THE ROLE OF RAPID ADAPTATION IN PLANT POPULATION ESTABLISHMENT

By

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ABSTRACT

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Rapid adaptation, or adaptation that occurs on an ecological timescale, has been documented across a wide range of taxa and in many biological contexts, and can potentially alter the outcomes of ecological interactions and ecosystem-level processes. Rapid adaptation is also hypothesized to influence the establishment of species in new habitats, as rapid adaptation can have important demographic consequences for a colonizing population that is not optimally suited to a novel habitat. Examining the relationship between rapid adaptation and establishment can lead to a better understanding of successful colonization events, such as biological invasions, range expansions, and successful establishment in ecological restorations. In this dissertation, I used manipulative field and greenhouse experiments to examine rapid adaptation, its potential drivers and trait changes that lead to adaptation, and its demographic consequences in two plant populations in recently restored prairies. I found evidence that one population rapidly adapted only six years after establishment, which could potentially influence population persistence. In addition, I found that the plant populations rapidly evolved different strategies of interacting with microbial mutualists, suggesting that these mutualists may act as agents of selection in this system. By providing evidence that rapid adaptation occurs in field populations and examining its potential drivers, my research expands our understanding of the potential causes and consequences of rapid adaptation in recently established plant populations.

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CHAPTER ONE:

INTRODUCTION

Introduction

Rapid adaptation (adaptation that occurs on an ecological timescale) can occur across a wide range of taxa and in many biological contexts, and can potentially alter the outcomes of ecological interactions and ecosystem-level processes (Carroll et al. 2007 and references therein). Rapid adaptation is also hypothesized to influence the establishment of species in new habitats (Colautti and Lau 2015), as rapid adaptation may be required for successful establishment when colonizing populations are poorly matched to local site conditions. Understanding the relationship between rapid adaptation and establishment could be important for understanding species' range expansions, biological invasions, or establishment in ecological restorations.

A colonizing population may need to rapidly adapt to its new environment if there is a mismatch between the population's traits and the environmental conditions in the new habitat. Both abiotic and biotic environmental factors have the potential to exert strong selection on a colonizing population and drive adaptation. For example, microbial symbionts, known to have important effects on plant communities (van der Heijden et al. 2008), can act as strong agents of natural selection on plants (Lau and Lennon 2011). Plants that rapidly adapt to new microbial partners, such as resource mutualists, might experience fitness benefits that could facilitate population establishment. Identifying the selective agents and traits responsible for adaptation can give insight into the factors that influence evolution in these systems.

Although theory predicts that rapid adaptation can influence the likelihood of population establishment (Jones and Gomulkiewicz 2012), and rapid adaptation is often cited as a factor leading to species establishment in novel habitats, there is sparse empirical evidence to support this (Colautti and Lau 2015). By looking for evidence of rapid adaptation in recently established populations and examining the effects of this adaptation on demography, we can determine whether rapid adaptation really does influence the establishment of field populations of colonizing species. My dissertation explores rapid evolution, potential drivers of evolution, and the demographic effects of adaptation in populations of the annual legume *Chamaecrista fasciculata* in recently restored prairies. Specifically, I ask:

Chapter 2) Is there evidence of rapid adaptation in plant populations in recently restored prairies?
Chapter 3) Do plant populations rapidly adapt to microbial mutualists in novel habitats?
Chapter 4) Are recently established plant populations locally adapted, and if so, is adaptation likely to affect population persistence?

In 2010, two former agricultural fields in southwest Michigan (approximately 15km apart) were planted as prairie restorations, using identical seed mixes that included *Chamaecrista fasciculata,* an annual legume native to eastern North America commonly found in prairies and disturbed grasslands (Galloway and Fenster 2000). *Chamaecrista* is self-compatible but predominantly outcrossing, and forms facultative mutualistic interactions with nitrogen-fixing bacteria in the *Bradyrhizobium* genus. While present at both prairie sites, *Chamaecrista* is highly abundant at one and less common at the other. These restorations provide a unique opportunity to examine rapid evolution in establishing *Chamaecrista* populations, as the populations originated from the same seed source but have been evolving independently in sites that vary in both abiotic and biotic factors (Stahlheber et al. 2016) over the past 6 years. In addition, seeds were saved from the original seed source, making it possible to determine not only if the two *Chamaecrista* populations have diverged from each other, but also whether they have differentiated from their source population.

Main results and significance

I used manipulative field and greenhouse experiments to examine rapid evolution, its potential drivers, and its demographic consequences in plant populations in recently restored prairies. I found evidence of rapid evolution in my two focal populations of *Chamaecrista*, indicated by trait differences between the populations and their original source population that were apparent within six years after the populations had been established. I detected no selection on these traits, which suggests these specific evolutionary changes may not be adaptive. One trait that differed between populations was nodule (structures on plant roots housing mutualistic rhizobia bacteria) production, suggesting that the populations may have evolved different ways of interacting with rhizobia mutualists. Further exploration of these interactions found that one population performs best when associating with rhizobia from its homesite (suggesting local adaptation to rhizobia), while the other population derives no benefit from rhizobia at all. These differences in plant-rhizobia interactions between the two populations could be driven by differences in rhizobia quality between the two restoration sites, and suggests that rhizobia may act as agents of selection in this system. Finally, by examining overall plant fitness in a reciprocal transplant experiment, I found that one *Chamaecrista* population is locally adapted to its homesite while the other is not, indicating that rapid adaptation did occur in at least one of the populations. However, this adaptation does not seem to influence population growth rates in a way that would increase the likelihood of population persistence. Although the locally adapted population performed better than the other populations at its homesite, it still had a population growth rate (λ) < 1. This suggests that rapid adaptation will not necessarily lead to population persistence in this system.

My dissertation research provides an empirical test of the importance of rapid adaptation for population establishment. My thesis makes three main contributions: First, I demonstrate that plant populations can rapidly evolve, and in one case adapt (in about six generations) in recently restored habitats. This could have implications for restoration, in that it suggests that commonly used seed sources may not be optimally adapted to restoration site conditions and that management practices that facilitate rapid adaptation (e.g., high seeding densities to maintain large population sizes) may be worth considering. Second, I add to the growing body of literature finding that microbial mutualists have the potential to influence the evolution of plant populations, by showing that the ways in which plant populations interact with microbial mutualists can rapidly evolve and that mutualist quality, not just presence, may influence the evolution of plant traits governing the interaction with mutualists. Third, I demonstrate the utility of linking local adaptation to demography to examine the ecological effects of adaptation, as well as the importance of using integrated fitness metrics (as opposed to fitness components) to assess local adaptation. Together, my findings suggest that while adaptation may be common in establishing populations, it may not always be sufficient to promote population persistence, at least in the early successional communities characteristic of restored ecosystems where environmental conditions change rapidly and adaptation must occur to both overall site conditions and the continuously changing competitive and resource environments that result from succession.

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CHAPTER TWO:

RAPID EVOLUTION IN ECOLOGICAL RESTORATIONS

Abstract

Mismatches between the traits of a colonizing population and a novel habitat can generate strong selection, resulting in rapid evolutionary change. Although many studies have found evidence of evolutionary changes in colonizing populations, few have determined whether these changes are actually adaptive rather than the result of founder effects. We combined a resurrection experiment, comparing populations of the annual legume *Chamaecrista fasciculata* in recently restored prairies to their original source population, with measures of natural selection on these populations to both measure evolutionary responses and determine whether these responses were predicted by contemporary estimates of selection. We found evidence of evolutionary changes in flowering time, root nodule production, and specific leaf area in the *Chamaecrista* populations. However, we detected no contemporary selection on these traits, suggesting that the observed evolutionary changes may not be adaptive. Our study demonstrates how resurrection approaches can be combined with measures of natural selection to understand rapid adaptation, but also illustrates the challenges in applying this approach given that selection may change over time, particularly in early successional, restored systems

Introduction

Populations colonizing new habitats typically encounter novel environmental conditions, which can act as strong selective agents and potentially lead to rapid adaptation of colonizing populations (Reznick and Ghalambor 2001, Prentis et al. 2008). This scenario is becoming more likely as species increasingly colonize new habitats in response to anthropogenic forces. For example, climate change is driving range expansions (Parmesan and Yohe 2003, Kelly and Goulden 2008, Chen et al. 2011), such that populations encounter novel conditions and new species as they shift in latitude or altitude (Alexander et al. 2016). Similarly, invasive species encounter novel herbivores, competitors, and mutualists (Mooney and Cleland 2001) or novel climates and soils within the range they are invading. In each of these scenarios, populations are likely to encounter environments to which they are not well-adapted and as a result experience strong selection. Rapid adaptation may aid in establishment in these novel environments, but it can be difficult to determine whether evolutionary changes observed in many colonizing populations are truly adaptive. Determining whether evolutionary changes in colonizing populations are the result of adaptation can give insight into whether rapid evolution has the potential to influence colonization success.

Evolutionary change can occur not just in response to selection (i.e., adaptation), but also due to stochastic processes such as founder effects. There are many examples of evolutionary changes occurring in colonizing populations, most of which come from studies of invasive species where mean trait values differ between native and invasive populations in common gardens (reviewed in Bossdorf et al. 2005, Colautti et al. 2009, Felker-Quinn et al. 2013) or trait changes in invasive populations are documented over time (Buswell et al. 2011, Flores-Moreno et al. 2015). Because often little is often known about the original source of these populations, it can be challenging to identify whether any differences between native and invasive populations are due to evolution. Moreover, because even less is known about the initial size of these colonizing populations, it can be difficult to determine whether evolutionary changes are due to selection or founder effects (Keller and Taylor 2008). One exception to this is studies of invasive species over geographic clines, which have shown that colonizing populations can adapt in response to climate. Colautti and Barrett (2013) demonstrated that *Lythrum salicaria* in North America rapidly evolved earlier flowering time as it invaded more northern latitudes with shorter growing seasons. Invasive *Drosophila subobscura* rapidly evolved latitudinal clines in wing length that replicate existing clines in its native range (Huey et al. 2000), and *Aedes albopictus* has undergone rapid adaptation of the photoperiodic response across latitude in its invasive range (Urbanski et al. 2012). For studies that do not involve geographic clines, there are several ways to investigate whether evolutionary changes in colonizing populations are adaptive. Keller and Taylor (2008) suggest testing the hypothesis of adaptation against appropriate null models using neutral and quantitative genetic information (Q_{ST}/F_{ST}) to separate effects of adaptation from stochastic events. Following these methods, Xu et al. (2010) found evidence of selection-driven evolution of reproductive strategy in the invasive weed *Phyla canescens*. However, this approach does not work for all taxa, particularly highly-selfing species (Porcher et al. 2006). An alternative approach is to use field studies of natural selection to determine if observed evolutionary changes in colonizing populations are adaptive (Colautti and Lau 2015). Evolution is likely adaptive if estimates of contemporary selection match the direction of observed past evolutionary changes in a population. This approach can also help identify the targets of selection, i.e., the specific traits contributing to adaptation, by controlling for correlations among measured traits. Such studies can be used for most taxa, but assume selection measured in the year of the study is typical, which might not be the case if selection fluctuates in direction across years, which has been documented but is not common (Siepielski et al. 2009).

While most studies of rapid evolution during colonization come from biological invasions, ecological restorations present an ideal opportunity to examine rapid evolution. The advantage of restorations is that colonizing populations come from a known source, and seeds from original restoration plantings can be saved and resurrected later to compare to extant populations (LaRue et al. 2017). Here, we compare extant populations to their resurrected source population in common environments and measure selection on plant traits to address the following questions: 1) Is there evidence of evolutionary change in recently established restoration populations? and 2) Are these evolutionary changes predicted by contemporary estimates of selection in the field? The use of a resurrection experiment combined with contemporary measures of natural selection in the field provides insight into whether observed evolutionary changes are likely adaptive, and which trait(s) may have contributed to adaptation.

Methods

Study System

Chamaecrista fasciculata Michx. is an annual legume native to eastern North America found in prairies and other disturbed sites, which is commonly planted in prairie restorations (Grman et al. 2015). *Chamaecrista* is predominantly outcrossing, its establishment can influence the composition of early successional plant communities (Keller 2014), and it forms facultative mutualistic interactions with nitrogen-fixing bacteria, which provide plants nitrogen in exchange for carbon. In 2010, two former agricultural fields (one 13 hectare and one 11 hectare) near Kellogg Biological Station in southwest Michigan, Lux Arbor (42°28’23” N, 85°26’50” W) and Marshall (42°26’37” N, 85°18’34” W), were planted with identical prairie seed mixes (containing 19 grass and forb species, including *Chamaecrista*, seeded at 0.28 kg/ha, roughly 26,700 seeds/ha) as part of a large bioenergy experiment being conducted by the Great Lakes Bioenergy Research Center (https://www.glbrc.org/). Biomass from each prairie is harvested every year using identical protocols (see Stahlheber et al. 2016 for full site details). Seeds were saved from the original seed mix.

We predicted that the original source *Chamaecrista* population may not have been optimally adapted to restoration sites in southwest MI; therefore, these populations might experience selection on phenological and morphological traits during population establishment, resulting in genetic differences between extant populations and the original source seeds. Our knowledge of the growing conditions of the original source population is somewhat limited, but we know seeds were produced on a seed farm and were supplied by Shooting Star Native Seeds in Houston County, MN, which is 1-2° higher latitude than our restoration sites in southwest MI, with slightly lower average rainfall. This latitude difference could lead to a mismatch in optimal phenology between the source and restoration sites, as higher-latitude populations of a species often flower earlier (Griffith and Watson 2006). In addition to the abiotic differences between the source and restoration sites, there are likely biotic differences that could lead to evolutionary changes in restored populations. Seeds for restorations are often grown in high-productivity monocultures, which can select for plants that are not optimally suited to restored habitats (Kulpa and Leger 2013). We therefore might expect traits related to competitive ability, such as plant height, to be under selection in diverse restoration sites.

We know that our two restorations sites differ from each other in both edaphic and biotic factors, which may influence natural selection on *Chamaecrista* and lead to differences between populations at these sites. For example, the Marshall site has roughly twice as much available soil ammonium and nitrate as the Lux site (Stahlheber et al. 2016), which could influence traits related to *Chamaecrista's* mutualism with nitrogen-fixing rhizobia. The two sites also differ in average soil moisture and light availability (Stahlheber et al. 2016), which could influence traits such as specific leaf area and height. Despite being planted with identical seed mixes, contemporary community composition differs significantly between the two sites, and *Chamaecrista* biomass is consistently higher at the Lux site, suggesting that these sites differ in ways that influence *Chamaecrista* demography and potentially in ways that influence the strength or direction of natural selection.

