
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<tr>
<td>Mass</td>
<td>0.060</td>
<td>0.56</td>
<td>0.58</td>
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<td><strong>Male movement</strong></td>
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<td>Lifespan</td>
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<td><strong>3.84</strong></td>
<td><strong>&lt;0.01</strong></td>
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<td>Mass</td>
<td>0.002</td>
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<td>0.98</td>
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<td><strong>Dispersal kernel</strong></td>
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<td>Total fecundity</td>
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<td><strong>2.23</strong></td>
<td><strong>0.03</strong></td>
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<tr>
<td>Mass</td>
<td>-0.011</td>
<td>-0.09</td>
<td>0.93</td>
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</table>
Figure 1. Schematic of the artificial selection design. Mesocosms are shortened for ease of visibility. The top panel represents a selection replicate, where the furthest eggs are located and used to found the next generation. The bottom panel represents a control replicate, where eggs are randomly selected based on their distribution, so most eggs will come from near the point of origin and only a few from further away.

Figure 2. Changes in the average distance of an egg from its starting location between treatments during selection, a measure of the dispersal kernel.

Figure 3. Changes in fecundity (number of eggs laid) between treatments during selection.

Figure 4. Differences in individual fecundity between treatments after selection. Symbols represent individuals used in nonspatial (petri dish) and spatial (mesocosm) analyses, respectively.

Figure 5. Emergence of adults over time as measured after selection.

Figure 6. Distributions of total movement by individuals after selection, measured in spatial mesocosms.

Figure 7. Density of eggs laid across distances by individuals after selection. A smoothing bandwidth was automatically estimated for each curve.

Figure 8. Correlation between total individual female movement and individual egg dispersal after selection. Line represents the slope of a linear regression between movement and egg dispersal.
Figure 1
Figure 2

A line graph shows the average egg dispersal distance over generations. The x-axis represents generations ranging from 0 to 20, while the y-axis displays the average egg dispersal distance ranging from 1.5 to 4.0. Two lines are depicted: one for the control group (black dots) and another for the selection group (gray dots). The graph illustrates the fluctuation in egg dispersal distance across generations for both groups.
Figure 3

![Graph showing the average fecundity over generations for control and selection treatments.](image-url)
Figure 4

Graph showing fecundity with error bars for control and selection groups, differentiated by nonspatial and spatial treatment.
Figure 5

![Graph showing the proportion emerged over days for Control and Selection groups. The graph plots the proportion emerged on the y-axis against days on the x-axis. The black line represents the Control group, and the gray line represents the Selection group. The graph illustrates the difference in the proportion of emergence between the two groups.]
Figure 6

- **Control males**
- **Selection males**
- **Control females**
- **Selection females**
- **Control**
- **Selection**
Figure 7
Figure 8

A scatter plot showing the relationship between Total movement and Average gene flow. The plot includes two groups: Control (black dots) and Selection (gray dots). A line of best fit is also shown, indicating a positive correlation between the two variables.
CHAPTER IV: Inter-individual variation in movement behavior and the presence of a behavioral syndrome in high-dispersing individuals of *Callosobruchus maculatus*

INTRODUCTION

The movement of organisms across landscapes is the result of numerous physiological, genetic, and behavioral processes, as well as natal habitat quality and availability (Nathan et al. 2008, Stamps et al. 2009, Zheng et al. 2009, Altizer and Davis 2010, Matthysen 2012, Debeffe et al. 2013). Movement from one location to another is typically divided into distinct stages of departure, transience, and settling (Bowler and Benton 2005, Clobert et al. 2009). Together, these stages jointly influence the movement of both organisms and their genes within and between populations, and are key factors in many ecological and evolutionary processes (Aars and Ims 2000, Ronce 2007, Hanski 2011). Therefore, an understanding of how the various components of dispersal ability and variation in movement behavior interact will lead to a more complete understanding of dispersal patterns in a population.

