Washington University in St. Louis

Washington University Open Scholarship

1-1-2011

Causes of rarity in glade-endemic plants: Implications for responses to climate change

Nicole Miller-Struttmann
Washington University in St. Louis

Follow this and additional works at: https://openscholarship.wustl.edu/etd

WASHINGTON UNIVERSITY IN ST. LOUIS

Division of Biology and Biomedical Sciences
Program in Ecology, Evolution, and Population Biology

Dissertation Committee:

Barbara Schaal, Chairperson
Peter Hoch, Advisor
Jim Cheverud
Kenneth M. Olsen
Kevin G. Smith
Alan R. Templeton

CAUSES OF RARITY IN GLADE-ENDEMIC PLANTS: IMPLICATIONS FOR

RESPONSES TO CLIMATE CHANGE

by

Nicole Miller-Struttmann

A dissertation presented to the
Graduate School of Arts and Sciences
of Washington University in
partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

August 2011
Saint Louis, MO

ABSTRACT OF THE DISSERTATION

CAUSES OF RARITY IN GLADE-ENDEMIC PLANTS: IMPLICATIONS FOR

RESPONSES TO CLIMATE CHANGE

by

Nicole Miller-Struttmann

Doctor of Philosophy

Division of Biology and Biology

Program in Ecology, Evolution, and Population Biology

Washington University

St. Louis, MO 2011

This dissertation documents the relationship between stress-adaptation and
reproductive specialization in three endemic plant species (*Delphinium treleasei*,
Echinacea paradoxa, and *Scutellaria bushii*) that are locally-abundant but restricted to
glade habitats and their closely-related congeners (*D. carolinianum*, *E. pallida*, *S. ovata*,
and *S. parvula*) that have broader habitat use, including glades. Glades are hot, xeric
environments with much exposed bedrock, limited soil development, herbaceous cover,
and are found in a matrix of more mesic woodland and prairie habitat.

Theory predicts that rare species are more likely to be self-compatible and have
generalist pollination systems in order ensure reproduction when pollen limitation is low.
However, if pollen is less limiting in larger local populations, other factors, such as
abiotic stress or resource limitation, could exert stronger selection pressure on floral traits
and plant-pollinator interactions. In Chapter Two, I test two hypotheses concerning

reproductive specialization in endemic plants and their common congeners (CC). I quantify morphological traits that are associated with stress-adaptation and reproductive specialization, pollinator behavior, plant specialization, and rates of autogamous self-fertilization. The locally-abundant, regionally-rare (LARR) species were significantly different from their CCs in vegetative traits associated with stress-adaptation (i.e., stem length and leaf area), but the direction of the differences was not consistent among genera or with predictions of stress-adaptation. On the other hand, two of the three LARR taxa had larger flowers and fewer dominant pollinators than their CCs, though there were no differences in autogamous selfing rates. This study supports a more nuanced theory of the relationship between reproductive specialization and rarity that addresses additional factors influencing rare taxa, such as stress-adaptation.

In Chapter 3, I examine the responses of the LARR and CC plants to drought and high heat conditions in order to explicitly test the prediction that the LARR species are more resistant to high-stress environments and allocate resources to fewer, higher-quality offspring in comparison to their widespread congeners. Plants that are adapted to stressful environment have a suit of traits that are thought to be adaptive and should lead to greater offspring quality in order to increase survival. I exposed plants of both species in a congeneric pair (*D. treleasei, D. carolinianum, E. paradoxa, E. pallida, S. bushii,* and *S. ovata*) to experimental manipulations of water availability and temperature regimes that were consistent with those experienced in the glade habitat. The LARR species were more resistant to stress is some morphological traits that are associated with adaptation to stressful environments, and the CC species were not. Moreover, the LARR species had fewer, higher-quality offspring, whereas the CC species have more seeds of lower

quality. This study indicates that plants that specialize on stressful environments differ from their CCs in morphological traits associated with stress-adaptation and in the allocation of resources to reproductive output, with implications for their interactions with pollinators.

I then test the alternative hypotheses that two LARR species are (1) poorer competitors for pollinators, as predicted by traditional theory of reproduction in rare species, (2) are better competitors for pollinators in stressful environments in comparison to a common congener, or (3) do not compete with their common congeners for pollination services (Chapter 4). Rare species are predicted to have floral traits associated with higher selfing rates, such as smaller flowers and lower reward output, and therefore, may be poorer competitors for shared pollinators than closely-related, widespread species. An alternative prediction is that stressful abiotic conditions should result in selection for traits that increase offspring quality, such as fewer, larger flowers or more specialized pollination systems, that may confer greater competitive ability for pollinators. Finally, pollinator abundance and behavior differs spatially and temporarily, and both the strength and direction of competitive superiority may vary accordingly. I conducted pollinator competition trials at multiple localities, in order to control for spatial variation in pollinator assemblage. Naïve plants were exposed to pollinators in two treatment arrays, either with conspecifics or heterospecifics, and constant density. I compared the pollination and reproductive success of the CC and LARR species in mixed groups and in monoculture. The congeneric pairs did not compete for pollinators but varied in their morphological traits and reproductive success across sites. The results of this study indicates that spatial variation in plant-pollinator interactions dominates

pollination success in years with low pollinator abundance. The LARR species, one of which has a more specialized pollination system than its CC, had greater pollination and reproductive success (defined as pollen deposition and pollen tube growth, respectively) than their CCs. This pattern suggests that more specialized pollination systems may lead to increased reproductive success per visit. This study enhances our understanding of the impacts of spatial variation and specialization on interspecific interactions, which is increasingly important as we attempt to conserve rare species and habitats in a rapidly changing world.

Finally, this dissertation explores the response of two LARR species and their CCs to regional climate change in order to test for the relative importance of intrinsic and extrinsic factors in determining species phenological patterns. Species responses to climate change have been well documented, but there is significant variation in responses both across and within taxa. I test the prediction that LARR species that specialize on stressful habitats are less responsive to climate change in comparison to their CC, since they are resistant to changes in abiotic conditions (intrinsic factors). On the other hand, local extrinsic factors may influence the degree of change at the scale of an individual. For instance, glade habitats have much exposed bed rock and low vegetative cover, which should increase the heat holding capacity and decrease cooling via evapotranspiration within the glade. All individuals that occur on glades will be equally likely to respond to climate change. Using herbarium specimens, I calculated a continuous variable to describe the development stage of all individuals and tested for the role of climate, long-term change (the time component), and habitat on the relationship between the development stage of the individual and the date it was collected. The

regional climate in Missouri and Arkansas is increasing in average minimum temperatures and precipitation, as indicated by an analysis of a composite climate variable. *Delphinium treleasei* (LARR) is responding to climate change by flowering earlier, and there is a similar trend in *S. bushii* (LARR), though low replication reduced my ability to rigorously test the response of this species. None of the widespread species exhibited a change in phenology with climate change. This study is the first to document phenological responses to climate change by describing the development stage of individuals in time, which is a more biologically realistic estimate of phenology. Moreover, the differential responses of LARR and CC plants indicating that intrinsic traits are driving their response to climate change. The stress-adapted species are more responsive to climate change, contrary to my prediction and may reflect a selective advantage for species that specialize on stressful habitats to more closely track climate. In light of current anthropogenic changes, understanding the influence of stress on reproductive systems, including local-adaptation and floral phenology, is of critical importance and warrants more in-depth study.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my husband, Justin (Benny) Struttmann, for all of his support, patience, and humor. I would not have been able to finish this dissertation without his encouragement, logistical support, and ability to make me laugh, which never failed to give me perspective. You inspire me to be the best scientist and individual that I can, and I am forever thankful for your love. I would also like to thank both of my families and my friends for all of their support and for accompanying me on this crazy journey that is graduate school.

I would like to thank my committee for their enormous gift of time and knowledge. To Barbara Schaal, I cannot thank you enough for supporting and believing in me throughout my graduate career. Your advice has always been insightful and much appreciated. I thank Alan Templeton for bestowing as much of his vast knowledge of the glade system, evolutionary ecology, and statistics as I was able to absorb. To Jim Cheverud, I thank you for your statistical insight and generous gift of time over which we discussed it. I thank Ken Olsen for his insight into the evolutionary underpinnings of my research. To Kevin Smith, I thank you for your in-depth analysis of my work. Your advice and feedback have been irreplaceable; they have improved my understanding of the results that I present here and how it pertains to the field of ecology as a whole. Finally, I would like to thank my primary advisor, Peter Hoch. I am truly grateful to you for all of your sage advice and patience. You are a true mentor, and I am here today because of your support, guidance, and balance of honest evaluation and encouragement. Your enthusiasm for science is infectious, and I have greatly enjoyed working with you.

TABLE OF CONTENTS

List of Tables

List of Figures

CHAPTER ONE

Pages 1 to 4

INTRODUCTION

A fundamental goals in ecology and evolutionary biology is to determine the mechanisms that influence species distributions, particularly among rare and widespread species. Both biotic and abiotic factors are thought to contribute to species distributions, particularly in light of their reproductive biology. There are several competing theories contributing to our understanding of the relationship between reproduction and rarity. The first is that of reproductive assurance, that rare species should maintain the ability to self-fertilize in order to buffer for variability in pollination success. Therefore, rare species are predicted to have higher outcrossing rates and to be self-compatible (Baker, 1955; Kelly et al., 1996; Neiland and Wilcock, 1998; Izco, 1998; Fausto et al., 2001; Murray et al., 2002; Domínguez Lozano and Schwartz, 2005; Jacquemyn, 2005; Moeller and Geber, 2005). This theory is realistic for locally rare species that may be infrequently visited by pollinators. However, species that are locally-abundant but regionally rare may not be limited by pollen availability, and other factors may play a more prominent role in determining their reproductive biology, such as stressful abiotic conditions (Armbruster and Reed, 2005; Fox and Reed, 2010). Rare species may have greater outcrossing rates in order to avoid negative genetic effects common to small population sizes (Wright 1922; Del Castillo and Trujillo, 2008; Firman and Simmons, 2008; Kennedy and Elle, 2008; Espeland and Emam, 2011).

My objective was to examine the relationship between rarity and reproduction in three locally-abundant, regionally rare glade plant species that are endemic to stressful habitats, in comparison to widespread, closely-related species that occur on glades but are not restricted to them. Glades are south to southwest facing, rocky outcroppings found throughout the Ozark region of Missouri and Arkansas. They characterized by high

quantity of exposed bedrock, low herbaceous cover, limited soil structure, and hot and xeric conditions (Baskin and Baskin, 1982; Nelson and Ladd, 1982; Yatskievych, 1999; Templeton et al., 2001). Glades have been of interest to ecologists and evolutionary biologists for decades due to their suit of endemic species, many with restricted ranges (Kucera and Martin, 1957; Baskin et al., 1974, 1982; Baskin and Baskin, 1985; Learn and Schaal, 1987; Jenkins and Jenkins, 2006) and their spatial structure, which makes them ideal for studies of dispersal (Brisson et al., 2003), spatial genetic structure (Learn and Schaal, 1987; Templeton et al., 2001; Brisson et al., 2003), metapopulation dynamics (Ryberg and Chase, 2007), and many other ecological and evolutionary topics (e.g., Van Zandt et al., 2005; Van Zandt, 2007).

Glades are also ideal for the study of rarity and reproductive theory, since they have several endemic plant species in sympatry with widespread, closely-related species. Therefore, the mechanisms that restrict one species, and not the other, to glades can be explored while controlling for the potential effect of evolutionary history on species traits (Bevill and Louda, 1999). In this dissertation, I test hypotheses concerning the relationship between stress-adaptation and reproductive biology in three endemic glade species (*Delphinium treleasei*, *Echinacea paradoxa*, and *Scutellaria bushii*) in comparison to their widespread, closely-related species (*D. carolinianum*, *E. pallida*, *S. ovata*, and *S. parvula*). Each of the following four chapters has an introduction to the principles behind the specific hypotheses to be tested, contain new data and analyses, and separate tables and figures that demonstrate the methodology of or present the result from analyses of the data therein.

In the first chapter, I document differences in the morphological traits, pollination biology, and breeding systems of three congeneric species pairs through an *in situ* field, observational study. In the second chapter, I explicitly test the three hypotheses, (1) glade endemic species are more resistant to experimental stress, (2) they have floral traits that are associated with decreased attractiveness to pollinators but increased outcrossing rates, and (3) reproductive output is allocated to fewer, higher quality offspring in comparison to their widespread congeners. The research presented in Chapter 4 tests the prediction that the floral traits of two stress-adapted endemic plants confer lower competitive ability for pollination services in comparison to their common congeners. The research in Chapter 5 documents the phenological responses of two the congenic species pairs to regional climate change. The final chapter concludes the dissertation with a discussion of the major results and their conservation implications, particularly in light of recent global changes.

Pages 5 to 31

REPRODUCTIVE BIOLOGY OF LOCALLY-ABUNDANT, REGIONALLY

RARE TAXA REVEALS A MORE COMOPLEX RELATIONSHIP BETWEEN

RARITY AND REPDOCTIVE SPECIALIZATION

INTRODUCTION

The relative importance of biotic and abiotic mechanisms in determining species'
ranges is an elemental debate in ecology and evolutionary biology. Competitive exclusion
(Hardin, 1960; Kunin and Gaston, 1997; Lloyd et al., 2002) and specialization to abiotic
or biotic conditions may restrict the biogeographical range of a species (Rabinowitz et al.,
1981; Kruckeberg and Rabinowitz, 1985; Kunin and Gaston, 1997; Gregory and Gaston,
2000), and rare plants are predicted to have self-compatible breeding systems and less
specialized pollination systems in order to maintain reproduction given low pollen
availability (Baker, 1955; Kelly et al., 1996; Izco, 1998; Neiland and Wilcock, 1998;
Fausto et al., 2001; Murray et al., 2002; Domínguez Lozano and Schwartz, 2005;
Jacquemyn, 2005; Moeller and Geber, 2005). While this prediction of reproductive
assurance may be relevant for those species that are locally rare and have small local
population sizes, locally abundant, regionally-rare (LARR) plants should be less
restricted by pollen receipt, allowing for factors other than pollen availability to influence
their reproductive biology (Rabinowitz et al., 1981; Kruckeberg and Rabinowitz, 1985;
Fenster et al., 2004; Williams et al., 2009), such as resource limitation or adaptation to
stressful environments. In 1981, Deborah Rabinowitz delineated seven forms of rarity
based on population size, geographic extent, and habitat specificity (Table 2-1;
Rabinowitz et al., 1981). Historically, the relationship between reproductive biology and
rarity has focused on the rare species with small populations sizes. However, LARR
plants may experience very different selective pressures, such as for increased
outcrossing rates, enhanced offspring quality, or decreased resource-loss in stressful
environments.

6

In response to xeric environments, plants often have similar suites of traits that are thought to be adaptive (Grime, 1977; Bryant et al., 1983; Chapin et al., 1993), each conferring a different advantage to the plant. While advantageous in more extreme abiotic habitats (Bryant et al., 1983; Chapin et al., 1993; Grime, 1977) stress adaptations often come at a cost, such as decreased competitive ability (Grime, 1977; Baskin and Baskin, 1988) potentially attractiveness to pollinators in more productive habitats. For instance, stress-adapted species are often shorter, with overall reduced floral output, and lower seed production (Aragón et al., 2008; Kudo et al., 2008). Plants that are taller (Dickson and Petit, 2006) and have larger floral displays are more attractive to pollinators and could be competitive dominants (Erhardt and Rusterholz, 1998; Naug and Arathi, 2007; Aragón et al., 2008), which may select for more generalized pollination systems and confer lower competitive ability for pollinators. Moreover, nectar production, an important floral attraction trait, is reduced in order to conserve water in xeric environments (Halpern et al., 2010) and therefore should confer reduced attractiveness to pollinators. As survival becomes increasingly important for population stability, resources allocated to reproduction (e.g., floral attraction traits) may be reduced (Silvertown et al., 1993), potentially resulting in lower floral output and competitive ability for pollinators.

Conversely if resources are limiting, investment in fewer, larger flowers that restrict the number and type of effective pollinators should increase outcrossing rates (Karron et al., 2004; Mitchell et al., 2004; Bell et al., 2005; Karron et al., 2009; Mitchell et al., 2009) and potentially enhance offspring quality. Plants with fewer flowers open concurrently are visited less frequently and for shorter periods of time, resulting in the

increased deposition of outcross pollen. Longer distances to floral rewards (i.e., spur or tube length) restrict the number and type of visitors that can access the reward, resulting in more specialized pollination systems (Anderson and Johnson, 2008), which should also increase offspring vigor and decrease resource losses through investment in lower-quality, self-fertilized offspring (Darwin, 1877; Whittall and Hodges, 2007). Despite their importance, plant-pollinator interactions have not been explicitly integrated into stress-competition theory.