Resurrection reciprocal transplant: Evidence of evolutionary change

To determine whether these recently established *Chamaecrista* populations have undergone evolutionary change, we conducted a reciprocal transplant experiment using seeds collected from the two extant populations, as well as resurrected seeds saved from the original seed mix. In September 2015 (likely six *Chamaecrista* generations after the populations were planted, as *Chamaecrista* has a limited seed bank (Fenster

1991)) we collected 5-20 seeds from each of 100 individuals from each site. To do this we established five 100 meter transects at each site, and collected seeds from the nearest *Chamaecrista* individuals to the transect at five-meter intervals. We grew seeds from these plants, along with seeds from the original seed mix (seeds had been stored mixed with all other species in the original seed mix in plastic mesh bags at room temperature since 2010), in the greenhouse for one generation to minimize maternal effects. For the two extant populations, we grew one seed from each of 96 of the 100 maternal plants. Each plant was randomly assigned to be a sire or a dam, and pollen from each sire was used to pollinate two dams, for a total of 64 full-sibling families (32 half-sibling families) per site. We did not include family structure when pollinating flowers of the original source plants, due to low germination of the original source seed (only 7 seeds out of 100 germinated; in contrast, approximately 95% of seeds collected from the two extant population germinated). Instead we used one plant as a pollen donor on a given day (for approximately 60 days), so that every plant was crossed with every other plant several times.

In May 2016 we germinated seeds produced by these plants in the greenhouse (we had approximately 95% germination success and no differences in germination among the three populations). We transplanted seedlings into six 4 x 4m plots (each divided into 16 1 x 1m subplots with seedlings spaced 16cm apart) in each prairie site {(2 seedlings/extant population full-sib family x 64 families/extant population x 2 extant populations + 64 original source population seedlings) x 6 plots x 2 sites; N=3840 total seedlings}. We fenced half of the plots to exclude deer and small mammals, to ensure that herbivores did not kill all of the seedlings (in a previous experiment, small mammal herbivory driven by a vole outbreak led to 95% plant mortality); however we detected little herbivore-induced mortality in this experiment. We disturbed existing vegetation as little as possible when planting seedlings. Conducting this experiment in the restored prairies as opposed to a greenhouse or non-prairie common garden allowed us to not only look for evidence of evolutionary changes in the *Chamaecrista* populations, but to also estimate selection on traits in these populations' home environments.

Over the course of the growing season we monitored survival and recorded day of first flower. In July 2016 we collected the third fully-expanded leaf from the top of each plant to measure specific leaf area (SLA). In September 2016, when most fruits were mature enough to count seeds, we harvested all aboveground biomass, measured plant height, and counted the number of seeds produced by each plant as a lifetime female fitness measure. We also harvested a root sample from each plant by digging up the top 5-10cm portion of the root, and then counted the number of root nodules to estimate the number of root nodules/length of root.

We compared trait values from each population using linear mixed models in R v. 3.5.1 (R Core Team 2018) using the lme4 package (Bates et al. 2015), with trait values (date of first flower, nodules/cm root, SLA, and height) as response variables. Source population (Lux, Marshall, Original), site (Lux, Marshall), fencing (present, absent), and all interactions between these variables were included as fixed effects, and plot nested within fencing, and subplot nested within plot were included as random effects. For the height model, we only included plants from fenced plots to avoid using plants that had been browsed by herbivores.

Due to low germination of the original source seed (we used seven viable seeds to generate all original source plants for the experiment), there was a risk that the mean trait values we found for this population were not accurate representations of the entire source population. A biased sample could result if the measured traits are correlated with seed viability, or if the small sample size led to founder effects. To estimate whether founder effects influenced our conclusions, we bootstrapped trait distributions for each trait in the extant populations by repeatedly drawing seven families at random from each population, to calculate a distribution of population mean trait values controlling for sample size. When we found trait differences between either extant population and the original source population in the experiment, we compared where the original source mean fell on the bootstrapped extant population trait distributions. In all cases, the original source mean fell outside the 95% confidence intervals of the distributions, indicating that trait differences were likely due to actual differences between source and extant populations and not the result of a founder effect in our sample of the source population (Fig. S2.3).

Genotypic selection analyses

To estimate the strength and direction of selection and determine whether any observed evolutionary responses in these populations can be predicted by estimates of selection, we conducted selection analyses on *Chamaecrista* at both sites. These analyses regress relative fitness on standardized trait values to estimate the strength and direction of selection on measured traits (Lande and Arnold 1983). We used family mean trait and fitness values to conduct genotypic selection analyses, which remove biases caused by environmental correlations between traits and fitness (Rausher 1992).

We used data from the two extant populations only (because we had no family structure for original source plants), and included only plants from unfenced plots as we wanted estimates of selection on traits under natural field conditions. We excluded height measurements from plants that had been browsed by deer from the analysis because we could not obtain accurate measurements for these individuals. These analyses included all plants grown at each site, regardless of population (that is, we combined data from Lux plants grown at the Lux site with Marshall plants grown at the Lux site to examine selection at the Lux site). This likely expanded the phenotypic distribution, which allow for better estimates of the overall fitness function (Conner and Hartl 2004).

Given that environmental conditions differ between the two restoration sites, we tested whether selection differed across sites, by calculating selection gradients using linear mixed models with family mean relative fitness as the response variable, standardized traits values (height, flowering time, root nodules, SLA) and their interactions with site as predictor variables, and plot nested within site as a random effect. We relativized fitness and standardized traits within sites. To estimate the strength and direction of selection on traits at each site we ran separate models for each site, similar to the one described above but without the trait x site interaction terms. To examine non-linear selection coefficients and correlational selection we used models with traits, quadratic terms (traits squared), and all trait cross-products as predictor variables (quadratic coefficients were doubled (Stinchcombe et al. 2008)).

Phenotypic selection analyses

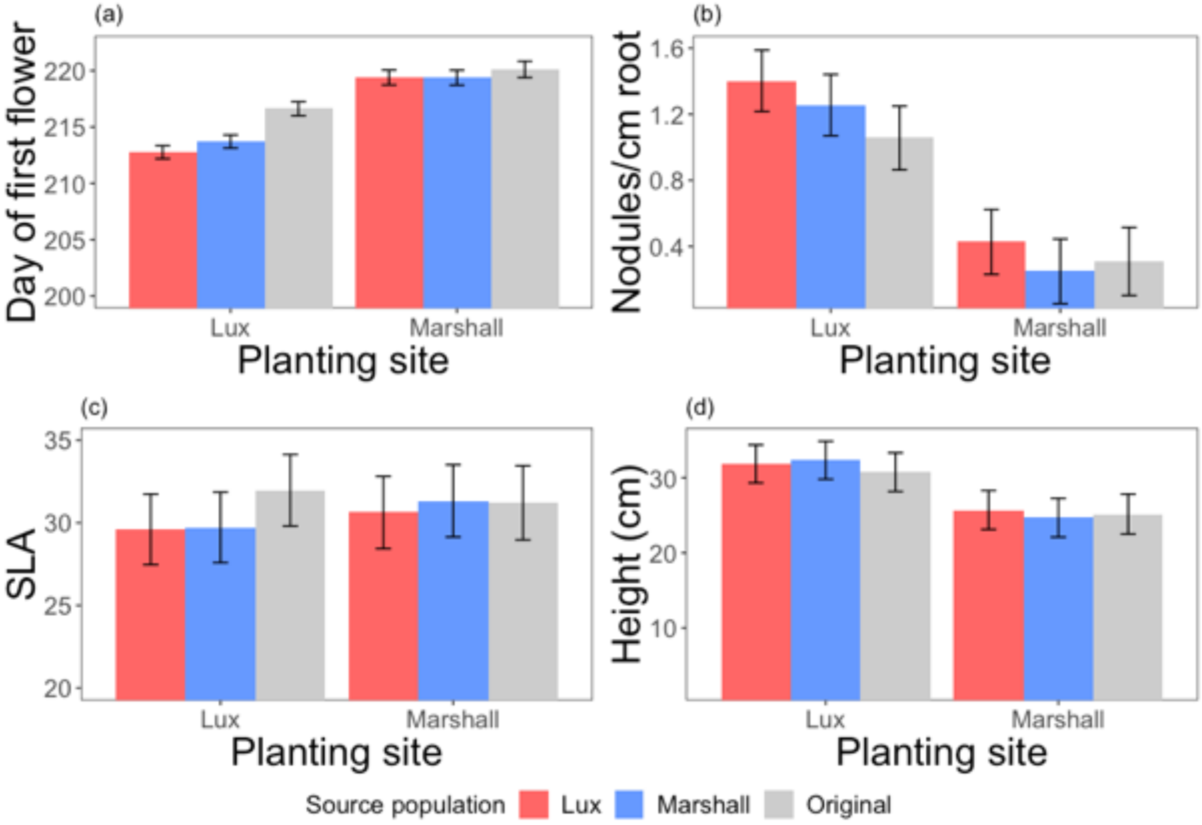
To explore selection on traits in different years, we also conducted phenotypic selection analyses on *Chamaecrista* trait and fitness data collected in 2014 and 2015 (we used phenotypic and not genotypic selection because we had no family structure for individuals measured in these years). We censused 100 and 200 plants from each site in 2014 and 2015, respectively (sampled along transects similar to those described above for seeds collections), and measured plant height, specific leaf area (2014 only), date of first flower (2015 only), and counted seeds to estimate fitness. We calculated linear selection gradients using separate models for each year. As with the genotypic selection analysis, we regressed trait x site interactions on relative fitness to determine whether selection differed between sites, then ran separate models for each site without the site interactions to estimate direct selection. We used models with the two traits measured each year, their quadratic terms, and cross-products, to examine non-linear and correlational selection. To determine whether selection on height differed between years (the only trait we measured in both years), we calculated a selection differential (which estimates total, rather than direct selection on a trait) for each site, with relative fitness as the response, and height, year, and their interactions as predictor variables.

Results

Evidence of evolutionary change

Populations differed significantly in flowering time, nodulation, and SLA (Fig. 2.1). Both Lux and Marshall plants flowered significantly earlier than original source plants, although this effect was only statistically significant when plants were grown at the Lux site (population*site: $F_{2,2075}$=10.63, p<0.0001; Fig. 2.1a), where Lux plants flowered an average of three days earlier and Marshall plants two days earlier than original source plants. Lux plants produced significantly more root nodules than Marshall and original source plants (34% increase compared to the original source population; population: $F_{2,2126}$=5.27, p=0.005; Fig. 2.1b) and had significantly lower SLA than original source plants (5% decrease; population: $F_{2,2096}$=3.54, p=0.03; Fig. 2.1c). There were no differences in height between populations (population: $F_{2,1223}$=0.76, p = 0.47; Fig. 2.1d).

Figure 2.1. Population trait means (LS means±SE) estimated from the resurrection/reciprocal transplant experiment. Panels show (a) day of first flower, (b) nodules/cm root, (c) specific leaf area, and (d) height.



Genotypic selection analyses and predicted evolutionary responses

Plants grown at the Marshall site had very low fitness compared to those grown at the Lux site, with many families not producing any seeds (Fig. S2.4). Selection on height tended to differ between sites ($F_{1,753}$=2.99, p=0.08), with selection for taller plants at Lux ($F_{1,376}$=14.0, p<0.001) and no significant selection on height at Marshall ($F_{1,377}$=0.39, p=0.53). We found no significant directional selection on flowering time, root nodules, or SLA at either site (Table 2.1). There was evidence of stabilizing selection on height at Lux (Table 2.1), although it was less in magnitude than directional selection. There was no significant correlational selection on pairs of traits at either site.

Table 2.1. Directional and quadratic genotypic selection gradient (β) estimates ± SE at the Lux and Marshall sites in 2016. Quadratic estimates are in parentheses. *P<0.05

	Lux β	**Marshall β**
Date of first flower	0.001±0.07 (-0.14±0.12)	-0.09±0.24 (-0.38±0.42)
Nodules/cm root	0.001±0.07 (0.14±0.12)	-0.19±0.23 (0.42±0.40)
SLA	-0.03±0.07 (-0.08±0.08)	0.01±0.25 (0.42±0.32)
Height	0.25±0.07* (-0.14±0.06*)	-0.16±0.25 (-0.16±0.48)

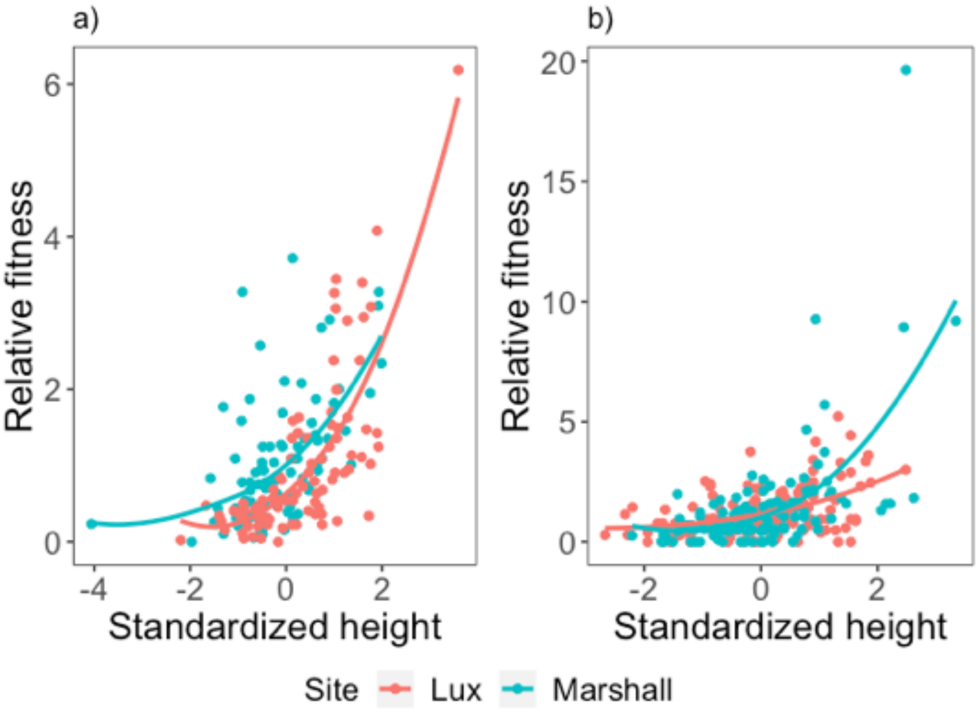
Phenotypic selection analyses

We detected selection for increased height at both sites in 2014 (Table 2.2), although it was stronger at Lux (height*site $F_{1,179}$=4.21, p=0.04). We also detected evidence for significant non-linear selection on height (Table 2.2; Fig. 2.2a), indicating that the strength of selection increases as height increases. There was no significant selection on SLA at either site (Lux $F_{1,89}$=0.03, p=0.86; Marshall $F_{1,90}$=1.90, p=0.17). We found a significant correlational selection on height and SLA at Marshall, suggesting direct selection for higher SLA when plants are tall but selection for lower SLA when plants are short ($F_{1,87}$=4.43, p=0.04; Fig. S2.5a). In 2015 we again found selection for increased height at both sites (Table 2.2), but it was stronger at Marshall than Lux (height*site $F_{1,264}$=20.98, p<0.001). We again found evidence that selection on height was non-linear, but quadratic coefficients were smaller in magnitude than the directional selection coefficients (Fig. 2.2b). There was no significant selection on flowering time at either site (Lux $F_{1,145}$=0.11, p=0.75; Marshall $F_{1,118}$=0.12, p=0.73). The was no significant interaction between flowering time and height at Lux ($F_{1,142}$=0.06, p=0.81), but this interaction was marginally significant at Marshall ($F_{1,115}$=3.85, p=0.052), with selection for later flowering when plants are tall (Fig.S2.5b). Comparing the strength of selection on height across years at each site, we found opposing patterns. At Lux selection on height was significantly stronger in 2014 (height*year $F_{1,237}$=7.68, p=0.006) while at Marshall selection was stronger in 2015 (height*year $F_{1,212}$=11.09, p=0.001).