Variation in movement behaviors, in addition to morphological and physiological traits, is increasingly recognized as an important component of the dispersal process. Variation in movement behavior can be driven either by variation in movement capacity, i.e. the speed or endurance of a disperser, or by variation in movement decisions, i.e. being more likely to disperse or move (Ducatez et al. 2012). Studies have found that dispersers are often more exploratory, more aggressive, and less risk-averse than their less mobile conspecifics (Dingemanse et al. 2003, Duckworth and Kruuk 2009, Cote and Clobert 2010, Debeffe et al. 2013).
In addition to single trait variation, there is also evidence that multiple traits may co-vary together, influencing the overall dispersal phenotype of an organism. For example, Ducatez et al. (2012) found consistent positive co-variation between the decision to move and movement patterns such as flight time and distance covered in the large white butterfly *Pieris brassicae*, connecting different components of the dispersal process to the actual distance covered by the organisms in different settings. The authors also found that dispersal traits co-varied with wing morphology and sex. In a related study, Larranaga et al. (2013) found that variation in flight direction also co-varied with mobility in *P. brassicae*. Co-variation in movement behavior may also be apparent when individuals are at the front of an expanding population. For example, cane toads (*Rhinella marina*) at an invasion front remained in a dispersive behavioral state for a longer period of time, exhibited increased directionality in movement, and covered a larger distance than individuals at the same site several years post-invasion (Lindström et al. 2013). In contrast, Lombaert et al. (2014) found that flight speed of *Harmonia axyridis* individuals increased at an invasion front, but without increases in two other movement-related traits, endurance and motivation to fly.

Despite increased interest in inter-individual variation and co-variation in movement behaviors, our understanding of how multiple movement-related characteristics might co-evolve in response to selection is less clear. In general, complex traits are expected to evolve more slowly and more constrained in their response compared to simple traits (Fisher 1930, Wagner 1988, Orr 2000). Because of this, if variation in movement is driven by multiple behavioral, morphological, and physiological components, we might expect responses to selection to be limited. This can be true even
if all components contain genetic variation, if the variation does not exist in the direction of multivariate selection (Blows and Hoffman 2005, Conner 2012, Dochtermann and Dingemanse 2013). Conversely, if the different components of movement are epistatically controlled in a modular framework, we might expect this genetic architecture to facilitate and perhaps augment the response to selection (Wagner and Altenberg 1996, Orr 2000). Thus, experimental studies are needed to understand the magnitude and direction of change in multiple functionally related traits.

In this experiment we measured the movement patterns of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) individuals that had been artificially selected for increased dispersal to investigate how behavioral syndromes are related to mobility and dispersal. Both males and females of these genetically divergent lineages exhibited increased movement over a lifetime as a result of 20 generations of selection for increased offspring dispersal distances. We measured the distance covered, speed, and proportion of time resting in individuals of both treatments in order to examine if traits associated with both movement decisions (resting time) and movement capacity (speed, distance covered) were different between treatments, and also to measure the presence and strength of correlations between the three movement traits. If these traits positively co-vary in a manner facilitating dispersal in the high dispersal treatment, this would suggest the existence of a behavioral dispersal syndrome.

**MATERIALS AND METHODS**

*Study System & Experimental design*
*Callosobruchus maculatus* is a cosmopolitan pest of legume seeds, with natural populations inhibiting stored products in the tropics and temperate regions. Individuals in our stock populations were originally captured on mung beans (*Vigna radiata*) and have been maintained on this host for hundreds of generations in a nonspatial environment (Messina 1991). Females are extremely fecund, laying approximately 70-80 eggs each in stock populations. After eggs finish development in 4-5 days, larvae burrow into seeds and complete development in 11-12 days when at 29.5 °C. Adults are capital breeders and require no food or water after eclosion. If given access to seeds, adults typically live 3-6 days. Males and females are sex-dimorphic, and there is some evidence of selection for sexual dimorphism as well as evidence of sexual conflict (Crudginton and Silva-Jothy 2000, Arnqvist et al. 2005, Rankin and Arnqvist 2008).

This experiment used replicate populations of the seed beetle *Callosobruchus maculatus* that had been previously selected for increased dispersal distance in an artificial selection experiment. Briefly, we created replicate experimental mesocosms made of 31 small (70 mm) jars each with ~150 seeds, connected by 3.1 mm i.d, tubing, to create one-dimensional, spatially extended habitats. We established 10 replicate populations consisting of 50 individuals with equal proportions from each of the three stock populations in a 50/50 sex ratio. Adult beetles were placed into the center of a replicate and allowed to mate and move at will. These replicates were placed into selection (high dispersal) or control treatments when all adults had died after the initial generation, with the five highest-dispersal replicates becoming the selection treatment and the remaining 5 replicates becoming the control treatment (one selection replicate was compromised during selection and so was excluded from all analyses). Successive
generations in each replicate were comprised of 50 offspring, picked as larvae. These larvae were picked in a frequency-dependent random manner for control replicates, whereas the selection replicate offspring were picked based on being furthest from the point of origin of the adults. Selected larvae were placed into the center jar and allowed to emerge, mate, and disperse at will. All replicates were kept at 29.5 °C ambient temperature.