I conducted observational and experimental studies to test six hypotheses regarding the relative importance of stress-adaptation verses reproductive specialization in three locally-abundant, regionally rare (LARR) plants that are endemic to the Ozark glades (i.e., geographically and edaphically restricted habitats characterized by hot and dry conditions) and congeners of these three species that occur on glades but are not restricted to them. First, I test the hypotheses that LARR plants have traits associated with stress-resistance or pollination specialization, such as smaller leaves and fewer, smaller flowers. Next, I test the predictions that LARR plants have lower rates of autogamy and are more dependent on their pollinators for their reproductive success (i.e., more pollen limited) in comparison to closely-related species with broader ecogeographical ranges. Finally, I test the hypotheses that visitation rates are lower and pollination specialization scores are higher in comparison to their CCs. In order to determine rates of autogamy and pollen limitation, I conducted breeding system and pollen-supplementation experiments. The hypotheses regarding pollination specialization and pollinator movement were examined through a three-year observational study of pollinator behavior. I show that two of the three LARR taxa are have more specialized

reproductive biology (morphology and pollination), but do not have traits associated with increased stressed-resistance. I then discuss the implications of these findings for species responses to climate change and conservation efforts in stressful environments.

MATERIALS AND METHODS

Study System—The glades of the Ozark Mountain Region are rocky, arid outcroppings that are spatially restricted within a matrix of more mesic oak-hickory forest matrix (Learn and Schaal, 1987; Baskin and Baskin, 1988; Templeton et al., 2001). They are dominated by herbaceous grasses and forbs and contain several endemic plant species (Nelson and Ladd, 1982; Yatskievych, 1999), many with restricted ranges. Temperature data recorded at three replicate glade, forest, and prairie sites between December 2007 and August 2008, indicate that glades are significantly hotter than the surrounding habitats during the spring and summer months ($DF = 3688$, $F = 2.36$, $p = 0.0017$; not shown), when many LARR glade plant species are photosynthetically active and blooming.

I chose three LARR glade species, *Delphinium treleasei* (Ranunculaceae), *Echinacea paradoxa* (Asteraceae - Heliantheae), and *Scutellaria bushii* (Lamiaceae) that are restricted to glades in the Ozark Region in Missouri and Arkansas but locally abundant (i.e., hundreds of individuals per glade). Each has a common congener (respectively, *D. carolinianum*, *E. pallida*, *S. ovata*, hereafter common congeners (CC)) that grows on glades but is not restricted to them. Comparing congeneric pairs provides insight into the factors that restrict one species to glades and not the other. Moreover, comparing several unrelated LARR species to their common congeners mitigates the potential influence of evolutionary history on the biological traits correlated with

9

endemism, such as specialized pollination. This provides a phylogenetically controlled study of the mechanisms contributing to the restricted ranges of these species (Kruckeberg and Rabinowitz, 1985; Bevill and Louda, 1999). Multiple congeneric comparisons allow for broader interpretation of the results from this study (Bevill and Louda, 1999) and a greater understanding of the relationship between stress-adaptation and reproductive biology, which has hitherto been not been explicitly explored.

Vegetative traits—I quantified selected vegetative and floral traits that I hypothesized to differ between the stress-adapted and non-stress-adapted species for ten to fifteen individuals per site for multiple sites per species (average = 4.82 sites and 144.5 individuals per species; Table 2-2; Figure 2-1). Over three field seasons (2007 – 2009), morphological traits were measured at no fewer than four glade sites for each LARR species and no fewer than one non-glade (i.e., prairie and woodland) and two glade sites for the widespread taxa (Table 2-2). I was not able to obtain above- or belowground biomass due to restrictions on the collection of the LARR plants and the required sampling techniques (including the substantial destruction of glade habitat). Therefore, biomass was estimated as the total number of leaves and total stem length (i.e., the product of the number of stems and stem length). In 2009, one medium-sized leaf per individual was measured for leaf thickness, pressed and digitized. The leaf area of each leaf was quantified in Sigma Scan (SYSTAT Software Inc., 2002), and total leaf area was calculated (i.e., average leaf area multiplied by the total number of leaves).

Floral traits—I measured floral attraction traits (i.e., display size, nectar volume) and floral morphological traits that I predicted to affected pollinator behavior and pollinator effectiveness. Due to morphological differences, some floral attraction traits

were quantified differently for each of the genera, particularly for the *Echinacea* species. For the *Delphinium* species, corolla area was calculated as the product of corolla width and corolla height, and distance to the nectar reward refers to the length of the floral spur. I measured corolla area of the *Scutellaria* taxa as the square of the lower corolla, often called the landing pad, and distance to nectar as floral tube depth. For both *Scutellaria* and *Delphinium*, I documented the number of flowers produced per individual and the number of flowers open concurrently. The proportion of flowers open was calculated as the number of flowers open concurrently divided by the total number of flowers produced per individual, and total display size was calculated as the product of corolla area, as defined above, times number of flowers open. In order to estimate nectar production, plants were bagged prior to anthesis, left undisturbed for several hours (4-6 hours later for *Delphinium* species and 5-6 hours for *Scutellaria* species, except *S. parvula* at one site where individuals were bagged for 29 hours, because nectar levels were too low to quantify after shorter time periods), and nectar was collected via micro-capillary tubes. Nectar production was then quantified as nectar volume produced per hour.

For the *Echincaea* species, corolla area was calculated as the product of the width and length of an average ray petal, and total display size is corolla area multiplied by the total number of ray petals. The number of flowers open per inflorescence was the sum of all florets that were shedding pollen or had receptive stigmas, and the proportion of florets open was quantified as the total number of florets open divided by the total number of florets produced. For all species, relative floral output was standardized by total stem length, in order to account for variation in individual plant size. Nectar was not

collected from Echinacea was not measured due to low nectar production and small floret size.

Reproductive success and Breeding systems—In order to estimate ambient reproductive success in a common habitat, fruits were collected from individuals at the glade sites, and seedset was quantified for up to three fruits per individual. For *Delphinium* and *Scutellaria* species, total reproductive success is defined as the average number of seeds per fruit multiplied by the total floral output. For *Echinacea* taxa, total reproductive success is defined as the proportion of achenes (florets) with a fertilized seed multiplied by the total number of achenes per capitulum (inflorescence). Since *Echinacea* species are known to produce unfertilized capsules that collapse when pressure is applied, thirty seeds per capitulum were gently pressed with forceps to confirm fertility and ensure accurate estimates of reproductive success. Seeds that did not collapse under the small amount of applied pressure were assumed to be fertile. For all species, relative reproductive success was standardized by total stem length.

I determined the degree to which each species is self-compatible via breeding system studies conducted under controlled greenhouse conditions or in natural populations when flowering could not be induced in the greenhouse. Wild-collected seed of the *Delphinium* and *Echinacea* taxa were germinated, transplanted and grown to flower in the greenhouse. I documented the phenology of reward presentation (e.g., corolla expansion, first nectar production, and stigma receptivity) under greenhouse conditions and in the field. I was unable to induce flowering of *Scutellaria bushii* in the greenhouse and conducted breeding system experiments for all *Scutellaria* taxa in the field. Thirty individuals per species were bagged before anthesis to exclude pollinators,

pollinated according to one of three treatments, and remained bagged until fruits developed. To demonstrate the role of pollinators in fertilization, the capacity of each species to self-pollinate, and the degree to which each species is or is not self-incompatible; I applied self-pollen, outcross pollen, and no pollen to 10 individuals of each species, respectively. Following maturation, I collected the fruit and quantified reproductive success as above. Degree of autogamy was calculated as the ratio of reproductive success in the bagged and in the pollen supplement treatments. Dependence on pollinators for self-pollination was calculated as the ratio of bagged and self-pollinated treatments.

Pollination biology— I conducted a pollen supplementation experiment at each field site to quantify ambient limitation of reproductive success via inadequate pollination. I applied outcross pollen to all receptive stigmas of 10 individuals, excluded pollinators from 10 individuals to estimate autogamous fertilization, and tagged 10 additional individuals which remained open to pollinators and acted as the control. Following maturation, all marked flowers or achenes were collected, and the reproductive success quantified. Seed fertility of the *Echinacea* taxa was estimated as noted above. Pollen limitation was calculated as supplemented reproductive success minus ambient reproductive success.

In order to document the pollination specialization of these target plant species, I recorded the number, type, and behavior (e.g., visit duration (s), anther and stigma contact) of all floral visitors through 30 minute observations during peak periods of activity and collected a representative number of each visitor for pollen counts and identification. Due to the spatial and temporal variation inherent in plant-pollinator

13

interactions, pollinator observations and insect collections were conducted at each site over two years, 2008 and 2009. In 2008, ten individual plants were observed for each species per site, and in 2009, fifteen individuals were observed. Replication was increased in 2009 in order to compensate for low visitation rates. Pollinator observations and collections were conducted at both glade and non-glade (i.e., prairies and woodlands) sites for all widespread species, which controls for potential differences in pollinator assemblages and behavior across sites. One inflorescence per individual was observed for 30 minutes, two to three times during the blooming period (10-15 individuals x 2 observation days x 3 sites per species = 60-90 individuals per species in 2008 and 2009, respectively; Table 2-2) in order to account for individual and temporal variation in insect activity. Since insect identification is often impossible during field observations, visitors were categorized into functional groups during field observations. Visitation rate of each species was calculated as the product of the visitation rate of the functional group per 30 minute observation and the proportion of individuals of that functional group represented by the given species.

Following collection in the field, visitors in Hymenoptera and Lepidoptera were identified by experts, where possible, to the species level (86.5%), and those in Coleoptera, Diptera, and Hemiptera were identified to family. Pollen was washed from insects with ethanol, mounted on slides and stained with Calberla's solution (Ogden et al., 1974; Dafni, 1993; Clinebell and Bernhardt, 1998). Pollen loads were determined for each insect species as the average number of pollen grains of the focal plant species found on the insect. Pollen was identified with the aid of a pollen reference library of all co-blooming species, and total pollen flow (L_j) of the focal plant species was calculated,

$$L_j = \sum_{i=1}^{Sj} (p_i v_i)$$

Eq. 1

where S_j is the total number of insect species visiting plant species j, p_i is number of species j pollen grains carried by visiting species i, and v_i is visitation rate of insect species i to plant species j. I measured the specialization of each plant species via the Generalization Index (i.e., the number of insect species that account for 95% of pollen flow), and the relative importance of all pollinators was quantified as the Dominance Index of Pollinator Importance (D_{PIj}, modified from Galloni, 2008). The Dominance Index is a modification of the Simpson's diversity index that accounts for both insect pollen load and visitation rate,

$$D_{PIj} = \sum_{i=1}^{Sj} [(p_i v_i/L_j)^2]$$

Eq. 2

(symbols are the same as in Eq. 1). The Dominance Index of ranges from zero (i.e., multiple pollinators each account for an equal proportion of pollen flow) to one (i.e., one pollinator accounts for all of the pollen flow), and it is a measure of the pollinator importance that accounts for pollinator richness within a defined pollinator community (Galloni, 2008).

Statistics—All morphological traits, reproductive biology, and pollination biology were normalized where necessary and tested for significant differences between congeners via t-test in R Statistical Software (R Development Core Team 2008). Variables with low replication (i.e., $N < 6$) that either had either marginally significant *t*-values or had variables that could not be normalized were analyzed via permutation

ANOVA (aovp in lmperm package; R Development Core Team 2008). All permANOVA results of non-normalizable data did not differ from the results of the *t*-tests; therefore I report only the *t*-test results.

RESULTS

Vegetative traits—Vegetative traits differed significantly between congeners but were not consistent among genera. Generally, there were no differences when non-glade habitats were included in the analyses, but for clarity, I discuss the differences within glades only, unless otherwise specified (all results are recorded in Table 2-3). *Delphinium treleasei*, the LARR taxa, did have thicker leaves than its CC as I predicted, but there were no differences in total stem length or in total leaf area, two estimates of biomass. Contrary to my predictions, the LARR *D. treleasei* had greater average leaf area (Table 2-3, Figure 2-2a) than its CC *D. carolinianum* and was not significantly shorter. In congruence with my hypotheses, the LARR *Echinacea paradoxa* was shorter than its CC, *E. paradoxa*, but there were no differences in any other vegetative trait measured, including average leaf area (Figure 2-2b), leaf thickness, or total leaf area. The LARR *S. bushii* was significantly different from its CC, *S. ovata*, in congruence with my hypotheses for vegetative traits, but not from *S. parvula*. *Scutellaria bushii* was shorter than *S. ovata* but taller than *S. parvula*, and *S. bushii* had significantly lower leaf area than *S. ovata* but not *S. parvula* (Table 2-3, Figure 2-2c). Contrary to my hypotheses, *S. bushii* was significantly taller in comparison to both *Scutellaria* CCs (*S. ovata* and *S. parvula*). There were no differences in leaf thickness or total leaf area between *S. bushii* (LARR) and *S. ovata* or *S. parvula*.

16

Floral traits—As predicted, *Delphinium treleasei* (LARR) had significantly larger flowers than *D. carolianum* (Table 2-3, Figure 2-2d) and marginally greater distance to the nectar reward (spur length) when individuals from prairie sites were included. On the other hand, total floral output, relative floral output, and the number of flowers open concurrently did not differ between *Delphinium* taxa (Table 2-3). There were no differences between *Echinacea* species, except in ray petal size. The LARR *E. paradoxa* had marginally larger ray petals (corolla area) in comparison to *E. pallida* on glades (Table 2-3; Figure 2-2e), in congruence with my predictions. *Scutellaria bushii* (LARR) had significantly larger flowers (Table 2-3; Figure 2-2f) and greater distance to the nectar reward than both CCs, as predicted. In comparison to *S. ovata*, *S. bushii* (LARR) also had lower relative floral output across all habitats (Table 2-3). On glades alone, there were no differences in total floral output, relative floral output (i.e., the number of flowers per cm total stem length), or nectar volume between *S. bushii* and *S. parvula*. Contrary to my predictions, *Scutellaria bushii* had more flowers open concurrently and marginally greater proportion of flowers open in comparison to *S. parvula*, though total display size (total corolla area) was marginally larger, as predicted. When compared to *S. ovata*, *S. bushii* had significantly lower relative floral output, but there were no differences in the number or proportion of flowers open.

Reproductive success and Breeding system experiments— Relative reproductive success (total seedset per unit total stem length) was significantly lower for the *E. paradoxa* (LARR) but not for the other two LARR taxa (Table 2-4) in comparison to their common congeners. There were no differences in total reproductive success

between any congeneric pair or in rates of autogamy, geitonogamy and outcrossing (Table 2-4).

Pollination biology— *Delphinium treleasei* (LARR) had a lower generalization score than *D. carolinianum* (Figure 2-2g) and was more pollen limited when non-glade habitats were included in the analysis (Table 2-3). Pollinators did not probe fewer flowers consecutively or visit for shorter durations when visiting *D. treleasei* in comparison to *D. carolinianum*, and there was no difference between species in pollinator dominance or fidelity (Table 2-3). *Echinacea paradoxa* (LARR) was more pollen limited than its CC, as predicted, but pollinator behavior and pollination generalization (i.e., the number of pollinators to account for 95% of pollen flow; Figure 2-2h) did not differ between species. *Scutellaria bushii* (LARR) did not differ from *S. parvula* in pollinator behavior or generalization score (Table 2-3; Figure 2-2i). The number of flowers visited consecutively did not differ between *S. ovata* and *S. bushii* on the glades, but when non-glade habitats were included, visitors probed fewer flowers of *S. bushii* than *S. ovata*, as predicted (Table 2-3). Due to low insect abundance, pollinator collections were insufficient at all but one of the glades where *S. ovata* was studied; therefore, I was unable to conduct the analysis with glade sites only. However when non-glade habitats were included, *S. bushii* had a significantly lower generalization score than *S. ovata* (Table 2-3; Figure 2-2i) but not in dominance or pollinator fidelity.