Table 2.2. Directional, quadratic, and correlational phenotypic selection gradient (β) estimates ± SE at the Lux and Marshall sites in 2014 and 2015. Quadratic estimates are in parentheses. Note that selection was not measured on all traits in all years. *P<0.05, ** P<0.01, ***P<0.0001

	2014		2015	
	Lux β	**Marshall β**	**Lux β**	**Marshall β**
Height	0.73±0.10*** (0.36±0.08***)	0.47±0.08*** (0.28±0.04***)	0.42±0.08*** (0.20±0.06)	0.92±0.12*** (0.72±0.16)***
SLA	-0.02±0.09 (-0.14±0.10)	-0.10±0.07 (0.06±0.05)	--	--
Height*SLA	-0.14±0.15	0.25±0.12*	--	--
Date of first flower	--	--	-0.03±0.08 (-0.08±0.04)	0.05±0.13 (-0.02±0.18)
Height*Date of first flower	--	--	-0.02±0.08	0.34±0.17*

Figure 2.2. Selection on plant height at the Lux and Marshall sites in (a) 2014 and (b) 2015. Graphs show the relationship between height and relative fitness, fitted with a LOESS curve.



Discussion

By combining a resurrection experiment with a reciprocal transplant experiment we were able to compare two recently established populations of *Chamaecrista fasciculata* both to each other and to their original source population to test for evidence of rapid adaptation. The two populations show clear evidence of rapid evolution in the six years since they were established (a maximum of six generations), with differences in flowering time, root nodule production, and SLA between extant populations and their original source population. While previous studies have found evidence of evolutionary changes in colonizing populations (Whitney and Gabler 2008), few have compared extant populations directly to their source population, or found evolutionary changes over such short timescales. Although these *Chamaecrista* populations appear to have evolved, we find little evidence that this evolution is adaptive based on contemporary estimates of selection. Both genotypic and phenotypic selection gradients showed no significant direct selection on the three traits that changed in these populations. However, we did find strong selection for taller plants at both restoration sites, yet no evolutionary response (that is, plants in the extant populations show no increases in height compared to their original source population).

There are several explanations for why we might find evidence of evolution but no selection on traits that appear to have evolved. First, it could be that the trait changes we found are not adaptive, but instead the product of stochastic processes such as genetic drift. However, this might be unlikely in our system given that drift is more likely to occur in small populations (Ellstrand and Elam 1993), and both *Chamaecrista* populations started out relatively large (approximately 300,000-350,000 seeds were originally planted at each site, and a rough estimate of population size based on biomass in 2010 suggests population sizes in the tens of thousands). Second, it could be that the trait changes are adaptive and but we do not detect directional selection because the traits are already at their evolutionary peak. In such a case, we might expect to observe stabilizing selection, which was not detected for any trait in which we observed evolutionary changes (Table 2.1). However, preliminary analyses show that selection at a single site may differ between populations: at Lux, we find only selection for increased height on the Lux population, but selection for increased height, later flowering time, fewer root nodules, and higher SLA for Marshall plants, suggesting

Lux plants may be optimally adapted to their homesite while Marshall plants are not. Third, and perhaps more likely in this system, it could be that the evolutionary changes we observed are (or at least were) adaptive, but there is temporal variation in selection on these traits. The evolutionary responses we observed are the product of past selection, while our selection gradients estimate current selection. Selection might change yearly due to stochastic events – for example, 2016 was an abnormally dry year, which may have influenced patterns of selection relative to other, wetter years. Alternatively, selection might change over the course of restoration establishment. For example, selection on *Danthonia spicata* traits in habitats at different successional stages differed in both the magnitude and direction of selection, depending on successional stage (Scheiner 1989). Prairie restorations tend to be dominated by weedy annuals in earlier years, followed by the establishment of perennial grasses and forbs in later years (Schramm 1990). These changes in plant composition during succession could lead to changes in competition and the abiotic environment that affect selective pressures on *Chamaecrista* (which may be likely, given that *Chamaecrista* is an early-successional annual). Conducting selection analyses starting during initial establishment and continuing over the course of succession would help determine if this is the case.

While we found no significant contemporary selection on the *Chamaecrista* traits exhibiting historical evolutionary changes in these populations, we did find strong selection for increased height at both sites. In a review of studies that estimated selection in natural populations, the median $|\beta|$ value for linear selection via fecundity was 0.16 (Kingsolver et al. 2001). Our β values for height were all > 0.16, indicating that selection is sometimes strong in this system compared to other natural populations, and supporting the idea that restored plant populations will likely experience strong selection (LaRue et al. 2017). Despite such strong selection, we found no changes in plant height in either *Chamaecrista* population, relative to the source seeds. Such a disconnect between a selection coefficient and an evolutionary response may suggest an environmental correlation between height and fitness (that is, height might be very plastic). However, even genotypic selection coefficients, which should minimize the effects of such correlations, indicated strong selection for increased plant height at the Lux site, suggesting that trait-environment-fitness correlations are not solely responsible for the strong selection on height. The lack of evolutionary response could be due to genetic constraints.

While it is unclear whether the rapid evolutionary changes we found are adaptive, the two *Chamaecrista* populations notably differed in the magnitude of these changes. While both populations flower earlier than their original source population (at least when grown at the Lux site), only the Lux population differs significantly from the original source population in root nodule production and SLA. If the evolutionary changes in these populations are truly not adaptive, the differences we observed between traits in the two extant populations are likely due to stochastic processes. If the changes are adaptive, the differences in evolutionary responses between the Lux and Marshall populations could be due to environmental differences between the two restoration sites. As noted in the site descriptions in the methods, available soil nitrogen is roughly twice as high and the light availability is lower at Marshall than at Lux in some years (Stahlheber et al. 2016), which could explain why Lux plants produce significantly more root nodules than the original source and Marshall plants, as forming mutualistic relationships with nitrogen-fixing bacteria is more beneficial to plants grown in lower-nitrogen soil in high light environments (Heath and Tiffin 2007, Lau et al. 2012). The fact that Lux plants are less shaded could also explain why they have lower specific leaf area, given that higher light conditions are correlated with lower SLA (Poorter et al. 2009). Population size might also influence the magnitude of evolutionary response in these populations, if there was selection in previous years. The Marshall population has been 2-12 times smaller than the Lux population (based on yearly biomass estimates from the Great Lakes Bioenergy Research Center) ever since they were planted, which could mean the Marshall population has a decreased ability to respond to selection (Gillespie 1998, Willi et al. 2006).

Conclusions

This study demonstrates that evolution can occur in just six years in populations of an annual plant in recently restored habitats. It also shows the utility of ecological restorations, which can be easily manipulated and monitored, for field studies of rapid evolution. Rapid adaptation in restorations likely has ecological consequences (LaRue et al. 2017), and future work should address whether rapid adaptation has demographic effects that can facilitate population establishment, or whether plant adaptation can occur rapidly enough to lead to evolutionary rescue of declining populations. There is also the potential to investigate the amount of genetic diversity or minimum population size needed in a restored population to facilitate adaptation. Understanding the consequences of rapid evolution during colonization not only has the potential to give insight into how to make restorations more successful, but also will help us better understand the evolutionary processes occurring during other colonization events such as invasions and range expansions.

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APPENDIX

Figure S2.3. Bootstrapped trait distributions of (a) flowering time of the Lux and Marshall populations, (b) Lux nodules/cm root, and (c) Lux specific leaf area. Original population means are shown for each trait. Shading indicates 95% confidence intervals.

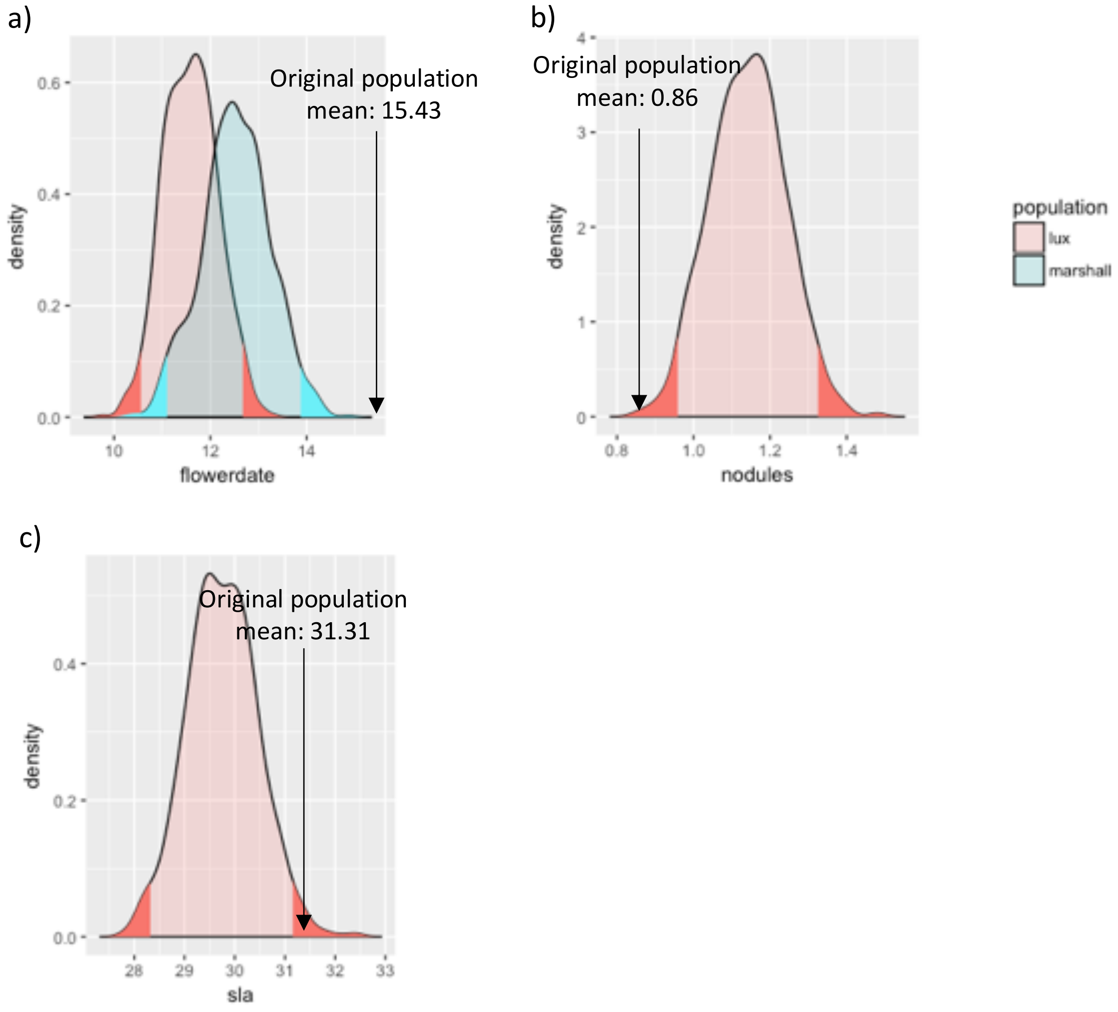


Figure S2.4. Histograms showing the distribution of relative fitness values of plants grown at (a) the Lux site and (b) the Marshall site.