Following selection, we kept all replicates in a common nonspatial environment for one generation and then measured individual-level divergence in dispersal and life history traits between treatments. As measured in one-dimensional mesocosms, selected individuals moved twice as far as control individuals over their lifespans and moved further between consecutive observation points (see Chapter II).

*C. maculatus* individuals from selection and control replicates were selected haphazardly from the nonspatial environment and isolated individually as larvae inside seeds. Adult beetles were used for tracking within 16 hours of eclosion. We recorded individual movement patterns that took place in an arena made of a 56 cm x 71 cm (22” x 28”) white poster board with masking tape at the edges to encourage beetles to stay within the arena. All trials took place in a well-lit open room without disturbance at 29.5 °C ambient temperature. Beetles were recorded for 15 minutes using a Canon Powershot A610 set on a tripod placed 3’ above the arena. Each trial used 5 individuals of the same sex, released simultaneously in the center of the arena. We conducted 3-5 trials for each sex for each replicate (5 control and 4 selection replicates) for a total *n* = 68 trials.

Movement pattern data were extracted from trials using the software program Multi-Worm Tracker (MWT; Swierczek et al. 2011). This program, initially designed to
track multiple *Caenorhabditis elegans*, can more generally be used to track many moving and nonmoving objects across a stationary, contrasting background. Individuals that touch during trials are removed automatically rather than trying to discern between individuals. The MWT software creates output files summarized for each video, as well as for each individual object tracked during the video, that are designed to be analyzed by the companion Java program Choreography, available under an open source license as part of the MWT package at http://sourceforge.net/projects/mwt. Choreography computes a variety of metrics, including speed, angular speed, linear distance moved, and changes in direction, as well as simple x-y coordinates, and can also incorporate object selection criteria such as minimum distance moved and minimum persistence time for an object to be considered in an analysis.

We used MWT and Choreography to estimate three movement parameters over the course of each trial: total linear distance moved (hereafter ‘path length’) over time, speed of individuals over time, and proportion of time spent resting. We used a cutoff for persistence of 5 seconds for our data; that is, objects had to be tracked for at least 5 continues seconds to be considered in our measurements. The data for path length over time represent a cumulative measure of the total distance covered, summed across any individuals that moved, over the course of the trial. This sacrifices knowledge about the average distance covered by any single individual in order to focus on differences in the total exploration over time by all individuals (i.e., do selected individuals cover more territory during the trial and how does the rate of exploration change over time). Summarized speed measurements produced by Choreography are averages, composed of any individuals that are being tracked at that particular point in time. This again sacrifices
knowledge about any given individual’s speed in order to estimate the average speed and also to estimate changes in speed over time. Measurements of the proportion of time spent not moving come from the object-level data, for which we have speeds of each object tracked during a given frame. For this metric, we used a cutoff of a speed of 0.2 mm/s to mark an individual object as not moving, based on visual inspection of individual object speeds for both treatments. We then used the sum of observation points where any object was measured as not moving divided by the total number of observation points summed across all objects as the proportion of time that objects were not moving during the trial.

To estimate the presence and strength of co-variation between different aspects of movement, we created summary statistics for each movement parameter for each trial, and measured the two-way correlations between all three parameters. For path length, we used the total cumulative path length at the end of the trial. The time spent resting during a trial was calculated as described above. For speed, we created a separate estimate of individual ‘running’ speed by taking the average speed of individuals for any data points with a speed greater than 0.4 mm/s. This essentially captures the average speeds of individuals when moving at full speed and eliminates the ‘noise’ of resting time, acceleration and deceleration.

Statistical Analyses

All analyses were conducted using R 3.0.3 (R Core Team 2014). Analyses measuring differences between treatments in path length and speed over time were conducted as linear mixed-effects model analyses (LME) using the MCMCglmm function in the MCMCglmm package (Hadfield 2010). These analyses used path length or speed as
the response, with treatment, time, and sex as fixed effects including all interactions, and replicate and trial as random effects, with trial nested within line. Similarly, we used \textit{MCMCglmm} to test for differences in the proportion of time spent not moving between treatments. Because this is a summed measure, treatment and sex are the only main effects along with their interaction, with the random effect of trial again nested within replicate.

We measured the two-way correlations between the maximum distance covered during the trial, average top speeds of individuals, and proportion of the trial spent not moving, using the \textit{cor.test} function in the \textit{stats} package (R Core Team 2014).