DISCUSSION

Species of each of the seven forms of rarity (Rabinowitz et al., 1981; Kunin and Gaston, 1997; Gregory and Gaston, 2000) may experience different selective pressures resulting in a wide range of pollination systems, given the appropriate abiotic or biotic

environment. Here I explore the reproductive biology of three locally-abundant and regionally-rare (LARR) plant species that are endemic to stressful environments. The floral traits and pollinator behavior documented in two of the three species in this study support the hypothesis that glade LARR species have more specialized reproductive systems. While these results generally support my prediction that persistence in stressful environments may result in pollination specialization, there is little evidence for stress-adaptation per se.

Glade endemics did not consistently share morphological traits associated with stress-adaptation, and differences in a few traits, such as leaf area (Figure 2-2b), had conflicting patterns across genera. For instance, leaves of *S. bushii* (Figure 2-2c) were significantly smaller than *S. ovata* but not than the other CC, *S. parvula*. *Delphinium treleasei* (LARR) had larger leaves than its CC (Figure 2-2a), which is the opposite of my prediction, and *E. paradoxa* was not different from its CC (Figure 2-2b). Height has a similar, contradictory pattern. *S. bushii* is shorter than *S. ovata* but taller than *S. bushii*; *Echinacea paradoxa* is shorter than its CC, and there is no difference between *Delphinium* species. One key trait that I was not able to assess due to collection restrictions was below ground growth, and it should be explored in order to have a more complete understanding of the vegetative traits associated with stress-adaptation in plants that are endemic to stressful environments, such as glades. However, the lack of consistent differences (both statistically and directionally) between congeners in the selected traits measured here suggests a lack of adaptation to the hot, dry conditions on glades.

On the other hand, all three LARR species had larger flowers (i.e., corolla area) than their common congeners (Figure 2-2 d–f), although *Echinacea paradoxa* was only marginally larger than *E. pallida*, (CC; Table 2-3). Corolla area was the only trait that was consistently different among the genera, which confirms the hypothesis that reproductive specialization plays an important specializing on stressful environments but not the hypothesis that stress-adapted plants are poor competitors for pollinators. Moreover, *S. bushii* (LARR) species had longer floral tubes than both of its common congeners (CC), and *D. carolinianum* had longer spurs, though only marginally when the non-glade habitats were included. I predicted that the restricted access to nectar reward would reduce the number of pollinators to account for the majority of pollen flow. In congruence with this hypothesis, *Delphinium treleasei* (LARR) also had a more specialized pollination system than its congener (Figure 2-2g), and *S. bushii* had a more specialized pollination system than *S. ovata* (Figure 2-2i), though specialization could only be tested when all habitats were included due to low pollinator abundances. On the other hand, there was no difference in the dominance index, which controls for the richness of visitors, between any congeners. This suggests that while the number of species that account for the majority of the pollen flow (i.e., the generalization score) is significantly lower, the dominance of any individual pollinator was not different between LARR taxa and their congeners. Visitation rates and total seed production also did not differ between congeners. However, higher specialization in some of the LARR taxa could lead to greater outcrossing and presumably higher offspring quality. Offspring quality (e.g., germination rate) was not assessed in this study. Therefore, further study is required to explore the effects of these mechanisms on overall reproductive success.

Contrary to *Scutellaria* and *Delphinium*, the LARR *Echinacea* species did not

differ from *E. pallida* in pollination generalization, and it had lower relative reproductive

success and was more pollen limited than its CC. This disparity in reproductive success

could lead to the exclusion of the LARR *E. paradoxa* from habitats where competition

for resources is more intense or where total reproductive output is more instrumental in

population establishment (e.g., via founder effects). I have defined reproductive success

as total seedset, but there are other potentially important traits that could affect fitness,

specifically offspring quality (i.e., germination rate and establishment). While not

significant, there is also a trend toward more generalized pollination in *E. paradoxa*

(LARR; Figure 2-2h), a finding that warrants greater exploration to offset low replication

(N = 4) at the site level caused by low pollinator abundances in 2009.

In order to achieve reproductive assurance, rare plants are predicted to be self-

incompatible, assuming either low pollen availability due to local rarity or unpredictable

pollination. However, reproductive assurance may not be optimal for locally abundant or

stress-adapted taxa, which may be more limited by resources than by pollen. Rates of

autogamy of the LARR plants in this study reflect neither selection for reproductive

assurance nor reduction of inbreeding. Indeed, rates of autogamy appear to be conserved

across taxa. While there is mixed support for pollination specialization, all LARR plants

have larger floral displays (i.e., corolla area) than their congeners, which is congruent

with our predictions for optimal resource allocation in LARR taxa to increase

attractiveness and potentially outcrossing rates. More specialized pollination systems, as

seen in *D. treleasei*, may result in higher pollinator efficiency, higher outcrossing rates,

and lower stigmatic occlusion, which in turn may increase outcrossing rates and offspring

quality. If there is greater variation in floral traits, selection may act on those traits associated with pollination specialization, thereby increasing outcrossing rates while conserving the ability to self-pollinate. Additionally, inbreeding depression is predicted to be exacerbated in stressful environments (Wright, 1922; Hauser and Loeschcke, 1996; Cheptou et al., 2000; Armbruster and Reed, 2005; Waller et al., 2008) and could result in selection for increased outcrossing rates as well. The documentation of the mating systems and the strength of selection for traits conferring increased outcrossing rates are required to corroborate the hypothesis of higher inbreeding depression in stressful environments.

Despite the lack of support for a stress-reproduction tradeoff, the results of this study support a more nuanced approach to the relationship between rarity and reproductive specialization. The *Delphinium* and *Scutellaria* LARR species have traits in congruence with my predictions of increased specialization and outcrossing rates, but the *Echinacea* species does not, suggesting that both mechanisms (i.e., increased selection for outcrossing and reduced allocation to reproduction) may be acting concurrently in different LARR taxa. Further study of additional LARR species and larger sample sizes at the site level will be needed to determine the relative prominence of each mechanism. These endemic glade plants may not be more stress-resistant or adapted to local abiotic conditions, but they may be restricted from more productive habitats via competitive exclusion. It is unclear whether LARR glade plants are specifically adapted to glades or are simply restricted to glades due to low competitive ability in more productive habitats, and the relative importance of these mechanisms should be explored more in future research on the range restriction of species to stressful habitats.

The conventional paradigm of reproductive assurance in pollen-limited plants, such as locally rare plants, is an important theoretical prediction that has been supported by some studies (Fausto et al., 2001; Moeller and Geber, 2005). However, reproductive theory should be expanded to include more explicit predictions for each of the seven forms of rarity. The explicit integration of non-biogeographic factors, for instance stress-adaptation and competition for pollinator services, into this paradigm could greatly enhance our understanding of the factors that determine and are affected by species eco-geographical distributions. This study gains insight into stress-adaptation and pollination specialization as two potential factors restricting the ranges of three glade-endemic LARR species. The pattern of more specialized pollination systems (reflected in the LARR *D. treleasei*) and lower relative reproductive success (as in the LARR *E. paradoxa*) suggest a tradeoff between allocation to offspring quality and quantity that could affect the ability of rare plants to invade less-stressful habitats. Reduced reproductive success could inhibit the ability of LARR species to colonize new habitats and increase their risk of extinction. Moreover, specialization on insect pollinators found on or near glades could further restrict the ecogeographical range of these species.

In response to climatic change, many species are predicted to shift their ranges, adapt to their new environment, or go extinct. Low colonization potential and high habitat specialization could inhibit the ability of rare species to track their optimal climatic conditions or adapt to novel habitats and environmental conditions, resulting in an even greater restriction of their ranges and an increase in their risk of extinction. Insight into the relative importance of potential confounding factors related to each of the seven forms of rarity is imperative for our understanding of the mechanisms determining

the biogeographical ranges of all species and for the creation of effective conservation

and management plans for rare and endemic species. This study contributes to the

development of a more nuanced theory regarding the interaction between rarity and

reproductive specialization, which will inform our understanding and protection of rare

and endemic plant species.

Table 2-1. Description of the Rabinowitz's Seven Forms of Rarity based on geographic extent, population size and habitat specificity (modified from Rabinowitz et al., 1981). The bolded form of rarity represents the locally-abundant, regionally rare (LARR) taxa in this study.

| | *Geographic Extent (GR)* | | | |
	Large		Small	
Population Size (PS)				
Somewhere large	Common	Large PS Large GR Narrow HS	Large PS Small GE Broad HS	**Large PS (LA)** **Small GE (RR)** **Narrow HS**
Somewhere small	Small PS Large GE Broad HS	Small PS Large GE Narrow HS	Small PS Small GE Broad HS	Small PS Small GE Narrow HS
	Broad	Narrow	Broad	Narrow
	Habitat Specificity (HS)			

Table 2-2. Replication for each species by habitat for the number of field sites and the number of plants for which I documented morphological traits (Morph) and observed pollinator behavior, and the number of insects collected for pollen load analysis (Insects). * Field sites were not mutually exclusive; therefore, field sites per species do not sum to total number of field sites.

Plant species	Habitat	Field Sites*			Plants (no.)		Insects (no.)
		Morph	Observ	Insect	Morph	Observ	
Delphinium carolinianum	Glade	5	4	3	90	75	137
	Prairie	3	1	1	40	10	21
Delphinium treleasei	Glade	8	7	3	193	128	157
Echinacea pallida	Glade	4	4	2	90	72	40
	Prairie	2	2	2	73	20	213
Echinacea paradoxa	Glade	5	5	2	110	62	60
Scutellaria bushii	Glade	7	4	3	244	123	31
Scutellaria ovata	Glade	3	3	1	72	48	86
	Woodland	9	5	2	133	71	46
Scutellaria parvula	Glade	5	4	3	96	88	34
	Woodland	2	0	0	7	0	0
Total		31	23	18	1148	697	825

Table 2-3. Results (i.e., *t*-values) from analyses of morphological traits and pollination biology of three locally-abundant, regionally rare (LARR) taxa and their common congeners (CC) in glades and across all sites (including non-glade habitats, such as prairies and glades). Symbols represent significance levels († *P* < 0.01, *P < 0.05, ** *P* < 0.01, *** *P* < 0.0001), traits that were analyzed within a single site (α; *Echinacea* corolla area and total display size only), and traits for which only comparisons included all habitats could be conducted (ø; *Scutellaria bushii* vs. *S. ovata* pollination biology only (i.e., generalization score, dominance, and pollinator fidelity)). Numbers in parentheses denote significant *P*-values from permutation ANOVAs that were conducted for marginally significant *t*-test results and N < 6.

| | *Delphinium* | | *Echinacea* | | *Scutellaria* | | | |
| | | | | | S. ovata | | S. parvula | |
	Glades	*All sites*	*Glades*	*All sites*	*Glade*	*All sites*	*Glade*	*All sites*
Vegetative traits								
Height	0.93	1.19	**2.84***	2.04[†]	**8.31***	**6.49***	**-4.27**	**-3.31**
Leaf area	**-14.07***	--	1.62	--	**12.48***	--	-2.66	--
Leaf thickness	**-4.82**	--	1.64	--	2.28	--	-2.12	--
Total stem length	-0.53	-0.38	-0.79	-0.21	**-2.76***	-2.41	**-4.58**	**-3.15**
Total leaf area	-4.09[†]	--	0.77	--	-0.79	--	-3.05	--
Floral traits								
Corolla area	**-3.13**	**-3.36**	-1.79[†α]	--	**-4.82***	**-4.83**	**-12.69***	--
Distance to nectar	-1.64	-2.07[†]	--	--	**-5.88**	**-5.49**	**-26.3***	--
Nectar volume	1.58	--	--	--	--	--	-1.11	--
No. flowers open	1.19	0.95	-0.38	0.01	1.26	2.16[†]	**-4.24**	--
Proportion of flowers open	2.25[†]	1.67	-2.08	--	1.35	1.10	2.13[†]	--
Relative floral output	0.28	-0.29	0.52	--	**3.80***	**3.84**	-0.44	-0.37
Total display size	-0.25	-0.06	-0.48[α]	--	-1.43	-1.11	-5.33[†]	--
Total floral output	0.29	0.79	0.39	--	1.32	1.98[†]	-4.24	**-3.26**
Pollination biology								
Visitation rate	1.00	0.63	0.85	0.47	0.64	1.99[†]	1.84	--
Visit duration	-0.23	-0.48	0.08	0.24	-0.50	0.56	0.14	--
Flowers visited consecutively	0.75	0.51	--	--	1.66	**2.64***	-0.26	--
Pollen limitation	1.92	**2.89[†]** **(0.0002)**	**6.14***	--	-1.10	-2.45[†]	-1.98	--
Generalization score	**2.24[†]** **(0.004)**	**2.10[†]** **(0.0002)**	-1.18	-0.57	--	**2.68[†Ø]** **(0.001)**	1.00	--
Dominance	-1.90	-1.49	1.45	0.64	--	-2.06[Ø]	-1.67	--
Pollinator fidelity	-0.15	0.39	-1.40	0.38	--	-1.97[Ø]	-2.38	--

Table 2-4. Comparison of reproductive success and breeding systems between three locally-rare, regionally-restricted (LARR) taxa and their common congeners (CC). Results shown are *t-values*, and significance levels are denoted with symbols († $P < 0.10$, *$P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$). Breeding system studies of the *Delphinium* and *Echinacea* taxa were conducted in the greenhouse. *Scutellaria bushii* could not be induced to flower in the greenhouse; therefore, breeding system studies were conducted in the field. Numbers in parentheses denote significant *P*-values from permutation ANOVAs that were conducted for marginally significant *t*-test results and N < 6).

	Delphinium	*Echinacea*	*Scutellaria*	
			S. ovata	S. parvula
Relative reproductive success	0.34	**2.33†(0.004)**	2.45	0.58
Total reproductive success	0.43	1.95	1.70	-0.04
Autogamous seedset	0.93	0.40	-0.22	-0.68
Geitonogamy seedset	1.65	0.60	--	0.001
Outcross seedset	-1.63	0.05	0.25	-0.40

Figure 2-1. A map of 31 glade and non-glade (woodland and prairies) field sites (○) in Missouri at which I quantified morphological traits and observed pollinators for three locally-abundant, regionally-rare species (*Delphinium treleasei*, *Echinacea paradoxa*, and *Scutellaria bushii*) in comparison to their common congeners (*D. carolinianum*, *E. pallida*, *S. ovata*, and *S. bushii*). Glade density was coded in to a heat-density map from Nelson and Ladd (1982). The original data represented a range in glade density per 7.5 degree minute quadrats (approximately 144 km^2 in Missouri); therefore the heat map reflects a rough estimate of glade density per 144 km^2 as of1982.
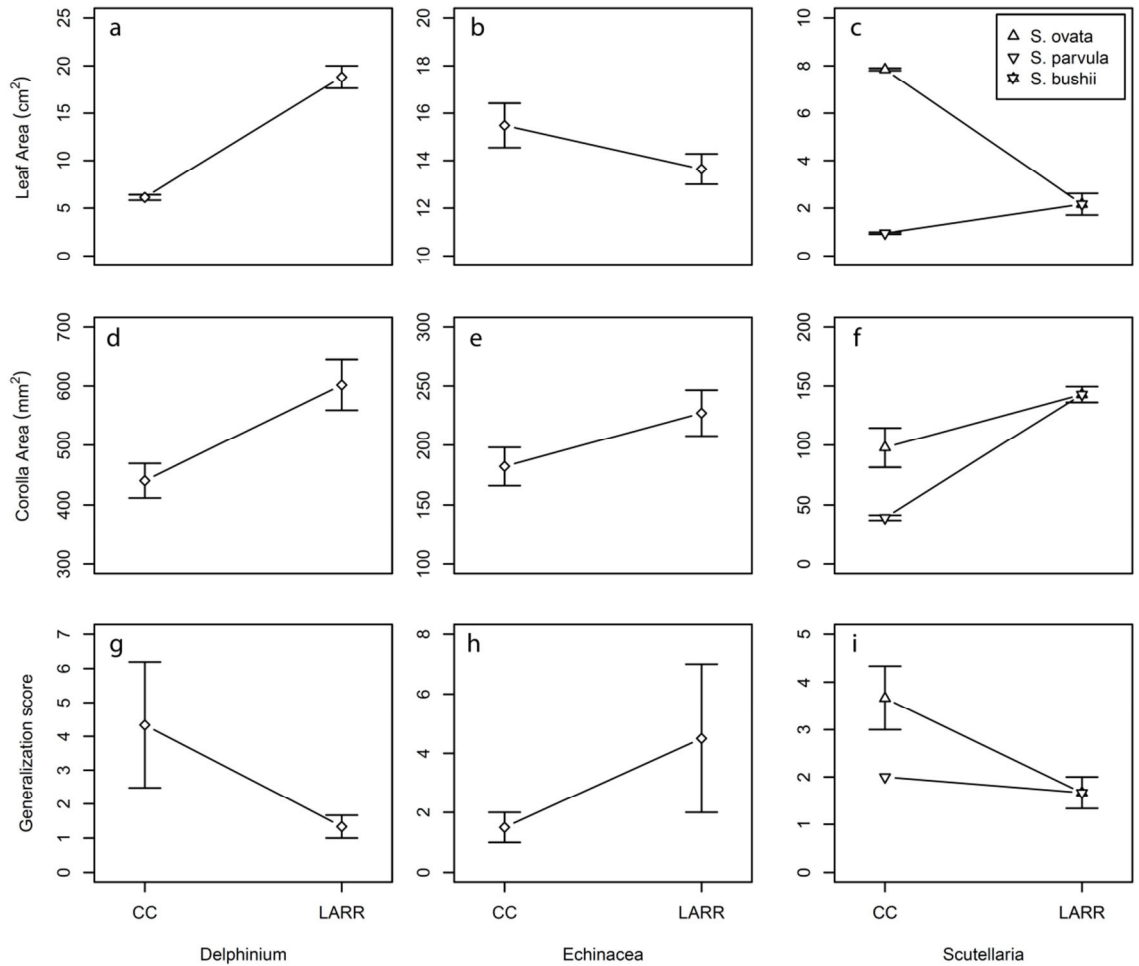
Figure 2-2. Morphological traits and pollination biology of three locally-abundant,
regional-rare plant species (LARR; *Delphinium treleasei* (◇)*, Echinacea paradoxa* (◇),
and *Scutellaria bushii* (✿)) in comparison to their common congeners (CC; respectively,
D. carolinianum (◇), *E. pallida* (◇), *S. ovata* (△) and *S. parvula* (▽)) as quantified in the
field and graphed by distribution and genus; Leaf area (cm^2; a – c), Corolla area (mm^2; d
– f), and Generalization score (g – i; no. species to account for 95% of pollen flow; the
comparison between *S. bushii* and *S. ovata* was calculated with data from glade and non-
glade habitats due to low pollinator densities on glades; *S. parvula* does not have standard
error bars, because it's generalization score was identical across all sites). All other
results shown were calculated from within-glade comparisons.