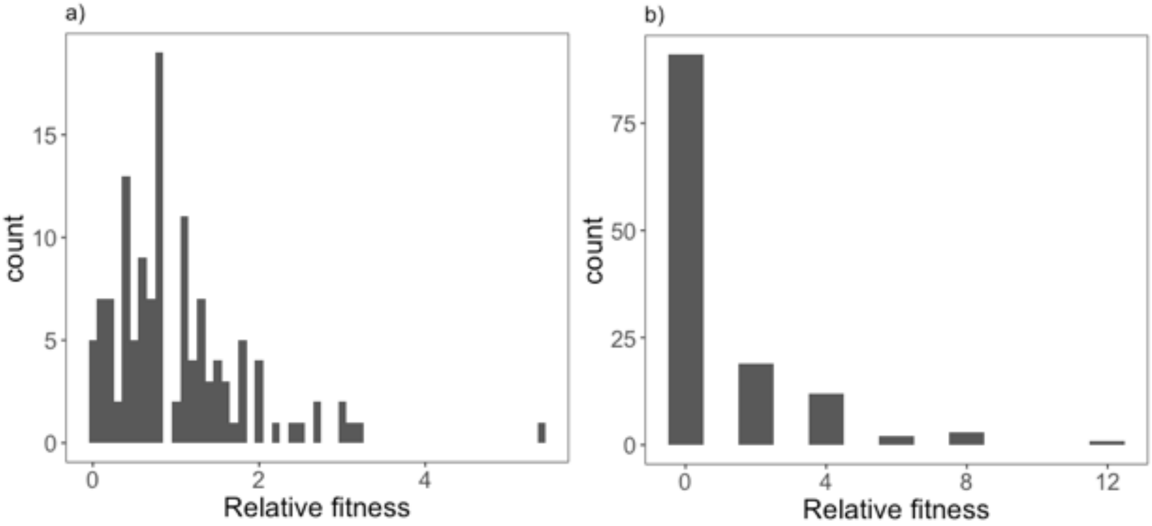
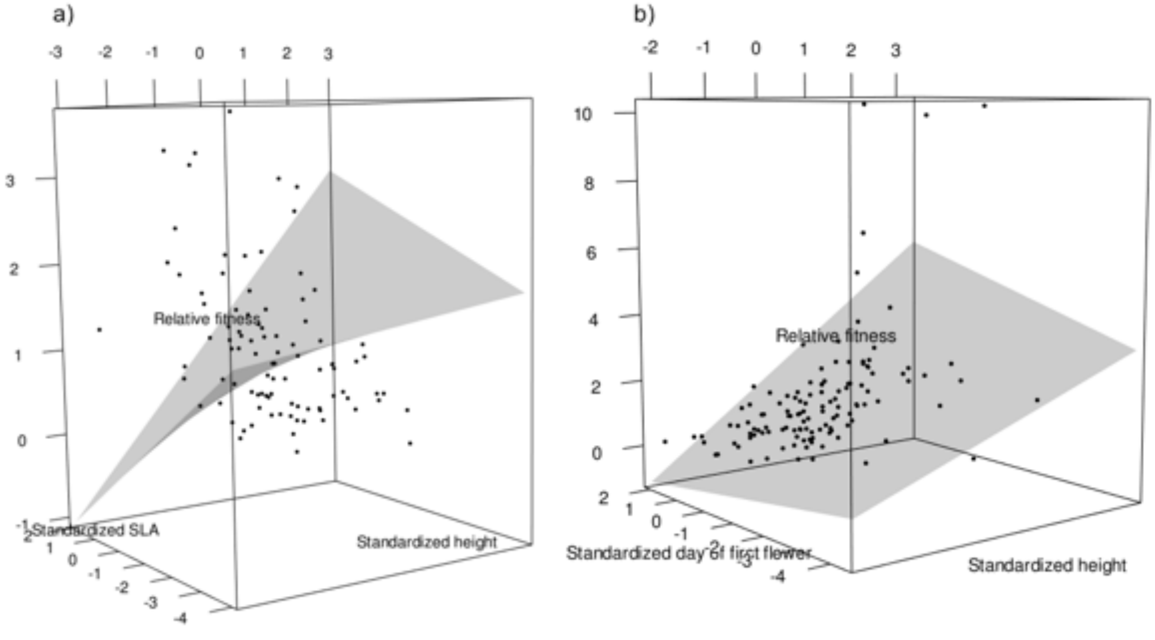


Figure S2.5. Response surfaces showing correlational selection on pairs of traits. Panels show (a) the relationship between height, specific leaf area, and relative fitness and (b) the relationship between height, day of first flower, and relative fitness.



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CHAPTER THREE:

NOVEL PLANT-MICROBIAL INTERACTIONS: RAPID EVOLUTION OF A LEGUME-RHIZOBIUM MUTUALISM IN RESTORED PRAIRIES

Abstract

When plants colonize new habitats, the novel interactions they form with new mutualists or enemies can immediately affect plant performance. These novel interactions also may provoke rapid evolutionary responses and can be ideal scenarios for investigating how species interactions influence plant evolution. To explore how mutualists influence the evolution of colonizing plant populations, we capitalized on an experiment in which two former agricultural fields were seeded with identical prairie seed mixes in 2010. Six years later, we tested for local adaptation between populations of the legume *Chamaecrista fasciculata* and their associated nitrogen-fixing rhizobia in a greenhouse reciprocal cross-inoculation experiment. We detected variation in rhizobia quality between sites, and plants from one site performed best when inoculated with their own rhizobia, suggesting plant local adaptation to co-occurring rhizobium genotypes. In contrast, plants from the other site derived no benefit from rhizobia, regardless of rhizobia origin. These results suggest that these plant populations have evolved different ways of interacting with rhizobia, potentially in response to differences in rhizobia quality between sites. Our study illustrates how microbial mutualists may shape plant evolution in new environments and highlights how genetically-based variation in mutualists may potentially select for different evolutionary strategies in plant hosts.

Introduction

Plant populations colonizing new habitats inevitably form novel interactions with mutualists, enemies, and competitors (Richardson et al. 2000, Levine et al. 2004, Traveset and Richardson 2014). While much work has focused on the ecological effects of these interactions (Mitchell et al. 2006), mutualists, enemies, and competitors also may act as strong agents of natural selection on colonizing populations. Microbial mutualists may be particularly important to the success of colonizing plants (Parker 2001, Parker et al. 2006, Rodríguez-Echeverría et al. 2009), and have the potential to act as strong agents of selection given their ability to influence plant fitness (Parker 1995, Rúa et al. 2016). These mutualists can benefit plants by increasing access to nutrients (Kiers and Denison 2008), mediating abiotic stress (Rodriguez et al. 2008, Dimkpa et al. 2009, Yang et al. 2017), and protecting plants from herbivory (Clay 1996). However, mutualist partners vary in quality, displaying both intra- and interspecific variation in the benefits they provide to their partners (Burdon et al. 1999, Heath 2010, Hoeksema 2010, Heath and Stinchcombe 2014, Weese et al. 2015), and the outcomes of plant-mutualist interactions may depend on the genotypes of both interacting partners (e.g., Heath and Tiffin 2007). As a result, colonizing plant populations are likely to encounter microbial mutualists that differ in quality or compatibility from mutualists at their home sites.

Many plant species also show intraspecific variation in the benefits they derive from their microbial mutualists. For example, several studies of legume-rhizobia interactions have shown that some plant genotypes or populations benefit more from rhizobia than others (Parker 1995, Heath and Tiffin 2007, Heath 2010, Keller and Lau

2018). Variation among populations in plant dependence on microbes (i.e., the magnitude of benefit from association) can evolve in response to variation in the presence or abundance of mutualists or from abiotic factors such as resource availability that influence the outcome of the interaction. For example, an invasive plant appears to have evolved reduced dependence on mycorrhizae due to lack of compatible mutualists in the introduced range (Seifert et al. 2009). Similarly, an *Andropogon gerardii* population growing in a high nutrient site where resource mutualists may be less necessary evolved reduced dependence on arbuscular mycorrhizal fungi compared to a population in a low nutrient site (Schultz et al. 2001).

Given spatial variation in the quality of microbial mutualists and the fitness consequences of associating with these mutualists, and given intraspecific variation in plant responses to microbial mutualists, we might expect plants to adapt to the microbes present at their home sites. However, empirical evidence for plant local adaptation to microbial symbionts is mixed. Some studies have found that on average, plants perform best when inoculated with microbes from their home sites (Parker 1995) or when grown with a combination of microbes and soil from their home sites (Johnson et al. 2010), which suggests plant local adaptation to microbes. In contrast, several other studies have found variation in microbe quality between sites but no plant local adaptation to microbes (Heath 2010, Barrett et al. 2012, Harrison et al. 2017), suggesting that plant local adaptation to microbial symbionts, while possible, is not ubiquitous, although in some of these studies within-population replication is minimal. Several factors can prevent plant local adaptation to symbionts, including gene flow between plant populations associating with different mutualists (Harrison et al. 2017), and temporal environmental variation that modifies the costs and benefits of mutualism (Heath 2010). Despite these barriers to local adaptation, for colonizing populations, in particular, the ability to associate with beneficial microbial mutualists and potentially rapidly adapt to local mutualist populations may be key to establishment success.

Here, we investigated plant responses to novel microbial interactions in recently restored prairies. Prairie restorations provide an ideal opportunity to examine novel plant-microbe interactions because plant species are typically planted into highly disturbed sites inhabited by populations of microbial mutualists with which they are unlikely to share a recent evolutionary history. We examined populations of the annual legume *Chamaecrista fasciculata* that originated from the same source population in two restored prairies, along with their associated nitrogen-fixing mutualist rhizobia, to determine whether plants have evolved novel interactions with local rhizobia in the six years since they were established. In a greenhouse reciprocal cross-inoculation experiment, we addressed the following questions: 1) Does rhizobium quality vary between restoration sites? 2) Do plant populations differ in the benefits they derive from rhizobia? and 3) Are plant populations locally adapted to rhizobia from their home sites?

Methods

Study system

Chamaecrista fasciculata Michx. is an annual legume native to eastern North America commonly found in prairies and disturbed sites. *Chamaecrista* forms facultative mutualistic interactions with rhizobia, such as *Bradyrhizobium* spp., which provide plants fixed nitrogen in exchange for carbon. For this study, we used *Chamaecrista* populations from two recently restored prairies in southwest Michigan, Lux Arbor (42°28’23” N, 85°26’50” W) and Marshall (42°26’37” N, 85°18’34” W). These two former agricultural fields were planted with identical prairie seed mixes (containing 19 grass and forb species) in 2010 using a no-till seed drill, and a portion of the seed mix was saved (hereafter referred to as the ‘original source’). The microbial community was not manipulated in either site. Despite the similar treatment of each site, they differ in community composition, and *Chamaecrista* biomass is consistently higher (2-12 times greater, depending on year) at the Lux site than at Marshall. The sites also differ in underlying abiotic factors, including available soil nitrogen, with the Marshall site having twice as much available soil ammonium and nitrate as Lux (see (Stahlheber et al. 2016) for full site details). A previous study of *Chamaecrista* populations at these sites found genetic differentiation between *Chamaecrista* populations in nodule production, with Lux plants producing significantly more root nodules than Marshall plants (Magnoli *Chapter 2*), suggesting that these plant populations interact differently with rhizobia.

In 2015 (a maximum of six *Chamaecrista* generations after the restorations were planted) we collected seeds from 100 haphazardly selected *Chamaecrista* individuals at each prairie site. We grew these field-collected seeds, along with seeds from the original source, for one generation in the greenhouse in 2017 to minimize maternal effects. We hand-pollinated plants, using one plant in each population as a pollen donor to all the other plants in its population on a given day, so that each plant was eventually crossed with every other plant in its population. We used the offspring from these greenhouse-reared plants in the experiment described below.

To isolate rhizobia strains, in summer 2017 we collected soil cores (2cm core to 10cm depth) at 10m intervals along a 200m transect through the middle of each site, and homogenized the samples from each site. We inoculated 10 Lux seedlings, 10 Marshall seedlings, and 10 original source seedlings grown in potting soil (Sunshine Mix #5; Sun Gro Horticulture Canada Ltd., Alberta, Canada) in the greenhouse with 2g of either Lux or Marshall soil (60 total seedlings). Four weeks after inoculation, we harvested two randomly selected root nodules from each plant, and attempted to isolate rhizobia strains from each by sterilizing individual nodules in ethanol and bleach, then crushing them and plating them onto tryptone yeast (TY) agar plates. We re-streaked strains onto additional TY plates until we obtained single colonies. We isolated strains from nodules produced by individuals from all three plant populations to avoid any biases in rhizobia selection by the different plant populations. Subsequent analyses showed that which plant population rhizobia strains were isolated from had no effect on rhizobia or plant performance (data not shown).

Reciprocal cross-inoculation experiment

To investigate variation among sites in rhizobia quality, variation between plant populations in benefits from rhizobia, and plant local adaptation to rhizobia, we conducted a fully-factorial greenhouse experiment manipulating plant population and rhizobia presence and source. Because sites differed in soil nitrogen availability and the outcomes of legume-rhizobium mutualisms can be sensitive to N availability (Thrall et al. 2007, Kiers et al. 2010), we conducted these experiments in two soil nitrogen levels simulating the different site conditions of the home environments. To test for variation in rhizobium quality and plant responses to rhizobia, we surface-sterilized *Chamaecrista* seeds from each population in 75% ethanol and germinated them in Petri dishes with distilled water. After germination, we transferred individual seedlings to 656 mL Deepots™ (Stuewe & Sons Inc., Corvallis, OR, USA) filled with potting soil. We grew rhizobia cultures in Modified Arabinose Gluconate (MAG) liquid culture at 30°C for 48 hours. One week later we inoculated seedlings with 1ml of a mixture of Lux rhizobia strains in liquid culture, a mixture of Marshall rhizobia strains, or sterile liquid media as a control (*c.* 2.5 x 10^6 cells based on OD670). Each rhizobia mixture was comprised of nine strains, three isolated from nodules produced by Lux seedlings, three from Marshall seedlings, and three from original source seedlings. Cell density was measured and standardized by diluting individual strain cultures with sterile media before combining. Plants were fertilized with ammonium nitrate at either low (1.3g/kg soil) or high (2.3g/kg soil) levels to approximate total available soil nitrogen at the Lux and Marshall sites, respectively. We fertilized in three intervals, starting three weeks after planting and every following two weeks. Each plant population/rhizobia/nitrogen treatment was replicated 30 times [3 plant populations (Lux, Marshall, original) x 3 rhizobia (Lux, Marshall, none) x 2 N (low, high) x 30 replicates = 540 total plants].

While inoculating plants with a mixture of rhizobia from each site simulates a diverse rhizobia community like those likely experienced in nature, it masks any differences between individual rhizobia strains. To determine if specific strains were driving site-specific effects of rhizobia on plants, we also inoculated plants from the two extant sites (Lux and Marshall) with each single strain used in the rhizobia mixtures. We did not include a nitrogen treatment, but fertilized plants at the low nitrogen level described above (2 plant populations x 18 rhizobia strains x 5 replicates = 180 total plants). Results from single strain inoculations were qualitatively similar to those from the multi-strain inoculations (with the exception of absolute fitness benefits), so we only present results from the multi-strain analyses in the main text (see Supplementary Material for detailed methods and single-strain results).

We harvested above and belowground biomass nine weeks after initial planting, just as plants were beginning to flower. We counted root nodules and haphazardly selected 10 nodules from each plant and weighed them to estimate total nodule mass. We dried above and belowground biomass at 60° C for 48 hours and weighed it to use as an estimate of plant fitness, as biomass has been shown to be positively correlated with seed production in this species (Galloway and Fenster 2001). We did not measure seed set directly because although *Chamaecrista* is self-compatible, seed production is substantially reduced in the absence of pollinators.