\textbf{RESULTS}

We found consistent differences between treatments in all three of our movement parameters. Selected individuals had a significantly higher path length, covering 30-35\% more territory over the course of the trial compared to control individuals, and females covered significantly less distance than males (Fig. 1, Table 1). In addition to the significant main effect of treatment, there was also a significant interaction between treatment and time, such that selected individuals covered proportionally more territory over the course of the trial compared to control individuals. There was also a significant main effect of sex, as well as a significant interaction between sex and time, with males moving further over the course of the experiment.

Selected individuals also moved faster than control individuals (Fig.2, Table 1). There was also a significant main effect of sex, with males moving faster than females. There was a significant treatment*time interaction, with selected individuals slowing
down over time compared to control individuals, who maintained a more constant speed. There was also a small but significant treatment*sex interaction, suggesting that selected females moved proportionally faster than their male counterparts.

Analysis of the proportion of time spent resting found that control individuals rested significantly more than selected individuals, and males rested significantly less than females (Fig. 3, Table 1). The interaction between treatment and sex was nonsignificant.

We found significant two-way correlations between all three of our movement variables (Fig. 4, Table 2). Average top speed of individuals and total distance covered during a trial were highly positively correlated, suggesting trials where individuals moved faster also covered more territory overall. Individual ‘running’ speed was negatively correlated with the proportion of time spent resting, indicating that in trials where individuals were slower when moving full speed, they also rested a larger proportion of the trial, as opposed to moving faster and resting more in-between. Finally, total distance covered was negatively correlated with proportion of time spent resting, confirming that individuals who rested more did not go as far.

DISCUSSION

Understanding how different components of the dispersal process co-evolve in response to selection has important implications not only for processes like responses to climate change or habitat fragmentation, but also for a basic understanding of how traits coevolve to manifest the observed response to selection. In this experiment we used replicate *C. maculatus* populations that had been artificially selected for increased
dispersal to measure the response and correlations between three different components of
the movement process: speed, total distance moved, and time spent resting. We found
significant differences in all three components, with individuals from the high dispersal
treatment showing significantly increased speed, reduced resting time, and larger linear
distance covered, and with individuals showing highly correlated responses between
behaviors. In addition, we found consistent differences between sexes in movement
characteristics, with females being less mobile and slower than males regardless of
treatment group.

There is an increasing appreciation for the hypothesis that the multiple traits
associated with a behavioral process do not evolve independently, but may co-vary
together for a number of reasons. For example, different components of these traits may
vary together due to a common selective pressure for increased or decreased movement
(Ronc and Clobert 2009). Conversely, individuals that do disperse may be under
different selection pressures in comparison to individuals who remain resident in an area
(Phillips et al. 2008, Clobert et al. 2009). These dynamics can lead to the genetic
covariation of certain physiological and behavioral traits, creating trait ‘syndromes’ that
vary in concert with dispersal characteristics (Dingemanse et al. 2003, Sih et al. 2004,
Aragon et al. 2006, Clobert et al. 2009, Ducatez et al. 2012). The nature of these
syndromes may vary due to habitat structure, and may vary between species with
differing movement constraints in the same landscape (Baguette and Van Dyck 2007,

We found clear correlations between all three movement traits, but the results
could have been otherwise. For example, beetles in a given trial could cover a larger
distance via moving more often, moving faster, or both. Thus, the negative relationship between resting time and speed is important in that slow individuals are not making up distance by moving more consistently, but are instead moving slower, leading to the correlations between both speed and resting time with total path length. These results are consistent with a genetically-based dispersal syndrome in this system, on a continuum from slow, sedentary individuals to fast, restless individuals.

The concept of behavioral syndromes has been fruitful in helping to elucidate many aspects of the dispersal process (Dingemanse et al. 2003, Clobert et al. 2009, Ronce and Clobert 2012). Perhaps most immediately, understanding trait covariation can help us predict which individuals will be more likely to disperse based on their phenotypes. In this study, we used genetically distinct populations, selected specifically for increased dispersal of offspring and known to move further over the course of a lifetime, in order to investigate if variation in movement decisions or movement ability influenced variation in the distance travelled. Our results provide strong evidence that all three traits we measured contribute to the increased movement of selected individuals over a lifetime compared to control individuals, and that females are generally less mobile than males.