31

CHAPTER THREE

Pages 32 to 62

RESOURCE ALLOCATION DIFFERS BETWEEN RARE AND WIDESPREAD

PLANT SPECIES IN RESPONSE TO EXPERIMENTAL STRESS

INTRODUCTION

In 1981, Rabinowitz and colleagues developed a conceptual matrix describing seven types of rarity based on geographical range, habitat specificity, and local population size. A recent review of studies that reference this framework indicates that species in each category of rarity have some similar traits (Espeland and Emam, 2011). For instance, species that are habitat specialists and have geographically restricted ranges are more likely to have outcrossing mating systems, which are thought to maintain genetic diversity and reduce the potential for genetic drift (Espeland and Emam, 2011). In order to maintain high outcrossing rates, species that specialize on restricted habitats should have mechanisms that restrict the movement of self-pollen and enhance the transfer of outcross pollen, including herkogamy (the separation of reproductive parts in space; Lavergne et al., 2004), more specialized pollination systems (Darwin, 1877; Rymer et al., 2005; Galloni, 2008), or a reduction in the number of co-blooming flowers (Karron et al., 2004; Mitchell et al., 2004). The reduction in co-blooming flowers, for instance, alters pollinator behavior, resulting in fewer within-plant movements and the deposition of more outcross pollen (Karron et al., 2004; Mitchell et al., 2004). Specialized pollination systems have fewer pollinating species that collect and transfer pollen between plants, which can increase outcrossing rates and decrease the deposition of heterospecific. Heterospecific pollen deposition interferes with the establishment and growth of conspecific pollen on the stigma and can lead to a reduction in seedset (McLernon et al., 1996).

In addition to buffering populations against genetic drift, higher outcrossing rates could also facilitate population viability is offspring quality is important, for instance if

inbreeding depression is prevalent (Cheptou et al., 2000; Fox and Reed, 2010).

Specifically, local adaptation and offspring quality (e.g., higher germination rates and

survivorship) should be particularly important for the maintenance of populations in

stressful (defined as high temperatures and water stress) environments where the

potential for mortality and negative genetic effects may be exacerbated (Hauser and

Loeschcke, 1996; Heschel et al., 2005; Armbruster and Reed, 2005; Riginos et al., 2007).

Many plants that specialize on stressful environments have a suite of traits that are

thought to be adaptive (Grime, 1977; Chapin et al., 1993; Walck et al., 1999, 2002; Van

Zandt, 2007), for instance by increasing water-use efficiency and increasing survival

rates. The presence of these convergent traits across a broad taxonomic range implies that

specialization on habitats with stressful conditions may require a relatively narrow set of

traits that should be conserved across generations. Moreover, recruitment from more

suitable habitats cannot buffer populations in the stressful environment via source-sink

dynamics, since few if any other populations exist in more benign habitats. Therefore,

species that specialize on stressful habitats should have less plasticity in their traits and be

more tightly restricted to the local environment (Ellison and Parker, 2002; Pohlman et al.,

2005).

However, traits that are adaptive in stressful conditions may also reduce the

attractiveness of stress-adapted plant species and their ability to compete for pollinator

services. For instance, plants that are shorter and have lower reproductive output may

have greater fitness in high-stress environments, but they are less attractive to pollinating

insects (Dickson and Petit, 2006; Mevi-Schutz and Erhardt, 2005; respectively), which

could lead to a reduction in pollen receipt. While a small floral display (fewer flowers

open concurrently) may reduce within-plant movements and increase outcrossing rates, it may also reduce visitation rate, pollination success, and total seedset. In order to optimize their investment in reproduction under stressful conditions, specialist plants should exhibit traits that will reduce resource loss while increasing offspring quality. When offspring quality comes at a cost to total reproductive output, lower seedset in a stress-adapted plants reduce their ability to colonize novel sites (Kelly et al., 1996b; MacDougall et al., 2006; Soberón, 2007), and therefore may contribute to range restriction of stress-adapted specialists. By identifying the mechanisms that restrict species ranges and contribute to their extinction risk, we will be better able to predict which species are affected by future climate changes and focus limited resources toward conservation of the species most vulnerable to extinction.

In this experimental study, I manipulate temperature and plant-available water in order to test two hypotheses concerning the relationship between stress-adaptive traits, pollinator attraction traits, and reproductive success in three locally-abundant, regionally rare species (LARR) in comparison to a common congeneric species (CC). The first hypothesis states that LARR species are more resistant to abiotic stress (i.e., drought and temperature) and have traits associated with stress adaptation (via differential resource allocation). Specifically, I predict that the CC species will have a reduction in key physiological traits, such as relative growth rate and specific leaf area, with an increase in stress, and that the LARR species will not. The second hypothesis predicts that LARR species have floral traits that are associated with decreased attractiveness to pollinators but increased outcrossing rates, and the widespread species will have traits that are more attractive to a variety of pollinators. Finally, I test the prediction that offspring quality

will be higher in the stress-adapted LARR species than their CC species, which may facilitate offspring recruitment in stressful environments.

MATERIALS AND METHODS

Study Species

In order to assess the prediction that locally abundant, regionally rare (LARR) species are adapted stressful abiotic conditions, I will compare the response of three LARR plants (*Delphinium treleasei, Echinacea paradoxa*, and *Scutellaria bushii*) and their common congeneric species (CC; *D. carolinianum*, *E. pallida*, and *S. ovata*, respectively) to experimental manipulations of heat and water availability that are characteristic of the habitats to which they are endemic. These three LARR species are all endemic to the Ozark glades in Missouri and Arkansas, which are hot, xeric environments with thin soils and exclusively herbaceous cover, located within a matrix of more mesic woodland habitat (Nelson and Ladd, 1982; Baskin and Baskin, 1988; Templeton et al., 2001; Yatskievych, 1999). All three CC species also occur on glades but are not restricted to them, which will offer insight into the mechanisms that restrict the LARR species and not the CC species to glades. Comparing closely-related species also controls for the potential effect of evolutionary history on the vegetative and floral traits of LARR species, and the multiple comparisons across a range of evolutionary history make the results of this study more broadly applicable.

Experimental manipulations

The stress resistance of three LARR species and their CC species to drought and high heat conditions was estimated via greenhouse and growth chamber experiments. Individuals of each congeneric pair were exposed to a series of manipulated abiotic

36

conditions, in accordance with *in situ* field observations and optimized in the greenhouse, and their fitness responses were compared. Seeds collected in the field during Summer 2008 were cold stratified at 4°C for 90 days and allowed to germinate. Forty-four to 50 seedlings of each species were planted in a 3:2:1 ratio of Metromix360:Turface athletics:New Plant Life Topsoil mix, to approximate the relatively rapid draining soils associated with glades. Individual plants were allowed to establish for one week and randomly assigned to one of two treatment within two separate experiments, one that manipulated water availability (low and moderate water) and temperature regime (high and moderate temperature), with 22 to 45 replicates per treatment depending on the species pair (Table 3-1). Due to low growth rates of the *Delphinium* taxa, both the drought and temperature experiment were conducted twice in order to increase replication (from 64 to 133 individuals for the temperature experiment and 91 to 193 replicates for the drought experiment). For the second experiment, established individuals were first cold stratified, then removed from the coldroom, and exposed to the same treatments as above. Individuals were randomly arranged in order to reduce potential spatial variation in abiotic conditions, and the temperature regimes were rotated weekly between two environmental chambers to diminish a potential chamber effect.

Drought experiment—Watering regimes were designed to reach soil water-holding capacity and were applied three times per week. The *Delphinium* and *Echinacea* species were first planted in small (7.6 cm diameter) pots, in order to maintain appropriate soil-moisture levels and where transplanted into large pots (11.4 cm diameter) after the first 49 days of treatment. The amount of water administered per treatment scaled with pot size (i.e., 50 mL for small pots and 100 mL for large pots) in

order to account for the increased soil volume. The *Scutellaria* species grew faster and were planted directly into the larger pots. The *Delphinium* and *Echinacea* species require vernalization, which simulate winter conditions, before they will bolt and flower. Following 49 days of treatment, all individuals were placed in an environmentally controlled coldroom (4ºC) and remained un-manipulated for 49 days. The individuals were then removed from the coldroom and allowed 10 days to acclimate. Cold stratification was repeated until the individual began flowering or until they had experienced four cold treatments, after which all individuals were harvested. During Fall 2010, the water treatments were increased to 100 mL and 150 mL, respectively, for the *Echinacea* taxa due to low relative humidity in the greenhouse. Plants were fertilized once a week with Peters Professional 15-16-17 Peat-Lite Special at 150 ppm nitrogen dissolved in water to reduce the effect of resource limitation on plant traits. All individuals were given equal amounts of fertilizer (50 and 100 mL for small and large pots, respectively) and the additional water required for the control treatment was added before the individuals were watered with fertilizer water, in order to reduce loss of nutrients via leaching.

Temperature experiment—The temperature regime for each species pair was determined via field observations of temperatures on and off glades during peak activity of each genus (Table 3-1) and incorporated diurnal temperature change. The temperature regime for the *Echinacea* species was reduced from 30/38 ºC after the first round of treatment, because growth was minimal and mortality was relatively high. For clarity, I focus on the response of these species following the first round of treatment. Temperature regimes commenced after an acclimation period during which the air temperature was

incrementally increased (two or three degrees Celsius every 3 or 4 days, for a total of 12 days). Since the *Delphinium* and *Echinacea* taxa require vernalization before flowering, all individuals were placed in a coldroom for 49 days as above. Before cold stratification, the high temperature treatment was incrementally decreased and was subsequently increased before re-administering the experimental treatments upon removal from the coldroom. Cycles between treatment administration and cold stratification were repeated until flowering commenced or for up to four cold treatments, after which all individuals were harvested. Plants were watered with fertilizer once a week in order to reduce the potential effect of resource limitation on plant traits.

Morphological measurements

Vegetative traits—In order to assess the response of the LARR and CC species pairs to the above treatments, vegetative and reproductive traits were quantified. Plant height, number of stems, and vegetative output (i.e., leaf size and number) were noted weekly. Three leaves per individual were tagged upon emergence, and leaf death noted in order to calculate leaf turnover. Leaf thickness was measured for up to five leaves per individual and averaged. In the drought experiment with the *Delphinium* species, many of the leaves were relatively small and fragile, and leaf thickness could not be measured without damaging the leaves; therefore, I could not test for differences in leaf thickness for this experiment.

Following fruit maturation, final morphological measurements were taken (e.g. final height, number of leaves, leaf thickness and trichome density), and plants where harvested, separated into vegetative, reproductive, and below ground biomass. One leaf per plant was removed, digitized for leaf area quantification in SigmaScan Pro 5.0

39

(SigmaScan Pro 5.0, 2002). The roots were washed thoroughly to remove soil particles, and all plant material was dried at 40-50ºC for no less than 48 hours and weighed. Total biomass, relative growth rate (total biomass divided by the age of the plant in days), leaf longevity, shoot to root ratio, and specific leaf area (SLA; the ratio of leaf area and dry mass) were quantified. Specific leaf area is a measure of leaf density; lower SLA indicates a more dense leaf, which is associated with lower evapotranspiration and photosynthetic rates. Relative reproductive output was calculated as the mass of reproductive material divided by total biomass (the sum of reproductive, shoot, and root biomass).

Reproductive traits—In order to simulate pollination and ensure seed set, I applied outcross pollen to three flowers per individual per week during blooming for the *Delphinium* and *Scutellaria* taxa and up to twenty florets for *Echinacea* species. Following maturation, the fruits were harvested. Due to morphological difference among genera, some traits, such as reproductive success, were quantified differently for each congeneric pair (see below).

Delphinium *congeners*

Vegetative traits—The *Delphinium* species have roughly round leaves that vary in the degree to which they are dissected. Therefore, I visually estimated the proportion of a circle filled by the leaf (i.e., 0.01, 0.25, 0.33, 0.5, 0.67, 0.75, and 1), and estimated the radius of the circle as the length of the leaf from the center (where the petiole and leaf blade merge) to the edge. Leaf area was then calculated as pi times the radius squared (π x (length of leaf blade)^2 x proportion of circle). Throughout the experiment, biomass was

estimated in a non-destructive manner as total leaf area (leaf number multiplied by estimated leaf area).

Reproductive traits—Individuals were checked three times a week for initiation of flowering, and reproductive phenology was defined as the age of the individual at the time of inflorescence initiation. Three flowers per plant were tagged while still in bud and monitored three times per week until flower senescence in order to determine flower longevity. At peak flowering, I measured the floral traits (i.e., sepal width, sepal height, floral spur length, and anther-stigma separation) of three flowers for each individual. Corolla area was calculated as the product of sepal area and height, and anther-stigma separation was measured from the top of stamen to the stigma of the lowest ovary. Nectar was collected from up to nine flowers per individual, and nectar volume was calculated. Nectar sugar content was determined using a Brix refractometer, and floral output was quantified as the number of flowers produced.

Reproductive success—Following the collection of fruits, the number of fruits that developed seeds and the number seeds produced per pollinated flower were quantified. Total seedset was calculated as the product of average seedset per pollinated fruit and total floral output. In order to estimate seed quality, up to thirty seeds per individual were weighed, and mass per seed was calculated.

Echinacea *congeners*

Vegetative traits—Throughout the experiment, biomass was estimated in a non-destructive manner as total leaf area (the product of leaf number and estimated leaf area) for the *Echinacea* species. Leaf area was calculated as the width times the length of an average leaf blade. Two individuals had an additional cold treatment and growth period

41

after the inflorescence senesced and were collected; none of the data collected after the inflorescences senesced (e.g., biomass measures) were used in the analyses.

Reproductive traits—Individuals were checked three times a week for initiation of inflorescence, and reproductive phenology was defined as the age (days) of the individual at the time of initiation. Corolla area was calculated as the number of ligules multiplied by the area of an average ligule size (i.e., the product of ligule width and length). The number disk florets were counted, and nectar volume and sugar content were quantified for three florets per sexual stage (i.e., male and female), since *Echinacea* species are protandrous. Few individuals of *E. paradoxa* bloomed in the temperature experiment; therefore, nectar production and quality could not be compared.