Statistical analyses

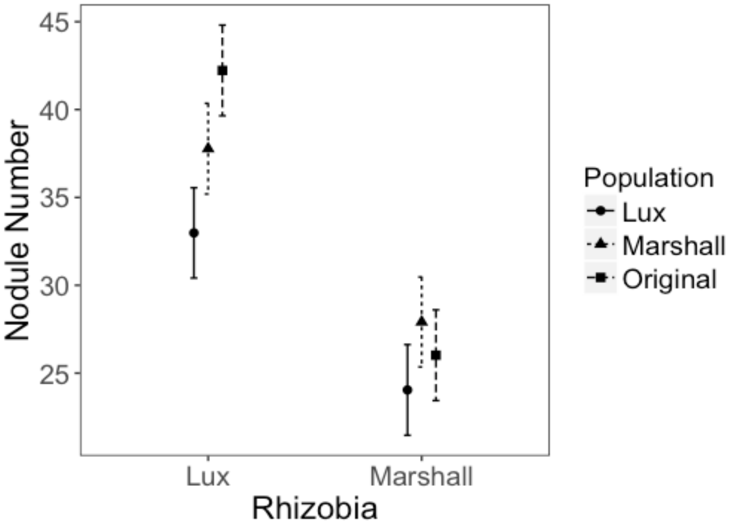
To determine whether Lux and Marshall rhizobia populations differ in traits relevant to their mutualism with *Chamaecrista*, we compared the number of nodules and mean and total nodule biomass plants produced when inoculated with different rhizobium populations. All analyses were conducted in R v.3.5.1 (R Core Team 2018) using linear mixed models in the *lme4* package (Bates et al. 2015), and we tested significance using type III sums of squares in the ANOVA function in the *lmerTest* package (Kuznetsova et al. 2016). We included nodule number, total estimated nodule mass, and mean nodule mass as response variables. We included plant population (Lux, Marshall, original), rhizobia origin (Lux, Marshall), and nitrogen (low, high) and all interactions between these variables as fixed effects, and block (where plants were positioned in the greenhouse) as a random effect. To compare the per nodule fitness benefits plants derived from each rhizobia type, we used a model with total plant mass (above + belowground biomass) as the response variable, and plant population, rhizobia origin, nitrogen, nodule number, and all interactions included as fixed effects, with block as a random effect. A significant rhizobia origin x nodule number interaction effect on plant biomass is consistent with variation in per nodule fitness benefits between Lux and Marshall rhizobium populations.

To compare the absolute fitness benefits plants derived from each rhizobia type and to determine whether plant populations differ in the benefits they derive from rhizobia, we compared plant total biomass when inoculated with Lux, Marshall, or no rhizobia. We included plant biomass as the response variable, with plant population (Lux, Marshall, original), rhizobia origin (Lux, Marshall, none), and nitrogen as fixed effects, and block as a random effect. To determine whether the extant plant populations are locally adapted to rhizobia from their home sites, we used the same model but excluded data from the original source population.

Results

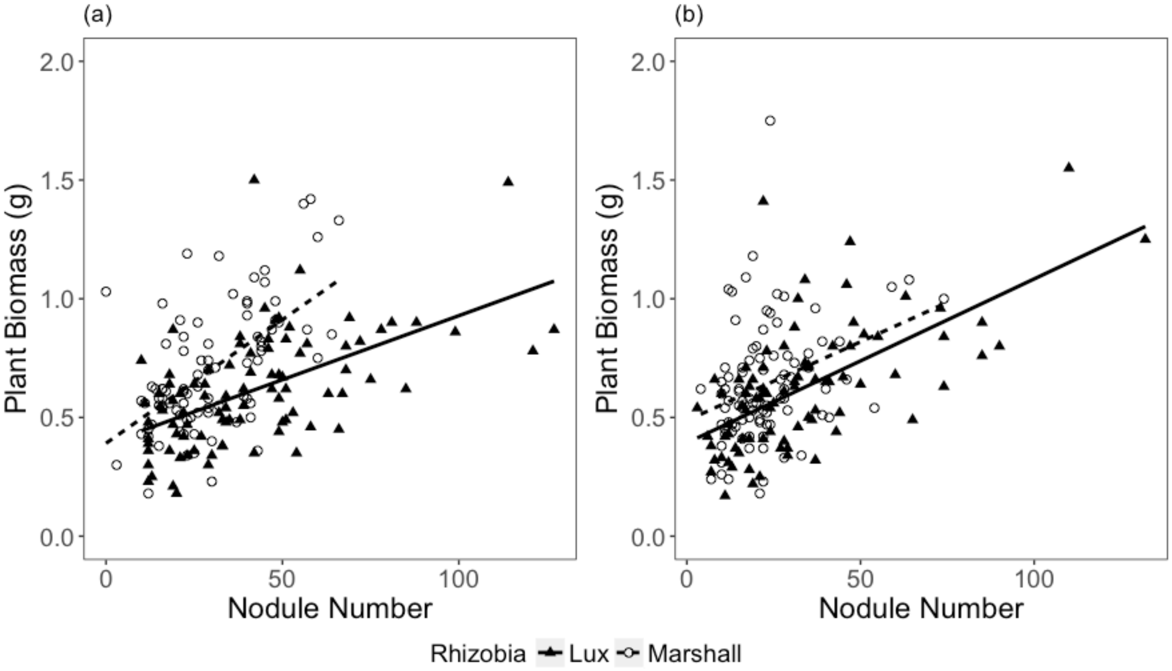
Plants inoculated with rhizobia from the Lux and Marshall sites differed in nodule production and the benefits they derived from rhizobia. Plants inoculated with rhizobia from the Lux site produced more, larger nodules (greater nodule number, total nodule mass and mean nodule mass) than plants inoculated with rhizobia from the Marshall site (nodule number: $F_{1,290}=33.1$, $p<0.0001$, Fig. 3.1; estimated total nodule mass: $F_{1,289}=111.1$, $p<0.0001$; mean nodule mass: $F_{1,441}=114.7$, $p<0.0001$). Nitrogen also

Figure 3.1. Mean estimated nodule number (LS means±SE) produced by plants inoculated with rhizobia from the Lux or Marshall site. Plants inoculated with rhizobia from the Lux site produced more nodules than those inoculated with rhizobia from the Marshall site.



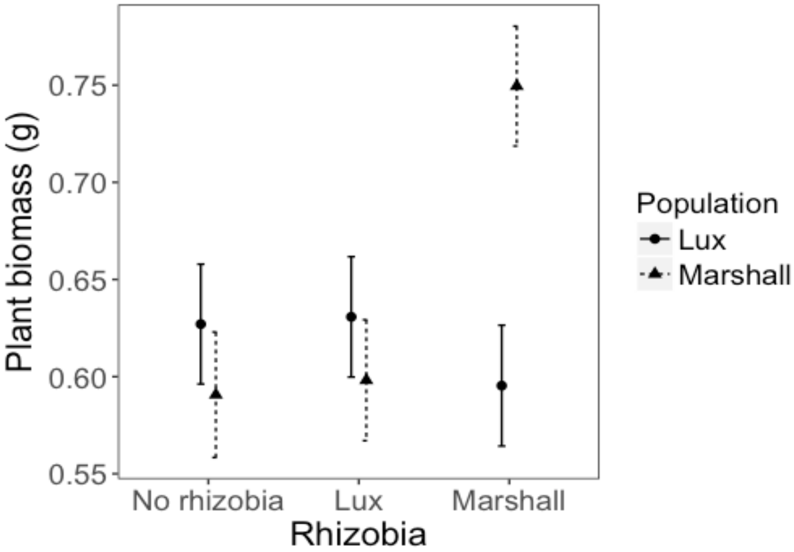
affected nodule number and mass, with plants fertilized at low nitrogen levels producing significantly more, but not larger, nodules than plants fertilized at high nitrogen levels, regardless of rhizobia origin (nodule number: $F_{1,324}$=12.7, p<0.001; estimated total nodule mass: $F_{1,324}$=7.1, p=0.008). Plant populations tended to produce different numbers of nodules, regardless of rhizobia origin ($F_{2,270}$=2.84, p=0.06; Fig. 3.1), with Lux plants tending to produce fewer nodules than original source plants. The per-nodule fitness benefits that plants derived from rhizobia differed depending on nitrogen (nitrogen*rhizobia origin*nodule number $F_{1,321}$=4.04, p=0.045). Under low nitrogen conditions, plants derived greater per nodule fitness benefits from rhizobia from the Marshall site, but this effect is most apparent at high nodule numbers (rhizobia origin*nodule number $F_{1,162}$=7.38, p=0.007; Fig. 3.2a). Per-nodule fitness benefits did not differ between rhizobia populations under high nitrogen conditions ($F_{1,162}$=.22, p=0.64; Fig. 3.2b).

Figure 3.2. Per-nodule fitness benefits derived from rhizobia from the Lux and Marshall sites under (a) low nitrogen conditions and (b) high nitrogen conditions. Marshall rhizobia tended to confer greater benefits to plant hosts, but only under low nitrogen conditions.



Marshall plants produced more biomass when inoculated with rhizobia from their home site than when inoculated with Lux rhizobia or no rhizobia (population*rhizobia $F_{2,297}$=6.58, p=0.001; Fig. 3.3), suggesting that Marshall plants are adapted to rhizobia from their home site. Rhizobia treatments did not affect biomass of Lux plants (Fig. 3.3), suggesting Lux plants derive no benefit from either rhizobia population and are not adapted to rhizobia from their home site. When the original source population was included in the analyses, as above, plant populations differed in the growth benefits they derived from rhizobia, but this effect depended on nitrogen treatment and the rhizobium population (plant population*rhizobia origin*nitrogen $F_{4,482}$=3.65 p=0.006). The response to nitrogen appeared to be due largely to differences in the original source population. Under low nitrogen conditions, the original source population performed significantly better without rhizobia than the two extant populations, and surprisingly produced more biomass when rhizobia were absent than when inoculated with Lux or Marshall rhizobia (Fig. S3.5a). In contrast, under high nitrogen conditions there were no significant differences in original source biomass between rhizobia treatments (Fig. S3.5b).

Figure 3.3. Mean plant biomass (LS means±SE) of Lux and Marshall plants inoculated with no rhizobia, Lux rhizobia, or Marshall rhizobia. Marshall plants produced significantly more biomass when inoculated with rhizobia from their home site, while Lux plants did not differ in biomass production between rhizobia treatments.



Discussion

Chamaecrista populations from two recently restored prairies, only 15 km apart, which were planted using the same source populations six years prior to this experiment, have rapidly diverged with regard to their dependence on rhizobia. The two prairies contain rhizobia communities that provide dramatically different benefits to their plant hosts; rhizobia from the Marshall site provide significant growth benefits to *Chamaecrista* from that site, while rhizobia from the Lux site provide little to no benefit to either host plant population. Our results suggest that the Marshall plant population has adapted to the beneficial rhizobia from its home site, producing more biomass when inoculated with its own rhizobia than when inoculated with rhizobia from the other site or no rhizobia. In contrast, Lux plants, which had been interacting with low quality rhizobia for six years, derived no benefit from rhizobia. Plants from both sites differ from their original source population, which does not benefit from rhizobia and even performs significantly better when uninoculated when grown under low nitrogen conditions.

These findings illustrate how populations can evolve divergent strategies of interaction with potential mutualists as they establish in new habitats. Given the variation in rhizobia quality between the two sites, and the rapid plant evolutionary responses in terms of benefits derived from rhizobia, it is likely that rhizobia are strong selective agents in this system. There is some evidence from studies of invasive plants that microbial resource mutualists (or a lack thereof) can influence plant evolution. For example, invasive populations of *Hypericum perforatum* evolved reduced dependence on mycorrhizae, which may have been driven by a lack of suitable microbial mutualists in the invaded range (Seifert et al. 2009). In our study, there is no lack of rhizobia (field grown plants at these sites nodulate extensively, although plants grown at the Lux site produce about 2x more nodules (Magnoli *Chapter 2*)), but a difference in rhizobia quality between restoration sites that could be driving our observed plant evolutionary responses. Specifically, the plant population evolving in the presence of non-beneficial rhizobia (Lux), appears to derive no benefit from rhizobia (even a rhizobium population that significantly increases the growth of a closely-related plant population), while a plant population that has evolved for 6 years in the presence of a high quality rhizobium population (Marshall) benefits from rhizobia to a much greater extent than the original source population.

The two extant *Chamaecrista* populations in this study seem to differ in the benefits they derive from rhizobia; however, we also detected some evidence that they may also differ in how the regulate interactions with rhizobia. Plants can regulate interactions in several ways, including reducing nodule formation with unfavorable partners (partner choice) (Simms et al. 2006) or by controlling resource allocation to nodules (sanctions) (Denison 2000). Lux plants tended to form fewer nodules than Marshall or original source plant populations, which might be indicative of greater partner choice capabilities or evolutionary changes in the likelihood of interacting with rhizobia in response to six years of coevolution with non-beneficial rhizobia; however, all plant populations formed more and bigger nodules with the low quality Lux rhizobia, which is inconsistent with both partner choice and sanctions. This is, however, consistent with empirical findings in other systems that plants form more and sometimes larger nodules when inoculated with poor quality rhizobia (Weese et al. 2015). There is a possibility that nodule number could have been affected by cell densities in the inoculation mixtures, as our single-strain inoculations showed several Marshall strains were ineffective (Supplemental Material Methods/Results), meaning the Marshall mixture may have had a lower cell density. However, cells were added at such high densities (*c.* 2.5 x 10^6 cells based on OD670) that it is unlikely that plants were rhizobium-limited. These findings also differ somewhat from a previous field reciprocal transplant study of these plant populations in which Lux plants produced more nodules than Marshall plants (Magnoli *Chapter 2*). Because legume-rhizobium interactions are notoriously context dependent (e.g., (Lau et al. 2012)), the difference between our field and greenhouse results may be due to variation in environmental conditions across studies. For example, intraspecific plant density has been shown to affect the costs and benefits of associating with microbial mutualists, with the cost of associating with mycorrhizae increasing as plant density increases (Allsopp and Stock 1992). *Chamaecrista* density in the field varies greatly, with a large population at the Lux site (*Chamaecrista* can comprise 50% cover in some areas) and a relatively small population at Marshall, which could explain the discrepancy between the field study and this study where plants are grown in the absence of competitors.

While we focus on plant evolution in response to rhizobia in this study, in tightly-linked symbioses such as the legume-rhizobium mutualism there is also the potential for coevolution, where rhizobia populations will also evolve in response to plant populations. In plant-microbial symbioses, microbes may adapt to plants more quickly than plants adapt to microbes, given their relatively short generation time and high population densities compared to that of their plant hosts (Gandon and Michalakis 2002). However, while we found some evidence of plant adaptation to rhizobia in this system, we did not find evidence that rhizobia populations perform better with plants from their home sites. Using total nodule mass as a fitness measure (for limitations of this as a fitness measure, see (Friesen 2012)), we found no effect of plant population on rhizobia fitness (see Results; Table S3.4, Fig. S3.6). Results from the single strain inoculations indicated that some strains did perform better with one plant population than the other, but there was no consistent pattern across rhizobium populations from a given site (Fig. S3.4a,c). Lack of rhizobia adaptation to *Chamaecrista* in this system could be a result of community context, given that environmental factors and symbiont specificity can affect mutualist coevolution (Thrall et al. 2007). *Bradyrhizobium* species associate with other legumes historically grown at these sites (soybean) and with non-legumes as free-living nitrogen fixers in the rhizosphere (Antoun et al. 1998), and therefore might be less likely to coevolve with *Chamaecrista* populations than if *Chamaecrista* were their only hosts. Further work examining rhizobia interactions with other plant species in this system would provide insight into whether this is the case.