A second area of discovery utilizing behavioral syndromes involves understanding how constraints influence trait variation that is under selection (Dochtermann and Dingemanse 2013). In general, we expect dispersal syndromes to be shaped by constraints imposed by resource allocation trade-offs due to antagonistic pleiotropy; and by selection on traits that maximize fitness in a given environment (Ronce and Clobert 2012). However, separating the relative influence of constraints versus multifarious selection on trait covariation depends largely on the genetic
architecture of the traits and the nature of selection (Conner 2012, Ronce and Clobert 2012). For example, differing Pgi genotypes in the Glanville fritillary butterfly Melitaea cinxia exhibit differing lifespans and clutch sizes, with more mobile individuals experiencing shorter lifespans and larger clutch sizes (Saastamoinen 2007a). However, the realized fitness between genotypes differs depending on the age of the population, such that more mobile females are more fit in new populations, but there is no relationship between mobility and fecundity in old populations (Saastamoinen 2007b). Thus, the trait covariation observed could be the result of one of two processes. It is possible that variation in Pgi genotype frequencies is due to pleiotropic constraints, where Pgi genotypes would be more frequent were it not for the trade-off with lifespan. On the other hand, this genotypic and phenotypic variation between M. cinxia populations could be the result of selection on multiple dispersal and life history traits, selecting on variants at the Pgi locus among other genes that to not have these pleiotropic effects.

More generally, these results bear on the evolvability of complex traits. If each component of movement-the decision to move, speed, endurance, morphology, and physiology-were evolving separately, but were all required for the evolution of organismal movement, we would expect to see a limited response in these traits due to the number of independent characters involved (Wagner 1988, Orr 2000). The correlations between our different movement traits and the clear differences between traits suggest these traits may be modular in some way, or at least co-vary in a manner that facilitates trait evolution. However, despite 20 generations of directional selection and evidence for increased movement as a result, there was no continuous trend for
increased offspring dispersal, either on average or at the far extreme of the distribution (Chapter II). Selected individuals also showed significantly reduced egg-to-adult survival and reduced fecundity (Chapter II). Combined with the results of this experiment, these data suggest that antagonistic pleiotropic trade-offs in C. maculatus are contributing to an overall dispersal syndrome including increased movement and higher speed, along with decreased fecundity and lower larval quality. By depending on behavioral traits and life history traits, it is possible the increased dimensionality of the dispersal process partly inhibited our populations from responding to selection with increased dispersal of offspring.

In conclusion, we found clear differences between control and high-dispersal C. maculatus individuals in both movement decisions and movement ability. These data suggest that different movement characteristics are correlated in this system, and that these traits can readily respond to selection. However, evolution in these traits did not result in an increase in the dispersal of offspring, suggesting that the additional complexity of that trait may constrain the response of populations to selection.

REFERENCES


URL: http://www.jstatsoft.org/v33/i02/


Table 1. Results of mixed-model analysis testing for differences between control and high dispersal individuals for the movement traits of path length, speed, and time spent resting. Posterior estimate is the parameter estimate as fit using the 
*MCMCglmm* package. Lower and upper 95% CI are credible intervals; and *p* is the proportion of MCMC runs with parameter estimates that are different from 0.

<table>
<thead>
<tr>
<th>Model</th>
<th>Posterior estimate</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th><em>p</em></th>
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</thead>
<tbody>
<tr>
<td>Path length</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>2.811</td>
<td>0.835</td>
<td>4.629</td>
<td>0.004</td>
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<tr>
<td>Time</td>
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<td>0.139</td>
<td>0.144</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
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<td>-6.679</td>
<td>-2.761</td>
<td>&lt;0.001</td>
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<tr>
<td>Treat*Time</td>
<td>0.062</td>
<td>0.059</td>
<td>0.066</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treat*Sex</td>
<td>-0.824</td>
<td>-3.345</td>
<td>1.934</td>
<td>0.566</td>
</tr>
<tr>
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<td>0.056</td>
<td>0.063</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treat<em>Time</em>Sex</td>
<td>0.008</td>
<td>0.003</td>
<td>0.013</td>
<td>0.006</td>
</tr>
<tr>
<td>Speed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
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<td>0.148</td>
<td>0.173</td>
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<tr>
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<td>-3.423E-05</td>
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<td>Sex</td>
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<td>0.144</td>
<td>0.169</td>
<td>&lt;0.001</td>
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<tr>
<td>Treat*Time</td>
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<td>-1.480E-04</td>
<td>-9.990E-05</td>
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<td>-0.072</td>
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<tr>
<td>Time*Sex</td>
<td>2.355E-05</td>
<td>-4.029E-06</td>
<td>4.507E-05</td>
<td>0.076</td>
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<tr>
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<td>1.424E-04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Resting time</td>
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<td></td>
</tr>
<tr>
<td>Treatment</td>
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<td>-0.173</td>
<td>-0.0366</td>
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<tr>
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<td>-0.242</td>
<td>-0.0878</td>
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<td>0.0189</td>
<td>-0.0901</td>
<td>0.112</td>
<td>0.714</td>
</tr>
</tbody>
</table>
Table 2. Correlations between various movement components. Correlation is the Pearson's product-moment correlation; t is the t-statistic, df is the degrees of freedom, and p is the associated p-value.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Correlation</th>
<th>t</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum distance</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average speed</td>
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<td>13.78</td>
<td>67</td>
<td>&lt;0.01</td>
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<tr>
<td>Resting time</td>
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<td>-9.24</td>
<td>67</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Average moving speed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting time</td>
<td>-0.282</td>
<td>-2.42</td>
<td>67</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Figure 1. Cumulative linear distance covered by males (top) and females (bottom) in the control and selection (high dispersal) treatments.