Reproductive success—Since *Echinacea* species are known to produce unfertilized capsules that collapse when pressure is applied, the pollinated capitula were gently pressed with forceps to confirm fertility. Seeds that did not collapse under the small amount of applied pressure were assumed to be fertile. Total reproductive success was quantified as the proportion of capsules that were fertile times the total number of florets produced. A virus infected some individuals resulting in the senescence of their inflorescences, and these individuals were excluded from the analyses of floral output and reproductive success. However, they were used in analyses of initiation of inflorescence, relative reproductive output, and total biomass.

Scutellaria *congeners*

Vegetative traits—Due to a large number of leaves and high variability of leaf size, the non-destructive estimate of biomass for the *Scutellaria* taxa was quantified as total stem length. One leaf per individual was collected, and the number of trichome was

counted in each of three, three by three millimeter squares haphazardly placed in upper third, middle third, and lower third of the leaf. Trichome density was quantified as the total number of trichomes per 27 mm^2.

Reproductive traits— Individuals were checked three times a week for initiation of flowering, and reproductive phenology was defined as the age of the individuals in days at the time flowering commenced. Three flowers per plant were tagged while still in bud and monitored three times per week until flower senescence in order to determine flower longevity. At peak flowering, I measured the floral traits (i.e., petal width, petal depth height, floral tube length) of three flowers for each individual. Floral output was quantified as the number of flowers produced. The LARR *S. bushii* produced only cleistogamous flowers, which do not open, and all fruits were fertilized internally. Therefore, I did not pollinate any flowers of either species in order to maintain a balanced comparison. All measures of reproductive success are based on self-pollinated fruits and should be interpreted with caution.

Reproductive success—During the breakdown process, the number of seeds per fruit was counted for ten fruits and averaged. Total seedset was calculated as the average number of seeds per fruit multiplied by total floral output, and offspring quality was determined via germination trails. Up to twenty and ten seeds for the drought and temperature experiments, respectively, were cold stratified for 90 days, placed in an environmentally-controlled chamber (21ºC), and allowed to germinate. Replication was different between the two experiments due to differences in total seedset, and replication of germination rates for the temperature experiment was biased (44 to 4, control to high temperature respectively) due to low fruit set in the high stress treatment. Since total

seedset could not be accurately quantified, I estimated total reproductive success as the proportion of fruits to set seed.

Statistical analyses

In order to control for multiple comparisons among congeners and treatments, vegetative traits of the *Delphinium* species were analyzed via two-way, nested MANOVA with Species and Treatment as factors and treatment nested within Experiment in order to account for replicate experiments. Only one individual of *D. trealeasi* (LARR) bolted in first experiments (i.e., the one without a cold treatment before manipulations commenced); therefore, the floral traits of the *Delphinium* taxa were analyzed for the second experiment only, and the experiment effect was removed. The morphological traits of *Echinacea* and *Scutellaria* taxa were tested via two-way MANOVA with Species and Treatment as factors. Since not all individuals flowered, analyses of vegetative and reproductive traits were conducted independently in order to account for the loss in replication that occurred. Some traits were only quantified for a subset of individuals, which would have decreased the replication of the MANOVA substantially and were analyzed separately. Those traits that were analyzed independent of the others, and therefore do not account for multiple comparisons, are indicated in the results section and corresponding results table (Table 3-2). Two traits of the *Delphinium* species had an exponential distribution in the temperature experiment and were analyzed via general linear model using the reciprocal link function in JMP Statistical Software (SAS Institute 2009).

Individuals were also ranked by whether or not they initiated inflorescences, and differences among congeners and treatments were tested via two-way logistic regression

with Species and Treatment as factors using JMP Statistical Software (SAS, 2009). Analysis of the *Delphinium* species accounted for replicate experiments as above. The nectar sugar content of the *Delphinium* species often reached the maximum of the Brix refractometer, which truncated the continuous variable at 50%. Therefore, I ranked nectar sugar content based on concentration (i.e., if the sugar concentration was greater than or equal to 50 percent then it was coded as one and if it was less than 50 percent it was coded as 0), and logistic regression was used to test for differences among treatments and congeners as above.

All data were transformed for normality, and if they could not be transformed, the analyses were repeated using permutation ANOVA (*aovp* in the R Statistical Package (R Development Core Team 2008)). I used permutation ANOVA in order to incorporate the nested experimental design of the *Delphinium* species into a nonparametric framework. Permutation ANOVA is a Monte Carlo resampling technique (without replacement) that estimates a null expectation based on a random resampling of the available data and compares each of 5000 runs to the null. Statistical significance is quantified as the number of runs for which the F-statistic is greater than or equal to the observed value.

RESULTS

Delphinium *congeners*

Drought experiment—Only three *D. carolinianum* and no *D. treleasei* individuals bolted and flowered in the precipitation experiments combined, despite the large number of individuals (193) and duration of the experiment (175 treatment days, 322 days including cold stratification events). Therefore, only vegetative traits could be analyzed. Relative growth rate, total biomass, and shoot to root ratio were lower in the LARR *D.*

treleasei in comparison to its CC congener (Table 3-2), as I predicted. However, there was no effect of treatment or an interaction between treatment and species in any trait measured, which suggests that there is no adaptive benefit to these traits in response to drought. No leaf traits (i.e., leaf area, specific leaf area, total leaf area, and leaf longevity; Table 3-2) differed between species or treatments.

Temperature experiment—The *Delphinium* congeners differed in their responses to stress, and the LARR, *D. treleasei*, was less negatively affected than its congener, in congruence with my hypothesis that the LARR species are more stress resistant. *Delphinium carolinianum* (CC) had lower relative growth rate and total seedset in the high stress treatment, but *D. treleasei* did not differ between treatments (Table 3-2; Figure 2-1A and C, respectively). Temperature regime had a significant effect on some traits in both *Delphinium* species, suggesting that they are either conserved, linked to evolutionarily stable traits, or do not represent an adaptive benefit in these plants. Height, total biomass, and shoot to root ratio were significantly lower in the high temperature treatment than the control treatment for both species (Table 3-2). In addition, the number of flowers open and floral display size decreased with an increase in temperature. Leaf longevity, on the other hand, was higher (Table 3-2), and relative reproductive output was lower in the high temperature treatment, as predicted by stress theory.

The *Delphinium* congeners differed in some vegetative and reproductive traits, regardless of temperature treatment. As I predicted, *D. treleasei* (LARR) was shorter, had thicker leaves, and produced fewer flowers total (Table 3-2). *Delphinium treleasei* also had fewer flowers open concurrently and higher nectar sugar content, which may affect outcrossing rates and offspring quality if pollinator behavior is affected. The difference in

the number of flowers open concurrently is not driven by reduced floral output *per se*, since the proportion of flowers open was marginally lower for *D. treleasei* than *D. carolinianum*. On the other hand, anther-stigma separation was lower in the LARR *D. treleasei* (Table 3-2), which I predicted to be higher in order to increase the potential for outcross pollination. Moreover, the longevity of *D. treleasei*'s leaves and flowers was shorter in comparison to *D. carolinianum*. I predicted that leaf and flower longevity would be higher in the LARR species in order to reduce loss of resources and increase the potential for outcross pollination, respectively. There were no or marginal differences in some leaf traits (leaf area, specific leaf area, and leaf longevity; Table 3-2) and some floral traits (inflorescence initiation, corolla area, and floral spur length; Table 3-2).

Echinacea *congeners*

Drought experiment—The *Echinacea* species did not differ in their responses to watering regime, contrary to my predictions. Vegetative and floral traits did not differ between species and treatments indicating the *E. paradoxa* is not more locally-adapted or specialized than its CC congener. Both species had shorter stems, lower relative growth rates, reduced total biomass, and fewer florets in the drought treatment than the control treatment (Table 3-2), indicating that the drought treatment was inducing a stress response.

Echinacea paradoxa (LARR) had thinner leaves and greater reproductive output (number of disk florets) than its CC, *E. pallida*, contrary to my prediction that LARR species would have thicker leaves and fewer florets. Moreover, *E. paradoxa* (LARR) bloomed later than *E. pallida* (CC). There were no differences in several traits (i.e., leaf area, leaf longevity, shoot to root ratio, corolla area, relative reproductive output,

reproductive phenology, total seedset, and the proportion of pollinated florets that were viable; Table 3-2), which suggests that these traits are either evolutionary conserved or both species are adapted to local conditions.

Temperature experiment—One trait, specific leaf area (Table 3-2), differed between species in response to the temperature treatments. *Echinacea paradoxa* (LARR) had higher specific leaf area (SLA) than *E. pallida* in the control treatment, but there was no difference in the high temperature treatment (Table 3-2; Figure 3-2A). This difference in SLA suggests that *E. paradoxa* responds to stress by reducing water-loss and photosynthetic rates, and *E. pallida* (CC) has an increase in evapotranspirative cooling, which results in similar SLA in the high stress environment.

Temperature treatment had a significant effect on leaf area, relative growth rate, and total biomass (Table 3-2), which indicates that the treatments were effective and that evolutionary history may be influencing the response of these species to an increase in temperature. Probability of flowering was the only trait that differed between species (Table 3-2), with *E. pallida* (CC) being more likely to flower than *E. paradoxa* (Table 3-2; Figure 3-2B). However, it is important to note that few individuals bolted and flowered, and the majority of them were *E. pallida* (CC; 17 of 20), which decreased the power with which to test for differences among species and treatments.

Scutellaria *congeners*

Drought experiment—*Scutellaria bushii* (LARR) and *S. ovata* responded differently to the watering regimes, and *S. bushii* was more resistant to drought stress, confirming my hypothesis of greater resistance in the LARR taxa. *Scutellaria ovata* (CC) had a reduction in height (Figure 3-3A), relative growth rate, and total seedset (Figure 3-

3B), but *S. bushii* did not (Table 3-2), which resulted in statistically significant differences between taxa in the control treatment only. In other words, the *Scutellaria* congeners did not differ in these traits under drought conditions. Reproductive phenology was only different between the congeners in the control treatment; *S. bushii* bloomed significantly later and was more variable than *S. ovata*. There was no difference between species across the drought treatments.

Total biomass and total floral output decreased for both species with a decrease in water availability (Table 3-2), though the response was more substantial in *S. ovata* (CC) than *S. bushii* (LARR). Both responded similarly to a decrease in water availability with a shift in resource allocation, specifically shoot to root ratio was lower for both species in the drought treatment in comparison to the control treatment. Leaf thickness (Table 3-2) was significantly lower and relative reproductive output (Table 3-2; Figure 3-3C) was higher in *S. bushii* than *S. ovata* (Table 3-2), contrary to my predictions. On the other hand, trichome density (Table 3-2) and germination rate (Table 3-2; Figure 3-3D) of *S. bushii* were higher than its common congener, *S. ovata*, corroborating my hypothesis of greater stress-adaptation and offspring quality in the LARR species.

Temperature experiment—The *Scutellaria* congeners responded differently to the temperature treatments, but the direction of those differences varies by trait. In congruence with my hypothesis, trichome density of *S. bushii* was higher than *S. ovata* and did not vary with temperature treatment. *Scutellaria ovata*, on the other hand, had an increase in trichome density with an increase in stress. Total floral output was lower for both species in the high temperature treatment, but the reduction was much larger in *S. ovata* (CC) than *S. bushii*. On average, *S. ovata* had a reduction of 388 (72.9%) flowers,

49

and *S. bushii* had 58 (59.2%) fewer flowers in the high stress treatment. Both species also had a decrease in height with an increase in temperature, resulting in the height of *S. ovata* in the high temperature treatment equaling that of *S. bushii* in the control treatment. Contrary to my predictions, specific leaf area, relative growth rate, and total biomass were not different between temperature treatments for *S. ovata* but were for *S. bushii* (LARR), which had a reduction in all three traits with an increase in temperature.

In response to an increase in temperature, both species had lower leaf area, were less likely to bloom, produced fewer seeds, and had lower relative reproductive output, which suggests that more resources are being allocated to survival rather than reproduction under high stress conditions. Some vegetative and floral traits differed between the taxa, which supports a role for trait differences in the maintenance of species range size. Leaves of *S. bushii* (LARR) were smaller (had lower leaf area) and thinner than *S. ovata*, offering contradictory evidence for stress adaptation in the LARR species. Relative reproductive output and germination rate was greater for *S. bushii* than *S. ovata*, implying that offspring quality may be important in order to specialize on these restricted habitats. *Scutellaria bushii* bloomed significantly earlier than *S. ovata* (Table 3-2).

<div align="center">DISCUSSION</div>

The relative importance of abiotic and biotic mechanisms in determining species ecogeographical distributions is a central and critical topic in ecology. Here, I document the potential for both factors to act synergistically in three locally-abundant, regionally-rare (LARR) species. The LARR species were less responsive to experimental stress in key physiological traits, and two of the three differed from their common congeneric (CC) species in floral traits and offspring quality, which confirms my hypothesis that the

<div align="center">50</div>

trade-off between resource allocation to vegetative and floral traits differs in LARR and CC species.

Each of the LARR species was more resistant to high stress environments in comparison to their widespread congeners, despite a stress response in both species, and only one CC species (*S. ovata* in the temperature experiment) had any traits that were less responsive than the LARR species. While all species were negatively affected by drought or high temperature conditions, the LARR species had fewer traits negatively affected by an increase in stress, particularly those important for water conservation. For instance, the relative growth rate (RGR) of *Delphinium treleasei* (LARR) did not differ between temperature treatments, but *D. carolinianum* had a significant reduction in RGR with an increase in stress (Figure 3-1A). Relative growth rate (RGR) is thought to be adaptive in stressful environments as it is associated with low photosynthetic rates and high water-use efficiency. Relative growth rate of *D. treleasei* was also lower than *D. carolinianum* in the precipitation experiment, which corroborates my prediction of stress-adaptation.

Similarly, the impact of stress on specific leaf area differed between the *Echinacea* species and potentially reflects two strategies for responding to abiotic stress. *Echinacea paradoxa* (LARR) produced more dense leaves (lower SLA) in the high stress environment, which is associated with reduced water loss, lower RGR, and lower resource turnover. *Echinacea pallida*, on the other hand, produces leaves with higher SLA in the high temperature conditions, which may reflect an increase in evaporative cooling. Contrary to my predictions, *E. pallida* was more likely to bolt and had fewer florets than *E. paradoxa* (Figure 3-3 B and C, respectively) in the precipitation experiment, which suggests that *E. pallida* has an accelerated life cycle and lower total

51

reproductive output in comparison to *E. paradoxa*. However, these *Echinacea* species are perennial and live longer than the duration of this study. Therefore, I could not test for this mechanism.

The *Scutellaria* species also differed in their response to an increase in stress, and *S. bushii* was generally more resistant to stress. For instance, *Scutellaria ovata* (CC) was shorter in the drought treatment in comparison to the control treatment, and *S. bushii* had no change in height (Figure 3-3A), which is associated with stress-adaptation. This pattern was consistent in several other traits (e.g., total biomass, relative growth rate, etc.), further corroborating my hypothesis that LARR species are more resistant to stress. Height is also an important attraction trait for many insect species and may result in higher competitive ability of *S. ovata* for pollinator services in less stressful habitats. In the high stress environment, there was no height difference between *S. ovata* and *S. bushii*, indicating that the competitive advantage of height may be reduced. The results from the temperature experiment with the *Scutellaria* species are less clear, with some traits differing in the direction I predicted and others in the opposite direction, particularly the vegetative traits. This variation in response to stress between temperature and drought stress may reflect the different physiological mechanisms that underlie resistance to these traits or it may reflect the simplicity of the experimental design. In order to pin-point which abiotic stressor dominated species responses given limited space, I conducted independent experiments and did not cross the water and temperature treatments. However, a combination of abiotic factors is likely contributing to the unique conditions on glades in concert. The rocky substrate and extreme abiotic conditions on glades make *in situ*, population-level, field manipulations difficult, and the establishment

of individuals from the species of interest is unreliable. Therefore, future studies should combine manipulations of abiotic factors in common garden experiments with *in situ* observational studies that document both biotic and abiotic factors at the individual level. The combination of common garden experiments and observational studies will contribute to a more biologically realistic understanding of abiotic and biotic factors in determining the reproductive success and population growth of LARR and CC species.