Although the rhizobia in this system do not appear to be adapted to the *Chamaecrista* at their home sites, the two rhizobium populations do differ in quality. Several factors could explain these differences. First, although both sites have a history of row crop agriculture prior to restoration (likely corn/soy rotations), meaning rhizobia compatible with *Chamaecrista* were likely present (soy and *Chamaecrista* both associate with *Bradyrhizobium*), the Marshall site was used for agriculture up to 1987, when it was planted with perennial grass species and put in the USDA conservation reserve program (Stahlheber et al. 2016). In contrast, the Lux site had been used for row crops continuously for at least 70 years prior to restoration, meaning it was likely growing with soybean hosts and receiving fertilizer inputs for over twenty years (1987-2009) when the Marshall site was not. Growing in the presence of soy hosts may have selected for strains that are less beneficial for alternate hosts like *Chamaecrista*, and while the Lux site now has lower soil nitrogen availability, prior fertilizer inputs could have led to the evolution of less beneficial rhizobia that still persist today (Weese et al. 2015, but see Schmidt et al. 2017). Second, edaphic differences between the two restoration sites could influence rhizobia quality. Differences in available soil nitrogen are particularly likely to affect rhizobia quality, as theory predicts that higher nitrogen conditions will lead to the evolution of less beneficial rhizobia (West et al. 2002, Denison and Kiers 2004, Akçay and Simms 2011). We find the opposite pattern in our system, however, with rhizobia at the higher nitrogen site (Marshall) providing more benefits to plants than rhizobia from the lower nitrogen Lux site, suggesting that current nitrogen availability is not responsible for differences in rhizobia quality. Third, differences in plant community composition between the two sites could also potentially affect rhizobia quality. A study of several *Acmispon* species inoculated with different *Bradyrhizobium* strains, found that an ineffective rhizobia strain sanctioned by one *Acmispon* species formed large nodules with a sympatric host species, which might maintain this strain in the system even though it is ineffective (Pahua et al. 2018). Similarly, if rhizobia in our system associate with multiple, different plant species at each site, this could explain the presence of ineffective rhizobia at one site and not the other. However, *Chamaecrista* is the only legume known to interact with *Bradyrhizobium* in these sites (although *Bradyrhizobium* may interact with other non-legumes as free-living nitrogen fixers in the rhizosphere), suggesting current plant community composition might not be a strong driver of rhizobia differences between these sites. Finally, although we see consistent differences in nodule production and per-nodule fitness benefits between sites, we also need to be cautious about the generalizations we make about rhizobia in this system, given the small number of strains from each site included. Because we focused on plant evolutionary responses, we opted for greater replication and sampling of the plant populations surveyed, so further work including more rhizobium strains may be necessary to adequately characterize genetic differences among the two rhizobium populations.

Conclusion

We examined two recently restored plant populations and the rhizobia they associate with to determine whether rhizobia vary in the benefits they provide to plants, and whether plants rapidly evolve with respect to their interactions with rhizobia. We found that the rhizobia at one site provide more benefits to plants than rhizobia at the other site, which seem to provide no benefit at all. The plant population that associates with the more beneficial rhizobia appears to be adapted to those rhizobia, while the plant population from the site with less beneficial rhizobia derives no benefit from associating with either rhizobium population. These findings suggest that as plants establish in novel habitats and encounter microbial mutualists with which they do not share an evolutionary history, the ways in which they interact with these mutualists can rapidly evolve. As anthropogenic forces alter the environment and lead to more colonization events such as range expansions, invasions and the need to restore degraded landscapes, mutualisms between plants and microbes will be disrupted and reformed in potentially novel ways leading to both ecological and evolutionary consequences.

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APPENDIX

Figure S3.4. Boxplots showing nodule number produced and per nodule fitness benefits derived by plants inoculated by single strains of rhizobia. Panels show (a) Lux plants and (c) Marshall plants inoculated with single strains of Lux and Marshall rhizobia (Lux rhizobia in shades of red, Marshall rhizobia in shades of blue), and the per-nodule fitness benefits derived by (b) Lux plants and (d) Marshall plants from each rhizobia strain.

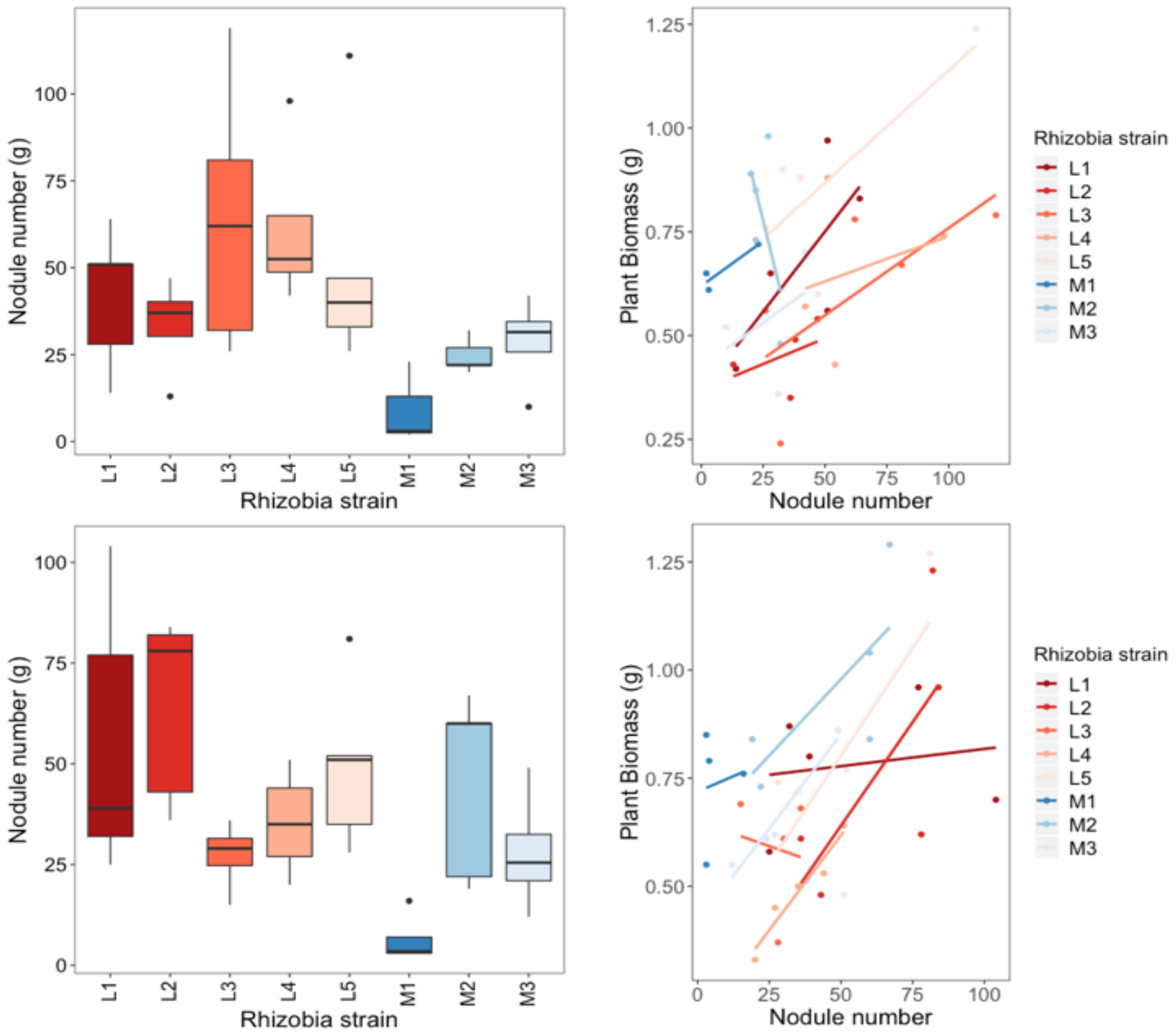

Figure S3.5. Mean plant biomass (LS means±SE) produced by *Chamaecrista* inoculated with no rhizobia, Lux, or Marshall rhizobia under (a) low nitrogen conditions and (b) high nitrogen conditions. Under low nitrogen conditions, original source plants had significantly higher biomass when grown without rhizobia than with Lux rhizobia, and marginally higher biomass than when grown with Marshall rhizobia, while Marshall plants performed best with their own rhizobia, and Lux plants showed no differences between rhizobia treatments (tests of rhizobia effects within each population: Original $F_{2,69}$=4.33, P=0.02; Marshall plants $F_{2,86}$=6.46, P=0.002; Lux plants $F_{2,59}$=0.96, P=0.39). Under high nitrogen conditions, original and Marshall plants showed no differences between rhizobia treatments, while the Lux population had marginally higher biomass when grown without rhizobia than when grown with Marshall rhizobia (tests of rhizobia effects within each population: Original $F_{2,84}$=1.04, P=0.36; Marshall plants $F_{2,58}$=2.27, P=0.11; Lux plants $F_{2,84}$=3.04, P=0.053).

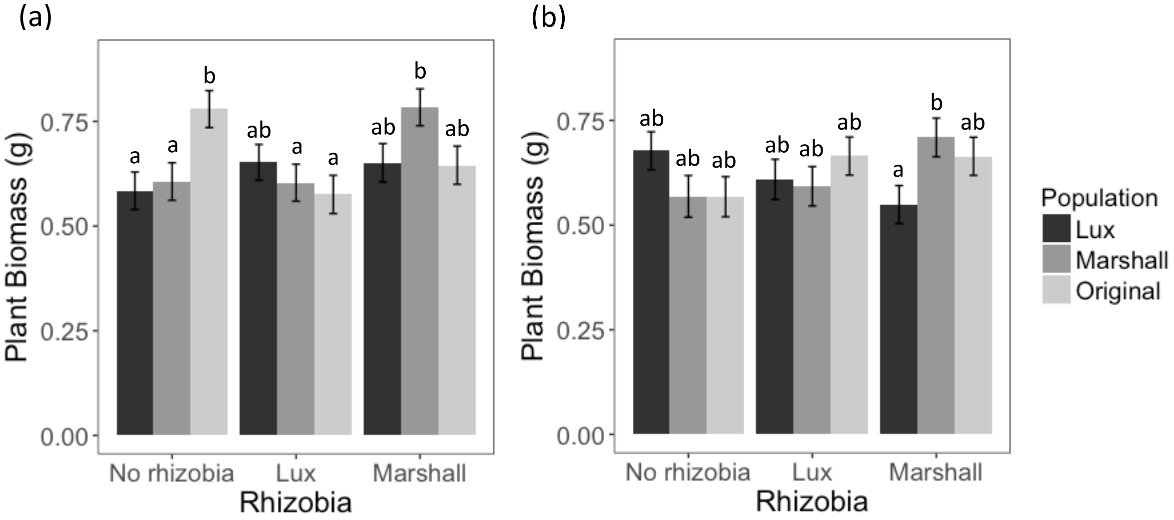

Figure S3.6. Mean estimated total nodule biomass (LS means±SE) produced by plants from each population inoculated with multi-strain mixes of Lux and Marshall rhizobia.

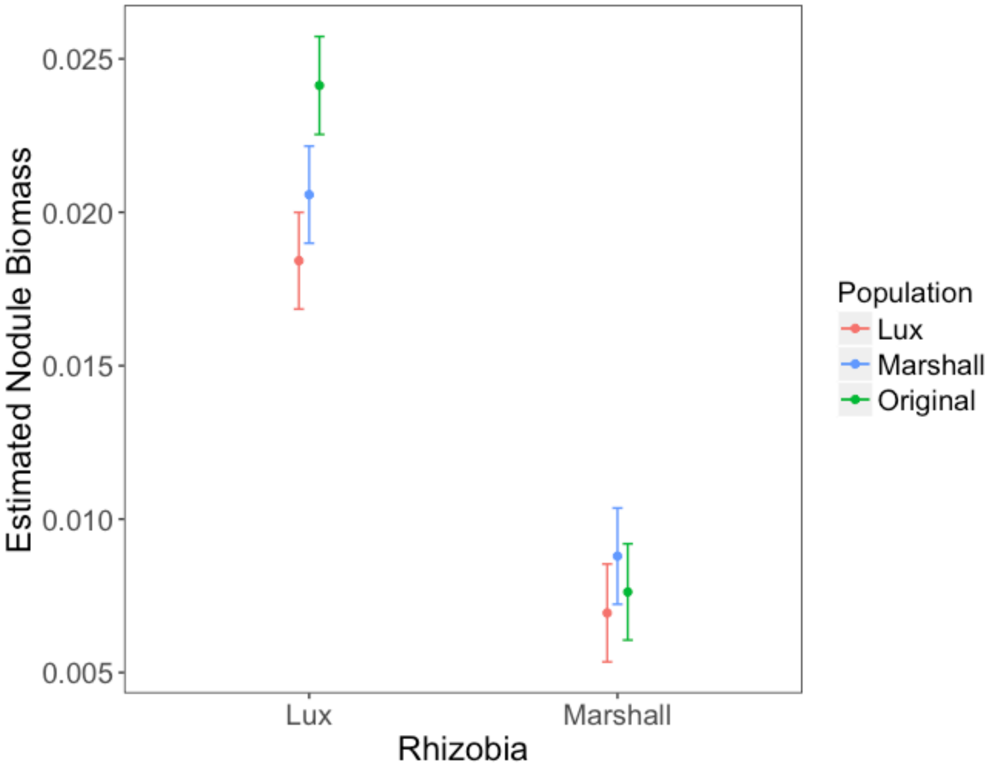

Table S3.1. ANOVA of the effects of plant population, rhizobia origin, and nitrogen on estimated total nodule biomass from the multi-strain experiment. Bold type denotes a significant effect ($p<0.05$).

	df	F	*P*
plant population	2,270	2.30	0.10
rhizobia origin	1,289	111.10	**<.0001**
nitrogen	1,324	7.06	**0.008**
plant population*rhizobia origin	2,306	1.56	0.1
plant population*nitrogen	2,330	0.3	0.88
rhizobia origin*nitrogen	1,315	0.97	0.33
plant population*rhizobia origin*nitrogen	2,327	0.08	0.93

Supplementary Methods/Results

Single strain rhizobia inoculations

While inoculating plants with a mixture of rhizobia from each site simulates a diverse rhizobia community, it masks any differences between individual rhizobia strains. To determine if certain strains were driving rhizobia effects on plants, we also inoculated plants from the two extant sites (Lux and Marshall) with each single strain used in the rhizobia mixtures. We did not include a nitrogen treatment, but fertilized plants at the low nitrogen level described above (2 plant populations x 18 rhizobia strains x 5 replicates = 180 total plants).