Figure 2. Average speed of males (top) and females (bottom) in both the control and selection (high dispersal) treatments over time. Error bars are not shown and are overlapping.

Figure 3. Proportion of time where objects are at rest during a trial for males and females in both the control and selection (high dispersal) treatments.

Figure 4. Correlations between maximum distance covered and average ‘running’ speed during a trial (top panel); maximum distance covered and proportion of time resting (middle panel); and average ‘running’ speed and proportion of time spent resting (bottom panel).
Figure 1
Figure 2

![Graphs showing speed over time for selection and control groups for both male and female participants.]
Figure 3

Proportion of time spent resting (+/- SE)

Control  Selection

△ Female  □ Male
Figure 4

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Proportion of time resting
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Average running speed
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Proportion of time resting
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Maximum distance (mm)
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Proportion of time resting
```

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Average running speed
```

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Maximum distance (mm)
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Proportion of time resting
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Average running speed
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Maximum distance (mm)
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Proportion of time resting
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Average running speed
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Maximum distance (mm)
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Proportion of time resting
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Average running speed
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Maximum distance (mm)
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Proportion of time resting
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Maximum distance (mm)
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Proportion of time resting
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Average running speed
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Maximum distance (mm)
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```
Proportion of time resting
```
CHAPTER V: Conclusions

This dissertation used the seed beetle *Callosobruchus maculatus* as a model organism to study the ecological consequences of genetic diversity, and the evolutionary consequences of evolution selecting on quantitative traits (dispersal) that are negatively correlated with important life history traits. These questions both benefit from the use of an experimental system by being able to manipulate genetic diversity and population size.

In Chapter II, we manipulated genetic diversity of replicate populations and measured the resource use of different hosts as well as population productivity. We found that populations that were intermediate in diversity were the most productive, and no change in resource use with varying levels of diversity. We also found major differences between individual host preference and population resource use, reflecting phenotypic plasticity in host preferences that may depend on the presence or density of conspecifics. These results highlight the fact that benefits of genetic diversity may not always be linear, and may also depend on other population characteristics that can augment or constrain the benefits of diversity.

In Chapter III we used an experimental evolution framework to investigate if selection on offspring dispersal can result in changes in individual movement and dispersal traits and/or life history traits, and how these interactions influence population dispersal distributions. The selection for offspring dispersal yielded distinct differences between the high dispersal and control treatments, but not continuous evolution of dispersal over time. However, there were large differences between treatments in both organismal movement and life history traits in a manner consistent with the presence of energetic constraints creating a negative trade-off between dispersal and life history traits,
and this trade-off potentially limited the ability of these populations to respond to selection in a consistent manner over time.

In Chapter IV we measured speed, resting time, and total path length in and control and selection (high dispersal) treatments of *C. maculatus* in order to measure the relationship between these traits and dispersal, as well as correlations between them to measure the presence of a dispersal syndrome in this system. We found significant differences between treatments in all three traits, with high dispersal individuals of both sexes moving faster, resting less, and moving covering more distance than their control counterparts. In addition, we found significant two-way correlations between all three traits, suggesting the dispersal ability of individuals co-varies predictably in the manner of a behavioral syndrome. These results provide strong evidence for a genetically based dispersal syndrome that varies predictably with sex and should facilitate increased individual movement over a lifetime.