Key floral attraction traits also differed between congeners in these experiments, as I predicted if resources for reproduction are allocated differently in LARR and CC species. *Delphinium carolinianum* was significantly taller than its LARR congener and could be a better competitor for pollinator services, if height dominates attractiveness. *Delphinium treleasei* (LARR) had fewer flowers open concurrently, which is less attractive to pollinators but often increases outcrossing rates via a reduction in within plant movement by the pollinator. While only marginally significant, the flowers of *D. treleasei* were larger than *D. carolinianum*, suggesting that the resources committed to reproduction are allocated to fewer, larger flowers. Previous research indicates that *D. treleasei* has fewer, larger flowers in the field and that they attract more specialized pollinators (Miller-Struttmann, Chapter 1). Nectar sugar concentration was also higher in *D. treleasei* than *D. carolinianum*, but there was no difference in nectar volume, which indicates that more concentrated nectar reward may be an important attraction trait for the species that specialize on water-limited habitats. Many nectar-collecting insects can detect nectar quality and choose flowers accordingly (Wilson et al., 2006), resulting in preferential visitation to those species with the greater reward. Moreover, higher sugar concentration is advantageous in stressful environments where water is limiting and may

compensate for the reduction in floral display. On the other hand, greater viscosity may make the nectar of the LARR species more difficult remove from the flower and may reduce the number of species able to extract the reward. Choice trials should be conducted with known pollinators of these species to determine if nectar sugar concentrations consistent with field measurements affect rate of nectar removal and the number of species able to capitalize on the resource. The LARR species, *S. bushii*, did not produce chasmogamous flowers, and I could not quantify floral attraction traits for this species. However, relative reproductive output was higher in *S. bushii* (LARR), contrary to the prediction of stress-resistance, but in congruence with my prediction that higher-quality offspring, which require greater resource investment, are required in order to species to survive and specialize on stressful habitats.

The measures of reproductive success in *D. treleasei* and *S. bushii* (LARR) documented here confirm my prediction that LARR species have fewer, higher-quality offspring, which may increase survival rates and population viability in more stressful habitats. Both LARR species had significantly higher offspring quality (i.e., seed mass and germination rate, respectively). Conversely, total seedset of both CC species (*D. carolinianum* and *S. ovata*) was significantly higher in the control treatments but was equivalent to their LARR congeners in the high stress treatments. The difference in total seedset between LARR and CC species in the control treatment may reflect an important role for propagule pressure in determining species ranges. High propagule pressure is thought to contribute to range expansion in some species by reducing recruitment limitation and increasing the number of individuals and genotypes with the potential to establish in a new habitat (Kelly et al., 1996; MacDougall et al., 2006; Soberón, 2007). In

less stressful habitats, the higher seedset of the CC species may allow it to establish more quickly than the LARR species, increase in population size, and/or outcompete it for abundant resources. However, in stressful habitats, high offspring quality may enhance survival and allow adaptation to local conditions, thus reinforcing the pattern of range restriction to specific, stressful abiotic conditions seen in these LARR species.

Given the rate of current climatic change and the prevalence of entomophilous pollination, understanding the relative importance of biotic and abiotic mechanisms in determining the biogeographical ranges of species is imperative. Few studies consider biotic and abiotic mechanisms in concert, despite a long history of inquiry into both, independent of one another. Future studies should work to understand how these mechanisms interact and in which cases they will predominate, rather than regard them as mutually exclusive. While biological information is lost by reducing continuous traits into categories, the seven forms of rarity offer a framework through which to make and test predictions concerning which mechanisms are most relevant for species with similar distributions. In this study, I explored the potential interaction between abiotic stress and reproductive allocation in three regionally-rare, habitat-specialist species, which are characterized by a type of rarity that is often ignored in the discussion of rarity and found that both may contribute to the restriction of their ranges to glade habitats. These results imply an important role for pollination biology in mediating the interaction between the stress-adaptation and reproductive success in stressful environments. In an era of increasing anthropogenic impacts on natural habitats, a more in-depth and nuanced understanding of the determinants of species biogeographical ranges will create more efficient and hopefully more effective conservation strategies.

Table 3-1. A description of the temperature regimes, number of cold stratifications required to induce flowering, and the floral traits that were quantified differently for each congeneric pair. Temperature regimes are based on field collected data, and reflect the night and day time temperatures, respectively. Temperatures were gradually changed manner over two hours in order to reduce heat shock. The *Echinacea* species had a temperature regime of 28/30ºC for the first application of the treatment. However due to slow growth, the temperature regime was changed to 27/35ºC, and analyses did not include data recorded from the first treatment application.

	Temperature Regime (low/high)	*Replication (Drought/Temp)*		*No. cold treatments*	*Corolla area (CA)*	*Display size*	*Floral output*	*Reproductive success*
		Total	Bolted					
Delphinium	21/29 ºC	193/133	0/62	0 - 2	Sepal width x height	CA x no. flowers open	No. flowers	Mass per seed
Echinacea	27/35 ºC	89/94	36/20	3 - 4	Ray petal width x length	CA x no. ray petals	No. florets	Fertilized florets: Pollinated florets
Scutellaria	28/38 ºC	90/103	87/70	0	Petal width x height	CA x no. flowers open	No. flowers	Germination rate

Table 3-2. Statistical results from analyses of vegetative traits, floral traits, and reproductive success between three congeneric species pairs and in response to experimental manipulations of water availability and air temperature. Analyses of the experiments with the *Delphinium* species have an additional term (Treatment nested within Experiment), since each experiment (drought and temperature) was repeated in order to increase replication. Few individuals (3) bolted in the drought experiment with the *Delphinium* species, and only vegetative traits could be analyzed. Significance values are indicated by symbols († denotes $P < 0.1$, * indicates $P < 0.05$, ** signifies $P < 0.01$, and *** symbolizes $P < 0.001$) and bolded. Some traits did not meet the assumption of normality for parametric tests, and a non-parametric resampling procedure (permutation ANOVA) was used to assess differences between species and treatments. When results of the parametric and non-parametric analyses differed, the significance levels from the non-parametric tests are indicated in parentheses, and those that are significant are bolded.

ø denotes that analyses were run independent of the other traits (i.e., not corrected for multiple comparisons) due to low replication of that trait.

‡ signifies analyses that were conducted using data from one of the two experimental replications (*Delphinium* species height and reproductive traits only)

• indicates that the analyses were conducted with data from both replicate experiments for the *Delphinium* temperature experiment.

^ signifies that the data have an exponential distribution and were analyzed independent of the other variables via general linear model using a reciprocal link function to transform the data.

º denotes that analyses were run independent of the other traits using logistic regression.

	Drought			Temperature		
	Species	*Treatment x Experiment*	*Species x Treatment*	*Species*	*Treatment x Experiment*	*Species x Treatment*
Delphinium						
Vegetative traits						
Height[‡]	--	--	--	**21.52*****	**10.53****	0.006
Leaf thickness	--	--	--	**13.43*****	2.88[†]	1.69
Leaf area[ø]	2.18	0.11	0.34	3.43[†]	2.86[†]	0.98
Specific leaf area	0.39	0.11	0.49	0.09	1.12	1.25
Total leaf area	0.30	1.23	0.62	--	--	--
Leaf longevity	1.48	0.092	0.031	(0.27)	(0.0627)	(0.84)
Relative growth rate[^]	**(0.0014)**	(0.41)	(0.92)	1.05	**6.53***	**5.24***
Shoot:Root ratio	**(<0.0001)**	(0.12)	(0.65)	**(0.0196)**	(0.094)	(0.34)
Total biomass[^]	**(0.0008)**	(0.87)	(0.96)	0.057	**11.41*****	1.04
Reproductive traits[‡]						
Inflorescence initiation[ø•]	--	--	--	0.89	2.96	1.30
Anther-stigma separation	--	--	--	**8.91****	0.005	1.03
Corolla area	--	--	--	3.04[†]	3.45[†]	1.11
Display size	--	--	--	3.09[†]	**6.62***	0.70
Floral output	--	--	--	**5.75***	**9.21***	0.29
Floral spur length	--	--	--	0.03	0.44	0.66
Flower longevity	--	--	--	**13.01****	2.24	1.17
Nectar volume	--	--	--	0.06	1.60	1.94
Nectar sugar content[ø]	--	--	--	**5.15***	**5.21***	0.66
No. flowers open	--	--	--	**11.88****	**4.70***	0.68
Proportion of flowers open	--	--	--	3.26	0.25	0.04
Relative reproductive output	--	--	--	0.58	**5.60***	0.63
Reproductive phenology	--	--	--	(0.90)	(0.10)	(0.077)
Reproductive success[‡]						
Seed quality	--	--	--	**13.80*****	**9.24****	1.49
Total seedset	--	--	--	(0.11)	(0.093)	**(0.051)**
	Species	*Treatment*	*Species x Treatment*	*Species*	*Treatment*	*Species x Treatment*
Echinacea						
Vegetative traits						
Height	3.09[†]	**17.05****	0.16	0.59	2.90	0.46
Leaf area	0.07	2.31	1.70	0.14	0.35	0.43
Leaf thickness	**12.42****	0.85	0.19	0.00	**5.44***	0.61
Leaf longevity	1.58	1.79	0.11	2.05	3.72[†]	1.80
Specific leaf area	1.39	0.06	0.22	3.83[†]	0.70	**6.83***
Relative growth rate	**6.69***	**13.85****	0.05	(0.18)	**(<0.0001)**	(0.67)

Total biomass	2.03	**16.61****	0.18	(0.43)	**(<0.0001)**	(0.42)
Shoot:Root ratio	(1.00)	(0.12)	(0.58)	(0.50)	(1.00)	(0.57)
Reproductive traits						
Inflorescence initiation°	1.00	1.91	0.61	**15.06****	0.01	0.96
Corolla area	0.16	1.63	0.02	0.00	2.75	0.07
Floral output	**19.15****	**12.69****	0.09	0.03	0.01	0.34
Nectar sugar content	0.03	0.40	0.01	--	--	--
Nectar volume	1.69	0.80	0.07	--	--	--
Relative reproductive output	(0.11)	(0.066)	(1.00)	0.21	1.16	1.35
Reproductive phenology	**(0.0261)**	(0.18)	(0.94)	0.45	1.87	4.51†
Reproductive success						
Proportion fertile capitula	0.58	1.63	0.58	0.60	0.10	0.44
Total seedset	0.60	3.16†	0.12	1.34	0.06	0.79
Scutellaria						
Vegetative traits						
Height	**23.61*****	**22.52*****	**4.33***	**198.2*****	**202.0*****	**29.03*****
Leaf area	**1158*****	**13.5****	1.97	**146.3*****	**26.47*****	2.11
Leaf longevity°	--	--	--	2.28	1.0	0.01
Leaf thickness	**308.0*****	0.01	0.07	**80.75*****	0.11	1.90
Shoot:Root ratio	0.61	**4.92***	0.75	0.04	0.33	2.18
Specific leaf area	0.20	1.88	0.24	0.42	3.02†	**5.24***
Trichome density	**12.17****	0.37	1.36	**27.71*****	1.83	**11.00****
Relative growth rate	**239.2*****	**35.51*****	**14.94****	**(<0.0001)**	**(<0.0001)**	**(0.0136)**
Total biomass	**(<0.0001)**	**(<0.0001)**	**(<0.0001)**	**(<0.0001)**	**(<0.0001)**	**(<0.0001)**
Reproductive traits						
Inflorescence initiation°	--	--	--	1.71	**33.77*****	0.02
Floral output	**101.6*****	**148.9*****	**54.97*****	(0.53)	**(<0.0001)**	**(<0.0001)**
Relative reproductive output	**7.94****	2.28	0.69	(0.058)	**(<0.0001)**	(0.34)
Reproductive phenology	(0.14)	(0.96)	**(0.0233)**	**5.13***	1.32	2.55
Reproductive success						
Total seedset	**79.25*****	**49.91*****	**6.15***	--	--	--
Total fruit set	--	--	--	2.10	**3.20****	0.07
Germination rate	**31.31*****	0.96	0.03	**(0.0286)**	(0.093)	(0.57)

Figure 3-1. Vegetative and reproductive traits of a locally-abundant regionally rare species (LARR), *Delphinium treleasei,* in comparison to its common congener*, D. carolinianum* and in response to experimental temperature treatments, (A) relative growth rate (mg/day; square root transformed), (B) total potential seedset (average number of seeds per fruit times the total number of flowers; square root transformed), (C) floral output (number of flowers; log$_e$ transformed), and (D) average seed mass (mg). Statistically significant differences between treatments and species in panels (A) and (C) are designated by having different letters (those that share letters are not significantly different). In panels (B) and (D), statistical significance is indicated by asterisks (* designates $P < 0.05$, ** indicates $P < 0.01$, and *** represents $P < 0.001$).
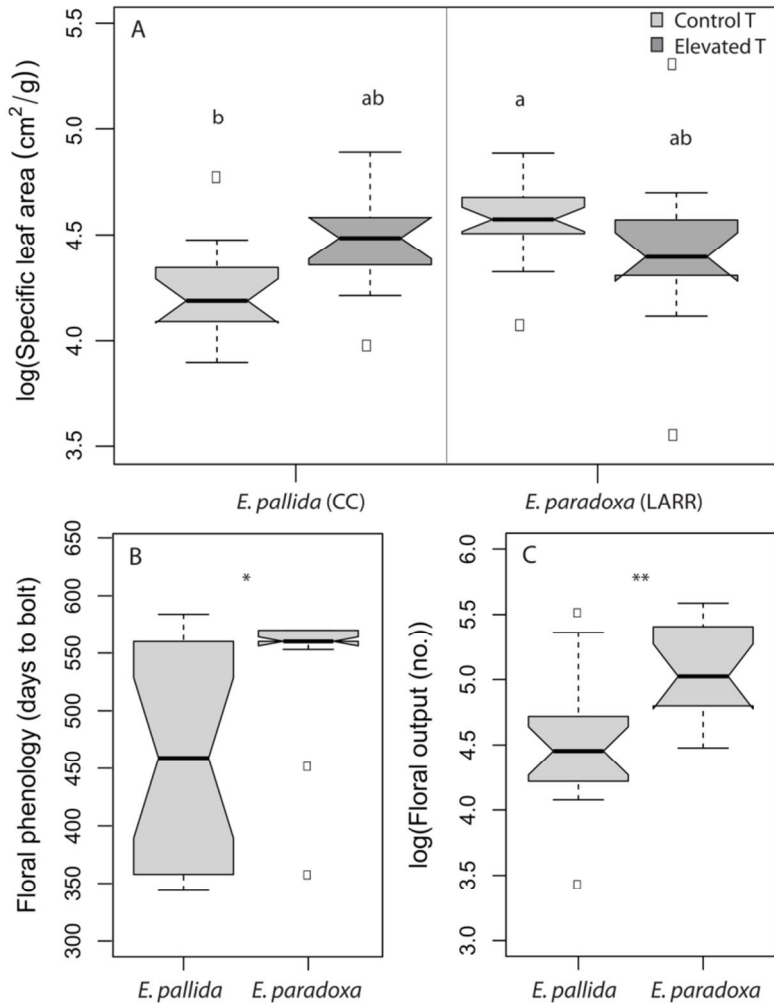
Figure 3-2. Vegetative and reproductive traits of *Echinacea paradoxa* (LARR) and *E. pallida* (CC) in response to temperature treatments and compared to each other (data represented in panels (B) and (C) are from the precipitation experiment), (A) specific leaf area (cm^2 per unit biomass; log$_e$ transformed), (B) floral phenology (age in days at date of inflorescence initiation), and (C) floral output (number of florets; log transformed). Statistically significant differences between treatments and species in panels (A) and (C) are designated by having different letters (those that share letters are not significantly different). In panels (B) and (D), statistical significance is indicated by asterisks (* designates $P < 0.05$, ** indicates $P < 0.01$, and *** represents $P < 0.001$).