To evaluate whether single rhizobium strains from each site differed in relevant mutualism traits, we used linear mixed models that included nodule number, average nodule mass, or estimated total nodule mass as the response variable, plant population, rhizobia origin, and the interaction as fixed effects, and rhizobia strain (nested within rhizobia origin) and block as random effects. To examine whether strains differed in the per nodule benefits or absolute fitness they provide to plants, we used a similar model, but with plant biomass as the response and nodule number included as a fixed effect (for per nodule fitness benefits only). To determine whether single strains differ from each other, regardless of site of origin, we used models with nodule number, mean nodule mass, or estimated total nodule mass as response variables, and plant population, rhizobia strain and their interaction as fixed effects, with block as a random effect.

Results

Not all plants inoculated with single strains produced nodules. Four strains from Lux and six strains from Marshall did not produce nodules and were excluded from further analysis. Consistent with the multi-strain results, plants inoculated with rhizobia strains from Lux produced more nodules and nodule mass than plants inoculated with strains from Marshall (nodule number: $F_{1,6}$=14.5, p=0.008, Fig. S3.4a,c; estimated total nodule mass: $F_{1,6}$=23.1, p<0.003; average nodule mass: $F_{1,6}$=11.1, p=0.01). There were no significant differences in per-nodule fitness benefits between plants inoculated with single strains from Lux and Marshall (rhizobia origin $F_{1,14}$=1.78, p=0.20, Fig.S3.4b,d), although the trends are consistent with the multi-strain results (average plant biomass per nodule for plants inoculated with Lux strains = 0.017g/nodule; Marshall strains = 0.071g/nodule) and might not be significant due to power issues stemming from low sample sizes. There were also no significant differences in absolute fitness benefits (rhizobia origin $F_{1,6}$=.10 p=0.76), although like in the multi-strain experiment, Marshall plants produced the most biomass when inoculated with their own rhizobia and Lux plants performed equally well with both types of rhizobia. In models including rhizobia strain as a fixed effect, nodule number varied between strains, but depended on plant population (plant population*rhizobia strain $F_{7,59}$=2.49, p=0.03), with some strains producing more nodules with Lux plants, and others producing more with Marshall plants. In contrast, estimated total nodule mass and average nodule mass varied between strains but did not depend on plant population (plant population*rhizobia strain: estimated nodule mass $F_{7,58}$=1.43, p=0.21; average nodule mass $F_{7,50}$=1.24, p=0.30).

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CHAPTER FOUR:

RAPID LOCAL ADAPTATION, BUT LITTLE DEMOGRAPHIC BENEFIT: EFFECTS OF EVOLUTION IN RECENTLY ESTABLISHED PLANT POPULATIONS

Abstract

Reciprocal transplant studies have long provided the opportunity to explore the environmental conditions and population attributes that lead to local adaptation, but they also offer the potential to examine the ecological effects of adaptation. Specifically, if linked with population demography, local adaptation studies can be used to examine the importance of adaptation to the success of populations establishing in novel environments. Here, we use a reciprocal transplant experiment to determine whether two plant populations, originating from the same seed source, have undergone rapid local adaptation in recently restored prairies, and how this adaptation influences population growth rates and, therefore, probabilities of population persistence. We found evidence of partial local adaptation, with one population performing best at its homesite while the other did not. This locally adapted population had a slightly higher population growth rate than the non-locally adapted populations, although all growth rates were substantially less than 1. Our study demonstrates that local adaptation can occur quickly (<6 plant generations), and can lead to small increases in population growth rate.

Introduction

Local adaptation, the adaptation of a population to its local environmental conditions, undoubtedly increases a population’s growth rate relative to non-locally adapted populations. But the extent to which local adaptation affects whether populations decline or persist can be unclear in studies that do not consider that absolute fitness benefits of local adaptation, or that measure a few fitness components rather than lifetime fitness. For example, differences in fitness between a local population and a foreign population indicate whether a population is locally adapted, but says little about population persistence (i.e. a population might perform best at its home site but still have a population growth rate < 1). In addition, studies that examine individual fitness components may assess local adaptation differently than if they used integrated measures of lifetime fitness, particularly if there are opposing patterns for different fitness components. The demographic consequences of local adaptation might be particularly important in situations where rapid adaptation is necessary for population persistence. This includes scenarios in which populations experience rapid environmental change in their existing habitats (such as shifts in biotic or abiotic factors due to global change) or when populations have recently colonized new habitats with novel environmental conditions (such as during biological invasions or when populations are establishing in restored habitats) (Kinnison and Hairston 2007). For example, in one of the few empirical studies that has examined demographic effects of local adaptation, Kinnison et al. (2008) found that local adaption in recently established salmon populations led to fitness increases that likely contributed to colonization success. Examining demographic effects in local adaptation studies like this gives insight into both evolutionary processes and their ecological effects.

While empirical studies specifically linking local adaptation to population growth rates of natural populations are rare, theory predicts that rapid local adaptation (or rapid adaptation more generally) can have meaningful demographic effects. In some circumstances, the ability to quickly adapt to novel conditions can influence population persistence (Gomulkiewicz and Holt 1995) and in the case of colonizing populations the likelihood of establishment (Jones and Gomulkiewicz 2012). Studies of evolutionary rescue, the process by which populations recover or persist through adaptation after experiencing sudden environmental changes, test this theory and provide good examples of when we might expect rapid adaptation to have meaningful demographic effects. These studies predict that population density, genetic variation, and gene flow (all of which also affect local adaptation (Holt and Gomulkiewicz 1997, Leimu and Fischer 2008)) will influence whether rapid adaptation can rescue declining populations (Kinnison and Hairston 2007, Bell 2017). Laboratory studies involving asexual organisms with short generation times tend to support these predictions (Bell and Gonzalez 2009, Ramsayer et al. 2013, Bell 2017). Evolutionary rescue of field populations of higher organisms is less well-studied, and findings from studies of unicellular organisms may not directly extend to multicellular ones (Bell 2017). However, the prevalence of herbicide and pesticide resistance (Neve et al. 2014) and the persistence of plant populations in heavy-metal contaminated soils (Wu et al. 1975, Antonovics 2006) suggests that evolutionary rescue can occur in the field. We can determine whether rapid adaptation can have similar demographic effects on populations that have recently established in novel habitats by looking for evidence of evolutionary divergence from original source populations and patterns of local adaptation and examine their effects on estimated population growth rates.

The reciprocal transplant experiments of Clausen, Keck, and Hiesey (Clausen et al. 1941) stimulated decades of research investigating local adaptation, fueling insights into fundamental issues in evolutionary biology and ecology. Reciprocal transplant studies have identified the environmental conditions and population attributes that lead to and maintain local adaptation (Reznick and Ghalambor 2001, Kawecki and Ebert 2004, Leimu and Fischer 2008, Hereford 2009), the capacity of populations to adapt to global changes ranging from biological invasions (Lau 2006) to warming climates (Etterson and Shaw 2001, Atkins and Travis 2010), and when coupled with genomic studies, the genetic mechanisms of local adaptation in nature (Lowry et al. 2009, Anderson et al. 2013, Leinonen et al. 2013). Here, we use a reciprocal transplant approach to test for rapid local adaptation and demographic effects of adaptation in populations of the annual legume *Chamaecrista fasciculata* in two recently restored prairies.

Ecological restorations present an ideal opportunity to examine local adaptation and demographic effects, as large numbers of seeds are often planted into novel, anthropogenically-disturbed habitats. These conditions (large population size and likely strong selection) may facilitate local adaptation on a timescale where positive demographic effects can influence establishment success. In our system, the two restored prairies are only 15km apart and were planted with the same seed mix in the same year, but differ significantly in community composition as well as edaphic characteristics. This allows us to follow the evolution of populations originating from the same source in two very different sites, and begin to look for generalities in how and when evolution may influence population dynamics. Using field reciprocal transplant experiments, we addressed the following questions: 1) Is there evidence for local adaptation in recently established plant populations, and if so 2) Does local adaptation affect population growth rate?

Methods

Study system

Chamaecrista fasciculata Michx. (hereafter Chamaecrista) is an annual legume native to eastern North America commonly found in prairies and disturbed sites. *Chamaecrista* is predominantly outcrossing, and its establishment can influence the composition of early successional plant communities (Keller 2014). In this study we used populations of *Chamaecrista* from two recently restored prairies in southwest Michigan, Lux Arbor (42°28’23” N, 85°26’50” W) and Marshall (42°26’37” N, 85°18’34” W). These two former agricultural fields were planted with identical prairie seed mixes (containing 19 grass and forb species) in 2010, and a portion of the seed mix was saved (hereafter referred to as the ‘original source’). The *Chamaecrista* seeds in this mix came from a commercial restoration seed supplier and were an ecotype from Houston County, MN, USA (Shooting Star Native Seeds, pers. comm.), which is 1-2° higher in latitude than the restoration sites in southwest MI, with slightly lower average rainfall.

Despite being only 15km apart, the Lux and Marshall restoration sites differ in both abiotic and biotic factors. The Lux site is less productive and more grass-dominated than the Marshall site, but *Chamaecrista* biomass is consistently higher at the Lux site (2-12 times greater, depending on year; Fig. S4.4). The sites also differ in underlying abiotic factors such as total available soil nitrogen (see Stahlheber et al.

2016 for full site details). A previous study of these *Chamaecrista* populations found evidence of genetic differentiation between populations in flowering time (the Lux and Marshall populations flower significantly earlier than the original source population), root nodule formation, and specific leaf area (the Lux population produces significantly more nodules and has significantly lower SLA than the Marshall or original populations) (Magnoli *Chapter 2*), suggesting that these populations have undergone evolutionary changes in the time since they were established.

Reciprocal transplant experiments

To determine whether *Chamaecrista* populations are locally adapted to their home sites and to determine whether any local adaptation increases population growth rates compared to their original source population, we conducted a reciprocal transplant experiment, growing Lux, Marshall, and the original source plants at both the Lux and Marshall sites. In 2015, we collected 5-20 seeds from 100 haphazardly chosen individuals at each site. We grew these seeds, along with seeds from the original population, in the greenhouse for one generation to minimize maternal effects. For the Lux and Marshall populations, we grew one seed from each of 96 of the 100 maternal plants. Each of these was randomly assigned to be a sire or a dam, and each sire was used to pollinate two dams, for a total of 64 full sibling families (32 half-sibling families) per site. Due to low germination of the original source seeds (only 7 seeds germinated), we did not include family structure when pollinating these plants, but instead used one plant as a pollen donor on a given day, so that every plant was crossed with every other plant several times. In May 2016 we germinated seeds produced by these plants in the greenhouse and one week later transplanted seedlings into three 4 x 4m plots (each divided into 16 1 x 1m subplots with plants spaced 16cm apart) at both sites [(2 seedlings/extant population full-sib family x 64 full-sib families x 2 extant populations + 64 original source population seedlings) x 3 plots x 2 sites; N=1920 total seedlings]. We disturbed existing vegetation as little as possible while planting seedlings. We monitored survival over the course of the growing season, and collected seeds produced by each plant at the end of the season in September 2016.

Because germination rate is an additional important fitness component that we were not able to measure in the reciprocal transplant described above, we conducted an additional reciprocal transplant with seeds from each population the following year. In November 2017, we planted seeds from each of the three populations into twelve 1 x 1 m plots (seeds spaced 10cm apart) at both the Lux and Marshall sites (3 populations x 2 sites x 375 replicates; N=2250 seeds). To keep track of individual seeds, we glued them to plastic swizzle sticks (Soodhalter Plastics Inc.) with water-soluble Elmer's glue, and placed the swizzle sticks in the ground so that the seeds were just below the soil surface. This way, the seeds detach from the swizzle stick in the moist soil, but germinate right next to the stick for easy identification. In May 2018 we censused each plot for germination success.

Statistical Analyses

To test for local adaptation we compared fitness among plant populations at each site using aster models (Geyer et al. 2007, Shaw et al. 2008) in R v.3.5.1 (R Core Team 2018), which allow for unified analysis of multiple life history stages with appropriate statistical distributions. Our aster model integrated two life history stages: survival and seed production (we did not include germination because our germination data came from a separate transplant experiment), to estimate lifetime seed production (our measure of fitness) for each population. We used a Bernoulli distribution for survival and a Poisson distribution for seed production. We fit aster models with plant population, site, and their interaction as fixed effects and plot as a random effect. We tested whether the population x site interaction improved the fit of the model by using likelihood ratio tests to compare models with and without the interaction. To examine differences between populations in individual fitness components we analyzed germination rate, survival, and seed production of surviving individuals separately using generalized linear mixed models in the lme4 package (Bates et al. 2015). For seed production we included only plants that survived in the analysis to avoid confounding the two fitness components. We used a binomial family distribution for germination rate and survival, and a Poisson distribution for seed production.

We estimated population growth rates of each extant population in its home site using lifetime seed production estimates from the best-fit aster models and germination rates from our second transplant experiment. To estimate λ, we simply multiplied lifetime seed production by the germination rate of each population from each site.

Results

We detected evidence of partial local adaptation. Aster models indicated that populations differed in lifetime seed production, although the magnitude and direction of effect depended on site (the addition of the population x site interaction term significantly improved model fit; Table 4.1). Specifically, the Lux population performed better at its home site than the Marshall or original source populations, producing 34%

more seeds on average than the Marshall population (Fig. 4.1). In contrast, the Marshall population did not perform best at its home site. When grown at the Marshall site, the original source population performed better than both the Lux and Marshall populations, which did not differ from each other, although fitness was very low overall at this site (Fig. 4.1). This suggests that the Lux population is locally adapted to its home site while the Marshall population is not. Analyses of individual fitness components showed no significant site*population effects (germinationχ^2=0.94, p=0.63; survival χ^2 =4.61, p=0.1; seed production given survival χ^2=3.08, p=0.21; Fig. 4.2), but the Lux population had higher seed production than the Marshall population, regardless of site (χ^2=46.9, p<0.001). This indicates that the local adaptation we detect at Lux is likely driven by increased seed production compared to the other populations rather than changes in survival.

Estimates of population growth rates based on lifetime seed production and germination suggest that while λ varied between populations and sites, λ for every population at each site was < 1 (Fig. 4.3).

Table 4.1. Model comparison to test the effects of plant population and site on lifetime seed production. The test deviance is twice the log likelihood ratio. A significant analysis of deviance indicates improvement of the model following the addition of a new factor or interaction. Bold type indicates best fit model.

Model	Model df	Model deviance	Test df	Test deviance	*P*
population + site	5	-1322			
population + site + population x site	**7**	**-1313.2**	**2**	**8.83**	**0.012**

Figure 4.1. Predicted values (mean± SE) of overall lifetime seed production. Values were predicted using a fixed-effect aster model, rather than the random effect model used to test significance of fixed effects, as parameter estimates from random effects models are difficult to interpret.

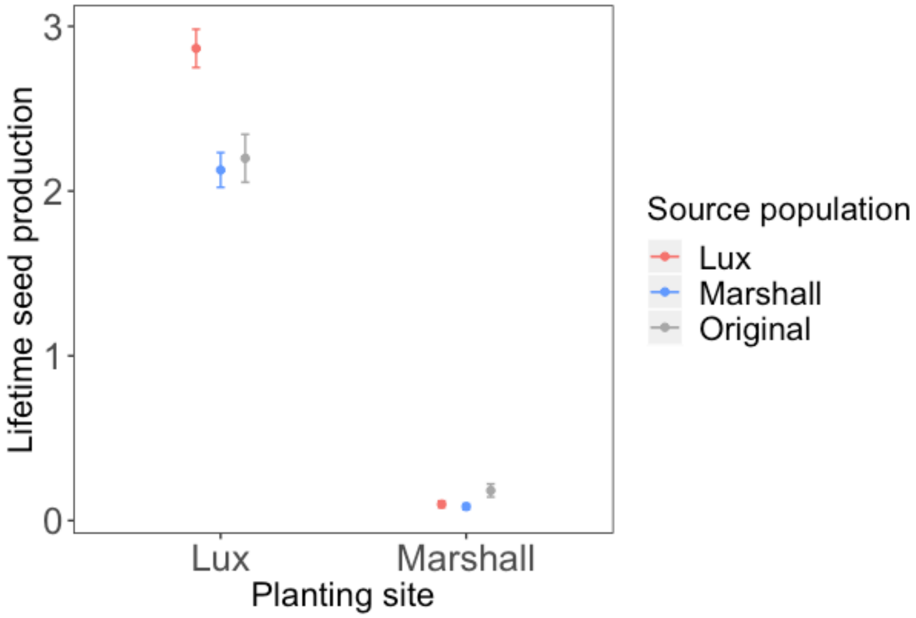


Figure 4.2. Mean (LS mean±SE) proportion of plants that survived to flower (a), number of seeds produced given the plant survived to flower (b) and proportion of seeds that germinated (c).

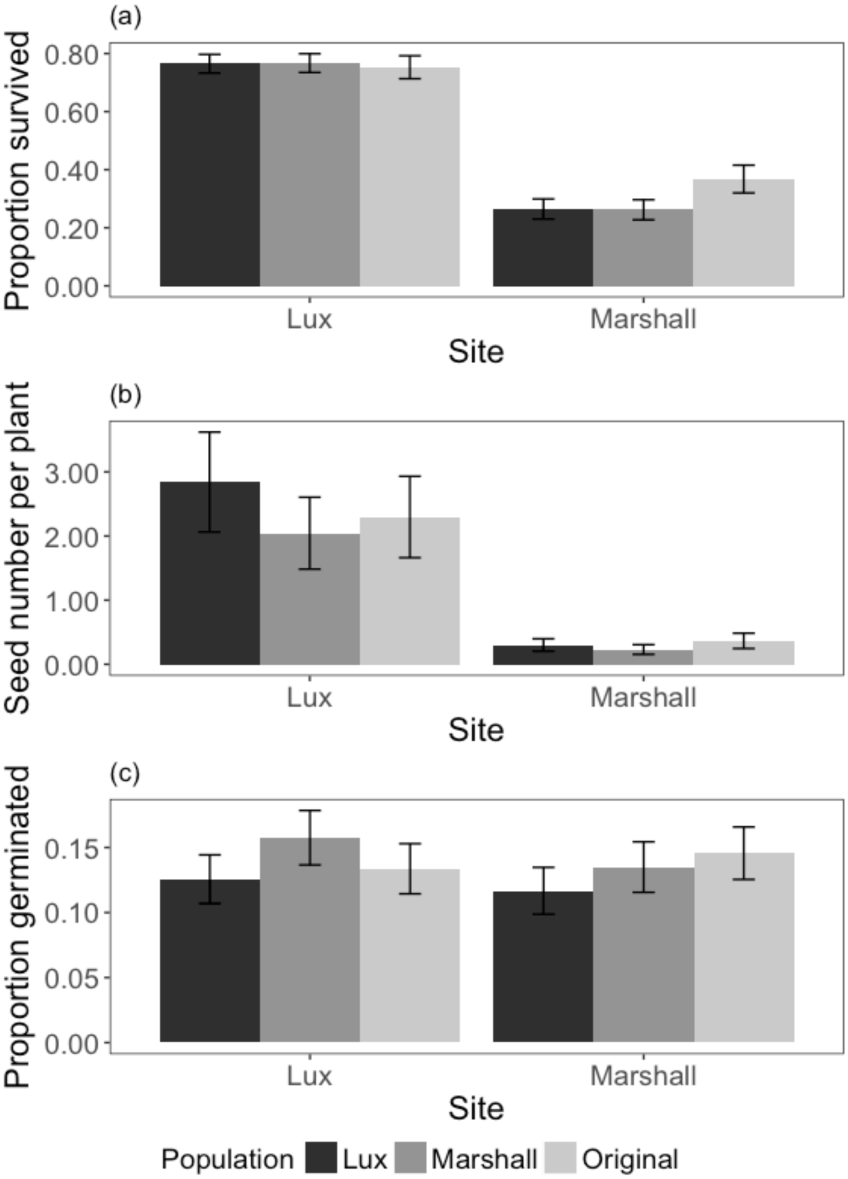
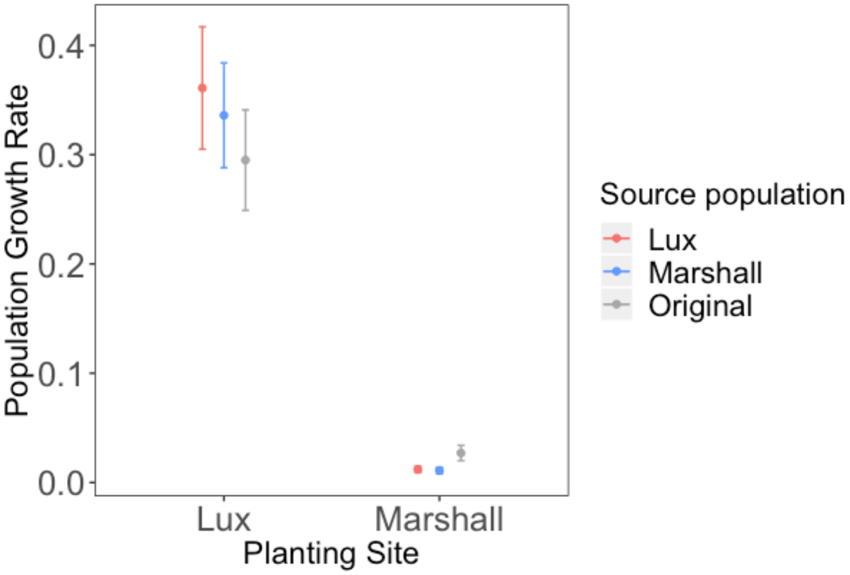


Figure 4.3. Estimated population growth rate (λ±SE) for each population grown at each site, based on germination rates and total lifetime seed production. Standard errors were calculated via error propagation, as the square root of the sum of the squared relative errors on fitness and germination rate.



Discussion

In a reciprocal transplant study of two recently established plant populations, we found evidence of partial local adaptation, and the locally adapted population had a 7-22% higher estimated population growth rate than other populations. Estimated population growth rates (λ) in even the locally adapted population were substantially less than 1, however, so whether the observed adaptation is large enough in magnitude to substantially tip the balance to population persistence remains to be seen. It appears that in this system the capacity of rapid adaptation to affect population persistence may be limited both by whether a population adapts at all (the Marshall population), and by whether adaptation has adequate effects on absolute fitness (the Lux population).

Adaptation can be limited by several factors, many of which are discussed at length in Antonovics (1976) and some of which may be relevant to our system. First, swamping effects of gene flow between populations can slow or inhibit local adaptation. This is unlikely to be a limiting factor in our system, as our *Chamaecrista* populations are 15km apart, making gene flow between them unlikely, and there are no known naturally-occurring *Chamaecrista* populations in the surrounding area. Second, a lack of genetic variation can inhibit adaptation. We do not know the variation present in the original source population, but there was clearly enough genetic variation to allow local adaptation to occur in the Lux population. Lack of genetic variation could be a limiting factor for adaptation at the Marshall site if the original source population was lacking variation for the specific traits that would contribute to adaptation to environmental conditions at the Marshall site. Third, adaptation could be limited by coevolutionary constraint. A plant population coevolving with competitors, mutualists, or pathogens could experience reductions in fitness gains needed to adapt to a new environment. This could potentially explain differences between populations in local adaptation in our system, as the two sites differ in several biotic factors (such as plant community composition (Stahlheber et al. 2016) and rhizobia quality (Magnoli *Chapter 3*), which could lead to coevolutionary resistance at the Marshall site but not the Lux site. Finally, trade-offs among fitness components could limit adaptation, for example if there is a negative correlation between survival and seed production. Preliminary analysis of correlations between family mean survival and seed production given survival for both populations grown at their homesites show negative correlations for both populations (Marshall: -0.04, Lux: -0.005). Although the correlation is stronger for the Marshall population, it is still fairly weak, suggesting trade-offs might not explain the lack of local adaptation.

By examining population growth rates in one year, our study provides a snapshot of potential effects of local adaptation on population persistence. Although we found that all populations had growth rates < 1 in the year of our study, the locally adapted population did have a slightly higher growth rate than the other populations, which could potentially have implications for population persistence over longer time scales. Biomass estimates of *Chamaecrista* at these sites from 2010-2016 (Fig. S4.4) show fluctuations in population size from year to year, indicating fluctuations in population growth rates over time. The overall low population growth rate estimates we found in the year of our study could be due to either environmental conditions in the year we conducted the reciprocal transplant experiment, or could be part of a pattern of overall population decline due to succession. In the summer of 2016 southwest Michigan experienced abnormally dry conditions (US Drought Monitor) which might explain both high mortality and low seed production, as seed production in 2014 and 2015, both years with normal rainfall, was much higher (observations of 100 and 200 plants at each site in these years, respectively, showed an average of 44 and 10 seeds per plant at Lux and 38 and 12 seeds per plant at Marshall, approximately 2-18 times higher than the lifetime seed production observed here). Alternatively, the low population growth rates we found may be driven by successional dynamics. *Chamaecrista* is an early successional species (Keller and Lau 2018), and may be starting to decline in these sites as the prairies mature, particularly if the positive effects of local adaptation do not outweigh the negative demographic effects of successional-driven environmental changes. This explanation may be less likely given that *Chamaecrista* biomass declined in 2016 after three years of increase (Fig. S4.4), which does not suggest an overall pattern of decline. However, estimating growth rates of these populations in future years would help determine whether the results we found were due to abnormally dry conditions in the year we conducted the experiment or whether they are the beginning of longer-term population decline.

Conclusions

Examining the ecological effects of adaptation in field populations of plants can provide insight into the role of rapid evolution in population persistence. In our system, we find no evidence that rapid local adaptation shifts population growth rates from negative to positive, but we do demonstrate the importance of considering integrated measures of fitness and absolute fitness when examining local adaptation. By using fitness estimates that integrate several fitness components (in this case both survival and seed production) we found evidence of local adaptation in one population, even though analyzing these two fitness components alone did not indicate local adaptation. Yet the vast majority of studies analyze fitness components; only five of the 35 papers included in Leimu and Fischer's (2008) review of plant local adaptation studies used integrated fitness measures (those that did typically used combinations of germination, survival, and/or reproduction measures). Considering an integrated measure of fitness also allowed us to explicitly link local adaptation to population growth and identify the population ecology consequences of rapid adaptation. While more challenging than only measuring fitness components, new statistical approaches, like the aster models used here, make the estimation of integrated components feasible. As a result, future studies of local adaptation, particularly those focusing on populations colonizing new habitats or inhabiting environments experiencing rapid environmental change, may be able to not only accurately assess local adaptation but also quantify the demographic effects of adaptation.

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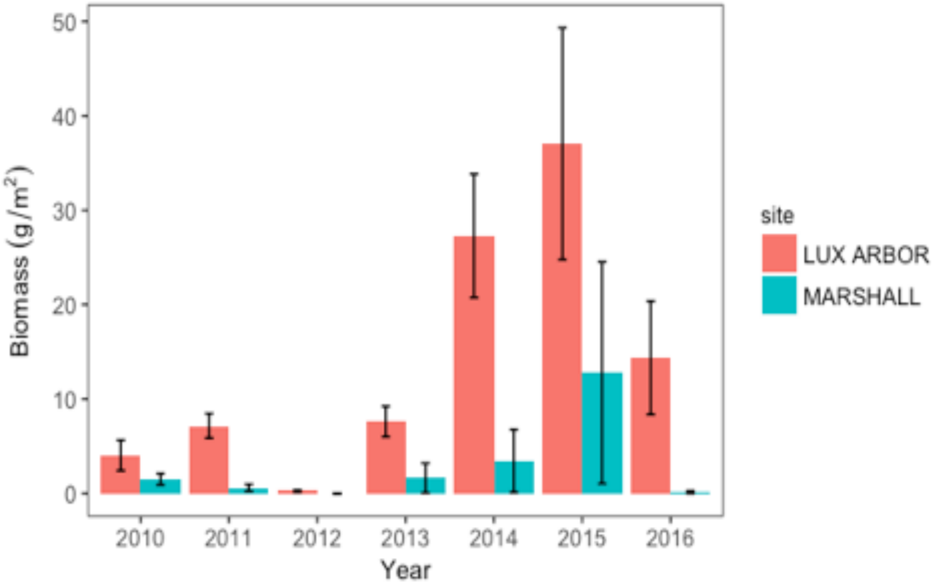
APPENDIX

Figure S4.4. Estimated *Chamaecrista* biomass (LS mean±SE) across years at the Lux and Marshall sites. Biomass was estimated by harvesting all aboveground plant material in ten 2 x 0.5 m quadrats at each site in August or September of each year. Plant material was sorted to the species level, dried at 60°C and weighed.



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