Figure 3-3. Vegetative and reproductive traits of *Scutellaria bushii* (LARR) in comparison to *S. ovata* (CC) and in response to watering treatments, (A) height (cm), (B) relative reproductive output (square root transformed), (C) total potential seed set (average number of seeds per fruit times total floral output), and (D) germination rate. Statistically significant differences between treatments and species in panels (A) and (C) are designated by having different letters (those that share letters are not significantly different). In panels (B) and (D), statistical significance is indicated by asterisks (* designates *P* < 0.05, ** indicates *P* < 0.01, and *** represents *P* < 0.001).
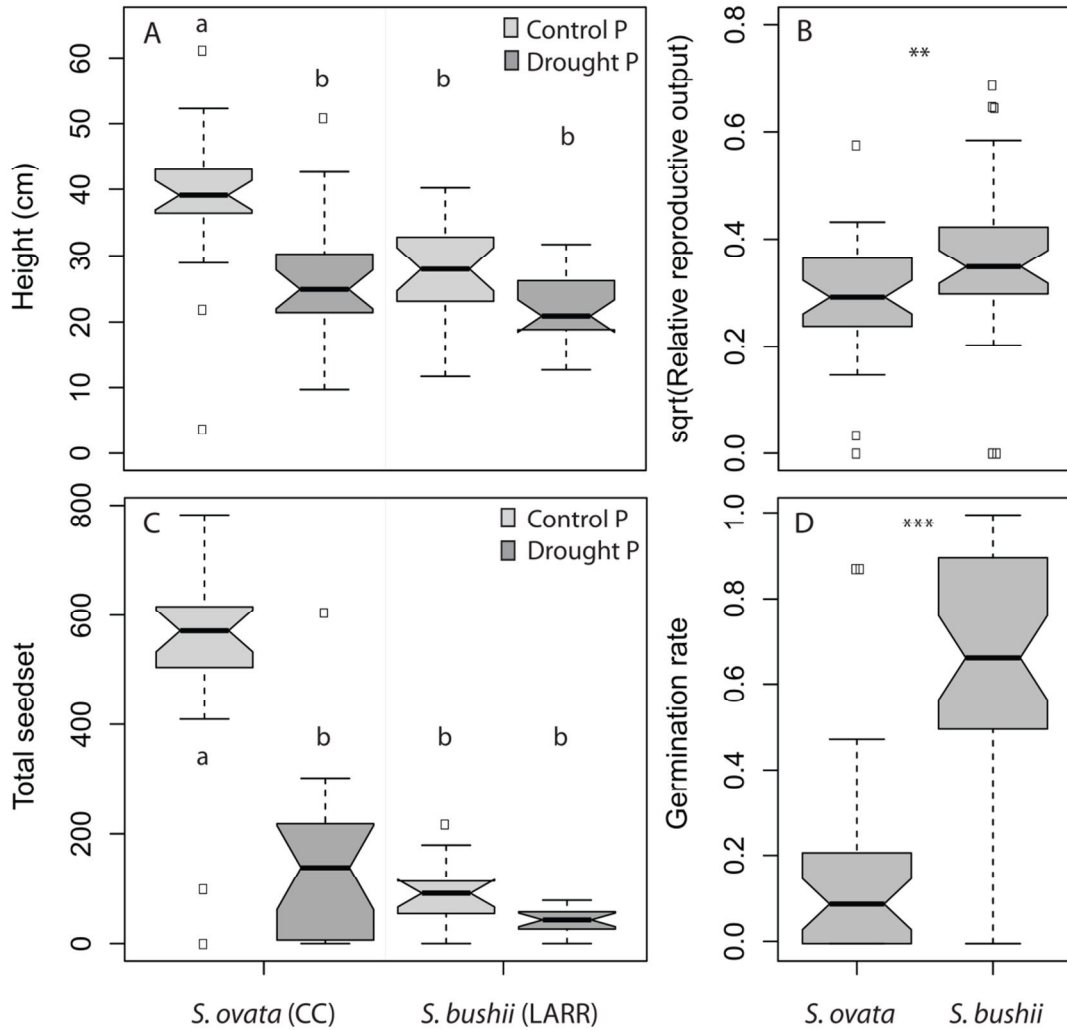
CHAPTER FOUR

Pages 63 to 87

SPATIAL VARIATION, NOT COMPETITION, AFFECTS POLLINATION SUCCESS

IN TWO ENDEMIC GLADE SPECIES

INTRODUCTION

A primary tenet of ecology is the role of competitive exclusion in determining species ranges (Tilman, 1976; Chase et al., 2002; Chase and Leibold, 2003; Fargione et al., 2003; Lau et al., 2008; Angert, 2009), although some studies challenge this tenet (Volkov et al., 2003; Muneepeerakul et al., 2008). Historically, the theory of competitive-exclusion has been conceptualized in terms of abiotic resources (Hardin, 1960; Tilman, 1976; Chase and Leibold, 2003; Rodríguez-Gironés and Santamaría, 2007; Abrams et al., 2008). Biotic resources, such as pollination services, are also limiting in many habitats (Totland and Eide, 1999; Knight et al., 2005; Fishman and Willis, 2006; Spigler and Chang, 2009; Martén-Rodríguez and Fenster, 2010; Wagenius and Lyon, 2010) and may lead to the extinction of local populations via reduced reproductive success. Many plants compete for pollinators (Brown et al., 2002; Bell et al., 2005; Devaux and Lande, 2009; Mitchell et al., 2009) and species with low relative abundance are often poorer competitors (Flanagan et al., 2010). Biotic resources could, therefore, contribute to the restriction of some rare species' ranges. An increase in competition for shared pollinators reduces the seedset of poorer competitors (Brown and Mitchell, 2001; Bell et al., 2005), and should restrict species from establishing populations in habitats characterized by competition for pollinators. Moreover, species that occur on stressful habitats, often have a similar suite of traits that are adaptive in stressful abiotic conditions (Grime, 1977; Chapin et al., 1993), specifically in hot and xeric environments, but may lead to reduced competitive ability for pollinators in more productive habitats, as is seen with competition for other resources (Maestre et al., 2009).

Rare taxa are thought to be less reliant on their pollinators by adopting self-compatibility (Darwin, 1877; Fausto et al., 2001; Moeller and Geber, 2005; Harder et al., 2008; Jacquemyn and Brys, 2008; Martén-Rodríguez and Fenster, 2010), which ensures reproductive success despite low pollination success. Plants that are self-compatible often have lower investment in floral traits to attract pollinators and often have smaller and less abundant flowers, and lower nectar and pollen rewards (Anderson and Busch, 2006; Fishman and Willis, 2006; Kennedy and Elle, 2008; Foxe et al., 2009); making them less attractive to pollinators (Kudo and Harder, 2005; Dickson and Petit, 2006; Ishii et al., 2008; Parachnowitsch and Kessler, 2010). Therefore, this hypothesis would predict that rare taxa are poor competitors for pollinators. The principle of reproductive assurance is based on one of the seven forms of rarity (Rabinowitz et al., 1981), which are defined by a combination of local population size, geographical extent, and habitat specificity (Table 4-1). One important assumption underlying this model, specifically low pollen availability, is applicable when local populations are small or pollination success is low or unpredictable. On the other hand, locally-abundant, regionally-rare (LARR) taxa may not be limited by pollen availability if pollination is sufficient and predictable. This leads to the possibility that other factors, other than low pollen availability, can affect selection on floral traits, such as stressful conditions or competition for pollinator services.

If rare species specialize on the stressful (defined here as hot and xeric) habitat in which they occur, they should be locally-adapted and better able to tolerate those conditions than species that are not restricted to these habitats or that are adapted to more benign habitats (Grime, 1977; Chapin et al., 1993). The widespread species may have a lower photosynthetic rates and a greater reduction in floral output and floral attraction

65

traits when stress is high. A reduction in floral attraction traits could diminish their ability to attract pollinators and compete for pollinator services under stressful conditions. Plants with larger floral displays and greater reward output are better competitors for pollinator services (Brown and Mitchell, 2001; Bell et al., 2005) and have greater reproductive success (Bell et al., 2005; Kudo and Harder, 2005). Stressful conditions may also select for higher offspring quality and outcrossing rates, in response to high mortality or inbreeding depression. Therefore, I predict that LARR species that are endemic to stressful environments should exhibit traits associated with increased outcrossing rates and competitive ability for pollinators. Individuals with fewer, larger flowers are known to have higher outcrossing rates (Karron et al., 2004; Eckert et al., 2009; Karron et al., 2009) mediated by pollinator behavior, and pollinators are attracted to individuals with greater floral display, either via flower number or flower size (Kudo and Harder, 2005; Dickson and Petit, 2006; Ishii et al., 2008; Parachnowitsch and Kessler, 2010). According to this hypothesis, and contrary to traditional theory, LARR taxa should be better competitors for shared pollinators than widespread, closely-related species when in high stress habitats.

In this study, I test the hypothesis that floral attraction traits affect the relative competitive ability of two locally-abundant, regional rare taxa (LARR) in comparison to their widespread, common congeners (CC) for pollinators in the stressful (i.e., hot and xeric) habitats of the Ozark glades. Specifically, I will test two alternative predictions. First, LARR taxa have traits associated with increased attractiveness to pollinators and are better competitors for pollinators than their common congeners (CC). Alternatively, LARR species have traits associated with reduced competitive ability (e.g., fewer,

smaller flowers) and are poorer competitors than their CC, as traditional competitive

exclusion theory would predict. I tested these hypotheses via a modified De Witt

replacement competition experiment that maintained density of inflorescences but

manipulated the relative proportion of conspecific and heterospecific individuals. I

introduce naïve individuals to pollinators in a common habitat to assess their ability to

attract pollinators as measured by visitation rate and reproductive success.

MATERIALS AND METHODS

Study system—I chose two plant species endemic to glades, *Delphinium treleasei*

(Ranunculaceae) and *Echinacea paradoxa* (Asteraceae), which are restricted to glades in

the Ozark Region in Missouri and Arkansas and have common congeners (CC; *D.*

carolinianum and *E. pallida*, respectively; vouchers of all species will be deposited in the

Missouri Botanical Garden) that can grow on glades but are not restricted to them. Glades

are hot, dry habitats with a high-proportion of exposed bedrock dominated by herbaceous

vegetation, which occur within a matrix of more mesic oak-hickory woodland (Kucera

and Martin, 1957; Yatskievych, 1999). Comparing congeneric pairs provides insight into

the factors that restrict one species to glades and not the other, and multiple congeneric

comparisons also make the results of this study more broadly applicable (Bevill and

Louda, 1999). Moreover, comparing restricted species to their widespread congeners

mitigates the potential influence of evolutionary history on the biological traits correlated

with competitive ability for coevolved pollinators.

Experimental design—I conducted a field competition experiment in order to

explicitly test the relative competitive abilities of the LARR and CC taxa. In order to

control for spatial variation in pollinator assemblage and abundance, I conducted the

experiment at multiple field sites (two for *Delphinium* and three for *Echinacea*; Table 4-2) across a broad geographic range relative to the distribution of the LARR taxa. Sites were chosen based on two criteria: (1) both species of a congeneric pair occur on the same glade and (2) the density of each species was roughly similar. Inflorescences from elsewhere in the same glade were bagged before flowering in order to exclude visiting insects. Because successful transplantation into glades is difficult due to shallow soils and extreme conditions, all inflorescences were collected, placed in glass containers filled with water and wrapped with a light, neutral-colored material in order to keep the inflorescence fresh and erect. Each individual was randomly assigned to one of three competition treatments: interspecific competition, focal individuals in the intraspecific competition, and non-focal individuals in the intraspecific competition treatment. In other words, each species competed with individuals of the same species (intraspecific, 0% proportion to heterospecifics) and with individuals of its congener (interspecific, 50% proportion to conspecifics) while plant density remained constant (Figure 4-1). The intraspecific competition treatment was subdivided into focal and non-focal individuals in order to maintain equal replication per species per treatment (i.e., two individuals per species per treatment replicate), despite a difference in relative abundance of conspecifics. Only those individuals assigned to the focal group were used for statistical analyses in order to maintain a balanced comparison between treatments. The experiment was repeated two to three times per site with different individuals, depending on availability of inflorescences, for a total of up to 36 individuals per field site [(4 individuals for the intraspecific competition treatment + 2 individuals for the interspecific competition treatment) x 3 replicates x 2 species; Figure 4.1].

Floral attraction traits—I measured the following floral attraction traits: plant height, number of open flowers (*Delphinium*) or florets (*Echinacea*), flower size (*Delphinium* only), and total display size (TDS). Due to morphological differences, TDS was quantified differently for each genus. For the *Delphinium* taxa, TDS is defined as the product of the total number of flowers open times corolla area (i.e., flower width x flower height), and TDS for the *Echinacea* taxa the product of number ray petals times corolla area (i.e., ray petal width times ray petal length). Pollinator attraction traits were measured following observations, in order to avoid damaging flowers or altering reward quality or quantity (e.g., pollen quantity).

Pollination and reproductive success—Plants were exposed to pollinators (i.e., bagging removed) after they were placed in the competition array in order to ensure that pollination occurred only when in the arrays, and they remained in the arrays for six to eight hours. I observed the number, type, and behavior (e.g., visit duration, anther and stigma contact) of all floral visitors during peak periods of activity for 30 minutes. The arrays were placed in a part of the glade where both species occur in equal abundance in order to reduce any potential density-dependence effect on pollinator behavior. Pollination was quantified as visitation rate, visit duration, and the number of flowers probed per visit (for *Delphinium* species only). Because seedset from cut inflorescences is variable and often unsuccessful, I quantified reproductive success as the number of pollen grains germinated and the number of pollen tubes reaching the ovary. Once the inflorescences were removed from the field, they remained undisturbed for 24 hours to allow for pollen tube growth, after which each ovary was dissected from the inflorescence. At one site (SNR; Table 4-2), the abundance of individuals blooming

69

concurrently was low and insufficient to conduct the experiment. Therefore, individuals that were collected at different (HHTSP) were randomly re-assigned to a new treatment and the experiment was repeated at SNR. Since *Echinacea* species have florets that are only receptive for one day, I was able to collect the achenes in between experiments and ensure that pollen deposition and pollen tube growth were not the result of previous exposure to pollinators. I also allowed two days between observation periods, during which the inflorescences were excluded from pollinators and kept in cool (roughly 23ºC) conditions to reduce wilting. I carefully removed those achenes that were exposed to pollinators after each treatment, without damaging the capitula.

Once removed, the ovaries were fixed in 3:1, 95% ethanol:glacial acetic acid for 2 hours, rinsed and stored in 70% ethanol. They were then immersed in 10% (w/v) solution of sodium sulfite and autoclaved for 30 or 40 minutes at 60 or 70°Celsius for *Delphinium* and *Echinacea* taxa, respectively. After cooling, each pistil was mounted on a slide, covered with 3-5 drops of decolorized aniline blue, refrigerated for 24 hours, and illuminated using a 100 W fluorescent source (Lipow et al. , 2002) on a Zeiss Universal microscope. In order to determine reproductive success, I counted the number of pollen grains on the stigma, the number of pollen tubes growing down the style, and the number of pollen tubes reaching the ovary (see Lipow et al., 2002). Competitive ability was quantified as differences in visitation rate, number of pollen on the stigma, number of pollen tubes germinating down the style, and number of pollen tubes to reach the ovary in response to the competition treatments.

Statistical analyses—I tested for differences in morphological traits between species of each genus and to ensure that the randomization procedure was not biased (i.e.,

traits did not vary by treatment) via a two-way, nested ANOVA with Treatment and

Distribution as factors. Distribution was nested within Site to control for potential spatial

variation in species' traits. All three treatments (i.e., intraspecific, interspecific focal

plants, interspecific non-focal plants) were included to determine the potential for a bias

in the randomization procedure. Upon finding a significant interaction term, I used a

Tukey's HSD *posthoc* test to determine paired differences between the significant factors.

Model reduction was conducted for all marginally significant results using the *step*

function in the R Statistical Package (R Development Core Team, 2008), in order to

refine the statistical result. The *step* function systematically removes higher-order terms

from the model structure, compares the Akaike Information Criterion (AIC; a measure of

relative goodness of fit that penalizes model complexity) between models, and reduces

the model to that with the best fit (i.e., lowest AIC). Following model reduction,

differences in the morphological trait was compared among the factors in the reduced

model (e.g., nested ANOVA with distribution nested within site).

The effects of competition treatment on pollination and reproductive success were

tested as a two-way, nested ANOVA with treatment and distribution nested within site.

Treatment was nested within site to control for among-site variation in pollinator

composition and abundance. Since some morphological traits varied spatially,

distribution was also nested within site, in order to differentiate between the effects of

site-level variation in morphological traits and pollinator assemblages. Due to low

visitation rates and pollen deposition, significant differences in the competitive ability of

congeners were also tested using a two-way nested, permutation ANOVA (*aovp* in R

Statistical Package (R Development Core Team, 2008)) with Treatment and Distribution

71

nested within Site as above. I used a Monte Carlo resampling approach (i.e., without replacement) to construct a null expectation based on a random sampling of the available data and compared each of 5000 runs to the null. Statistical significance is quantified as the number of runs for which the F-statistic is greater than or equal to the observed value. Response variables were tested for normality and transformed as necessary. Variables that could not be transformed to meet the assumption of normality for parametric statistics were also tested for differences using the permutation approach described above. Results did not differ between the parametric and nonparametric analyses; therefore, I report the results of the parametric tests.

RESULTS

Morphological traits—For the *Delphinium* taxa, morphological traits differed between congeners and across sites, but there was no treatment effect, which verified the randomization procedure. Height and display size of both *Delphinium* taxa differed significantly between sites (*DF* = 1, *F* stat = 6.33, *P* value = 0.0197; *DF* = 1, *F* stat = 26.0, *P* value < 0.0001, respectively; Figure 4-2 A and B), which means that the morphological traits differed between the replicates and could reflect different abiotic conditions between sites. There was no difference between congeners (*DF* = 2, *F* stat = 1.18, *P* value > 0.05; *DF* = 2, *F* stat = 1.98, *P* value > 0.05; respectively). There was a significant interaction between site and distribution for the number of flowers open (NFO) concurrently (*DF* = 2, *F* stat = 5.54, *P* value = 0.0105; Figure 4-3A). The LARR *Delphinium treleasei* had a higher NFO at one site (RBCA) than the other, but there was no difference between sites for the CC or between congeners within a site. Corolla height (CH), which includes the landing pad of the flower, also differed among species and sites

for the *Delphinium* taxa (*DF* = 2, *F* stat = 7.04, *P* value = 0.0043; Figure 4-3B). The LARR species had greater CH than its CC at DCA but not at RBCA, which suggests that CH is as variable by geography as it with between congeners. Distance to nectar was marginally different between *Delphinium* species at different sites (*DF* = 2, *F* stat = 2.87, *P* value = 0.0780), which was driven by a marginal difference between taxa at one site (DCA).

The *Echinacea* taxa are not different in their morphological traits among the interspecific, focal-individuals of the intraspecific treatment, and the non-focal individuals of the intraspecific treatment, again verifying the randomization procedure. They also differed in their morphological traits among sites and species; in other words, species traits were different at some sites and not at others. The number of flowers open and display size differed significantly among sites for both species, indicated the geographic variation is greater than any differences between congeners. There were more flowers open at PLH than either HHT or SNR (*DF* = 2, *F* stat = 19.15, *P* value < 0.0001), and total display size was successively larger at PLH, HHT, and SNR (*DF* = 2, *F* stat = 42.92, *P* value <0.0001). Height was significantly different between congeners among sites (*DF* = 2, *F* stat = 9.88, *P* value = 0.0005; Figure 4-4A), which was driven by differences between congeners at one site (HHTSP). *Echinacea paradoxa* (LARR) was significantly shorter than its CC at HHTSP, which is consistent with the prediction of greater competitive ability.

Competitive ability—Neither pollination nor reproductive success differed between the intra- and interspecific competition treatments for either genus. However, both genera had significant differences among sites and distributions. Both *Delphinium*

species had marginally lower visitation rates at DCA than RBCA ($DF = 1$, F stat = 3.48, P value = 0.0833; Figure 4-2C), but there was no difference between species or treatments ($DF = 2$, F stat = 0.54, P value > 0.05; $DF = 2$, F stat = 0.36, P value > 0.05; respectively). There were no differences in visit duration or the number of flowers probed per visit for site, distribution or treatment. The *Delphinium* species differed across sites in the number of pollen grains germinating on the stigma ($DF = 2$, F stat = 11.33, P value = 0.0017); the LARR species at RBCA had a greater number in comparison to the LARR species at DCA and to its CC at either site. The number of tubes developing down the style was significantly different between species within sites ($DF = 3$, F stat = 4.61, P value = 0.0327; Figure 4-3C); the LARR species at RBCA had more tubes in comparison to its CC at either site and to itself at DCA. However, there were no differences in the number of pollen tubes reaching the ovaries ($DF = 3$, F stat = 0.56, P value > 0.05), which may reflect low pollen quality or maternal effects, since the inflorescences were removed from the rest of the individual which could impede pollen tube growth.

The *Echinacea* taxa did not differ in visitation rate among sites ($DF = 2$, F stat = 2.36, P value > 0.05) or by distribution within sites ($DF = 3$, F stat = 1.80, P value > 0.05). The duration of the visits received was also not different among sites ($DF = 2$, F stat = 1.21, P value > 0.05) or by distribution within sites ($DF = 3$, F stat = 2.04, P value > 0.05). The number of pollen grains deposited on the stigma was significantly different between species across sites ($DF = 3$, F stat = 4.75, P value = 0.0232; Figure 4-4B), but there were no differences between congeners within a given site. There was also no difference in the number of pollen tubes reaching the ovaries at any site ($DF = 3$, F stat = 1.10, P value > 0.05).

DISCUSSION

For decades, the relative importance of biotic and abiotic factors in determining species distributions has been debated in ecology. Competition for shared pollinators may influence distributions via reproductive success. Here, I tested the alternative hypotheses that two rare species are (1) poorer competitors for pollinators in comparison to their common congeners (CC), based on the predictions of traditional reproductive theory which state that rare taxa have higher selfing rates and are therefore less attractive to pollinators, (2) better competitors for pollinators in the plant specialize for increased attractiveness in stressful environments, or (3) equal competitors for pollinators because pollination is not limiting or due to evolutionary history. There was no significant effect of the competition treatment on pollination or reproductive success of any species tested. However, there was significant spatial variation in the floral traits and reproductive success of endemics and their CC, which indicates the important role of scale on interspecific interactions and, potentially, the mechanisms restricting a species' range.

Contrary to my predictions, neither the CC nor the LARR species were consistently better competitors for pollinators. There were no significant differences in visitation rate, pollen deposition, or pollen tube growth between intra- and interspecific competition treatments. The lack of a treatment effect suggests that competition for pollinator services is not occurring between these congeneric pairs on glades. Overall, visitation and fertilization rates were very low, which could be the result of low insect abundance in 2009, when this experiment was conducted, or of generally low reproductive success in the hot, dry glade environment. In habitats with more abundant pollinators or less-stressful conditions, competition may be occurring. In order to

75

eliminate the potential effects of pollinator identity and experience, I conducted this experiment on glades at which both species occur. In order to fully understand the potential role of competition for pollinators in restricting the distribution of glade endemic plants, studies that test for differences in relative competitive ability for pollinators in non-glade habitats are required. While the LARR species' coevolved pollinators may not occur off of glades, a comparison of competitive ability for pollinators on and off glades will determine if the distributions of LARR species are influenced by their plant-pollinator interactions, either via a lack of effective pollinators or poor competitive ability for shared pollinators in comparison to their CC.

While there was no treatment effect on pollination or reproductive success, all species showed significant spatial variation in floral traits and pollination success. The traits of both *Delphinium* species indicate a tradeoff between allocation to reproduction and vegetative output that varies across space. Plants of both species were shorter but had greater floral displays at RBCA than at DCA. The insects respond to the increase in floral display seen at RBCA with higher visitation rates to plants at RBCA in comparison to DCA. Differences in allocation to reproduction could be a function of variation in soil nutrient content or habitat suitability between sites. For instance, if DCA is hotter and drier than RBCA, the difference seen in floral and vegetative traits would support the prediction that allocation to reproduction increases with stress. Higher visitation rates at RBCA than at DCA also indicate a role of larger spatial-scale variation in floral density on insect behavior. While I chose an area within each glade where both species were in equal abundance, density on the glade as a whole can vary considerable across glades. Anecdotally, relative abundance and floral density of the *Delphinium* species was much

lower at DCA in comparison to RBCA. Insects are known to travel large distances (van

Nieuwstadt and Iraheta, 1996; Pasquet et al., 2008; Elliott, 2009; Rader et al., 2011),

sometimes moving distances greater than the size of many glade habitat (e.g., more than

a kilometer; Osborne et al., 2008; Pasquet et al., 2008; Wikelski et al., 2010), which can

range in size from less than one hectare to greater than 200 hectares (Nelson and Ladd,

1982). Therefore, the pollinators may be responding to inflorescence density on the glade

and not directly to individual variation in floral traits. Future studies will document floral

density and abiotic conditions across glades in order to disentangle the relative

importance of these two mechanisms.

The *Delphinium* congeners also differed significant in reproductive success (i.e.,

number germinating pollen grains and pollen tube growth) at RBCA. While there was no

difference in visitation rate or the number of total pollen grains on the stigma, the LARR

plant had more pollen tubes that were germinating and developing down the style. This

pattern suggests that the visits to the LARR *Delphinium* species may have been more

effective and resulted in the transfer of higher quality or more conspecific pollen in

comparison to its common congener. The Delphinium congeners are known to hybridize

(Warnock, 1987), and germination rates may reflect differences in the ability of

conspecific pollen to cross-pollinate. However, pollen quality of each species, *per se*, was

not quantified, and further research is needed to determine the mechanism behind the

greater proportion of pollen grains that successfully fertilized ovaries in *D. treleasei*. In a

previous study conducted at a larger spatial scale (Chapter 1), I showed that the endemic

Delphinium species had a more specialized pollination system (i.e., fewer species of

pollinators accounted for the majority of pollen flow) than its congener, which could

account for the difference seen in pollen germination rate and pollen tube growth. Moreover, spatial variation in pollen tube growth is likely a result of low visitation rates and not variation in pollinator effectiveness. While differences were only marginally statistical significant due to low pollinator abundance and replication, visitation rates were nearly four times (3.8) higher at RBCA than DCA, and visit duration was more than two and a half times greater.

The *Echinacea* species also differed in their floral traits and reproductive success among sites. However, there was no difference within a given site, with the exception of height, which suggests that the differences were dominated by site-level effects. The LARR species was shorter than its CC at HHTSP. They were also shorter at SNR (which were individuals collected from HHTSP due to low abundance of blooming individuals at SNR), but the pollinators did not respond differently to the congeners at SNR. I predicted that the pollinators would be more attracted to the taller individuals, as has been shown in other taxa (Dickson and Petit, 2006), which would be the CC at both HHTSP and SNR. Contrary to my predictions, pollen deposition rate was higher to the LARR species at HHTSP, and there was no difference at SNR. Pollen deposition rate may be related to the variation in pollinator fidelity or effectiveness of the pollinators among sites, as seen in the *Delphinium* species. However, the LARR *E. paradoxa* does not have a more specialized pollination system than *E. pallida.* Moreover, the disparity in pollen deposition did not lead to differences in pollen tube growth or fertilization rates, which suggests there may be a trade-off between pollen abundance vs. pollen viability. In other words, *E. paradoxa* pollen may be more abundant but of lower quality, resulting in a negligible difference in reproductive success. On the other hand, the pollen deposited on

78

the stigmas of *E. paradoxa* could have had a greater proportion of heterospecific pollen, resulting in reduced fertilization rates. Unfortunately, the pollen of each *Echinacea* species could not be distinguished from one another, so I was unable to test for this mechanism. In future studies, pollen of each individual should be tagged with fluorescent dye in order to distinguish between the transfer of heterospecific and conspecific pollen deposition.

Conclusion

This study illuminates the importance of large-scale mechanisms for local reproductive success and pollination specialization for greater pollen transfer. In contrast to our hypotheses, there was no difference in competitive ability between two endemic glade plants and their congeners. Competition for pollinator services has been documented in some habitats (Brown and Mitchell, 2001; Bell et al., 2005; Internicola et al., 2007) but there is no evidence of competition for pollinator services between these congeners in glade habitats. While these congeners did not compete for pollinators, there were differences in the reproductive success of the *Delphinium* congeners when pollination visitation was relatively high. The *Delphinium* endemic glade plant has a more specialized pollination system and may have more effective pollinators. Pollination specialization is predicted to increase conspecific pollen transfer (Darwin, 1877; Rymer et al., 2005; Galloni, 2008) and increases reproductive success (Rymer et al. 2005; Galloni 2008) as documented in this study. Moreover, differences in pollination success and reproductive success appear to be influenced by habitat-level factors, such as inflorescence abundance or pollinator assemblages, and less by individual morphological traits. The structure of habitat within a landscape is known to affect the distribution and

behavior of pollinating insects in experimental and agricultural settings (Kremen et al., 2002; Ricketts et al., 2008). This study demonstrates the potential significance of landscape-scale factors on plant-pollinator interactions and plant reproductive success of LARR plants in natural habitats. Understanding the relative importance of landscape-scale factors on local interactions is increasingly important given high rates of habitat alteration and climatic change around the world.

Table 4-1. Description of the Rabinowitz's Seven Forms of Rarity based on geographic extent, population size and habitat specificity (modified from Rabinowitz et al., 1981). The bolded form of rarity represents the locally-abundant, regionally rare (LARR) taxa in this study.

| | Geographic Extent (GR) | | | |
	Large		Small	
Population Size (PS)				
Somewhere large	Common	Large PS Large GR Narrow HS	Large PS Small GE Broad HS	**Large PS (LA) Small GE (RR) Narrow HS**
Somewhere small	Small PS Large GE Broad HS	Small PS Large GE Narrow HS	Small PS Small GE Broad HS	Small PS Small GE Narrow HS
	Broad	Narrow	Broad	Narrow
	Habitat Specificity (HS)			

81

Figure 4.1. A schematic of a representative glade site (A) and the competition treatments (B; including intraspecific and interspecific competition treatments. In order to maintain equal replication between intra- and interspecific competition treatments only two individuals per intraspecific treatment (the *focal* individuals, solid and outlined circles) were used to test for differences between LARR and CC species in response to the competition treatments. The non-focal species (striped circles) were excluded from the analyses in order to maintain equal replication between treatments), and (C) a map of sites at which I conducted a pollination competition experiment with two congeneric species pairs, *Delphinium treleasei* and *D. carolinianum* (▫) and *Echincaea paradoxa*

and *E. pallida* (o ; LARR and CC, respectively). Glade density data was from Nelson

and Ladd (1982) coded in to a heat-density map. The original data represented a range in

glade density per 7.5 degree minute quadrats (approximately 144 km^2 in Missouri);

therefore the heat map reflects a rough estimate of glade density per 144 km^2 as of 1982.

Figure 4-2. Morphology and visitation rates of two *Delphinium* species at two field sites in south-western Missouri Drury Conservation Area (DCA) and Rocky Barrens Conservation Area (RBCA), including (A) Height (cm), (B) Display size (floral output x corolla area), and (C) visitation rate per 30 minute observation period. * denotes statistically significant differences.

Figure 4-3. Floral traits and reproductive success of a locally-abundant, regionally rare (LARR) *Delphinium treleasei*, and its common congener (*D. carolinianum*, CC) at two field sites in south-western Missouri (Drury Conservation Area (DCA) and Rocky Barrens Conservation Area (RBCA)), including (A) floral display (the number of open flowers), (B) corolla height, including the landing pad, and (C) the number of pollen tubes developing down the style of the experimental plants. Different letters denote statistically significant differences.

Figure 4-4. Height (A) and reproductive success (B; number of pollen grains on the stigma) of a locally-abundant, regionally rare (LARR) species, *Echinacea paradoxa*, and its common congener (*E. pallida*, CC) at three field sites in Missouri (HaHaTonka State Park (HHTSP), Private glade (PLH), and Shaw Nature Reserve (SNR)). Different letters represent statistical differences between

CHAPTER FIVE

Pages 88 to 105

PHENLOGICAL SHIFTS IN CONGENERIC PAIRS OF RARE AND WIDESPREAD

SPECIES: THE ROLE OF INTRINSIC AND EXTRINSIC FACTORS

INTRODUCTION

Phenological shifts in response to climate change have been well documented (IPCC, 2007, Badeck et al., 2004; Dose and Menzel, 2004; Ibanez et al., 2010; Kudo and Hirao, 2005; Lambert et al., 2010; Sherry et al., 2007). While species responses are consistent with the direction of climate change, there is considerable variation in the degree to which species are responding (Kudo and Hirao, 2005; Cleland et al., 2006; Miller-Rushing et al., 2007; Miller-Rushing and Primack, 2008; Miller-Rushing and Inouye, 2009; Forrest et al., 2010). For instance, many plant species in the same genus respond differently to changes in climate (Miller-Rushing and Primack, 2008; Miller-Rushing and Inouye, 2009), and differential responses may affect interspecific interactions (Forrest et al, 2010, Rafferty and Ives, 2011). Both intrinsic and extrinsic factors, for example pre-adaptation or micro-climate effects, respectively, may contribute to the variation in species responses to climate such. The mechanisms behind interspecies variation are rarely assessed (but see Kudo and Hirao, 2005; Miller-Rushing and Inouye, 2009) and could inform our understanding of the relative importance of the biological processes that underlie them.

Variation in species responses to climate change may be partially explained by historical factors, such as genetic composition or traits that are pre-adapted to climate change. For instance, species that are adapted to stressful (i.e., hot and/or xeric) climatic conditions or are relicts from previous periods of climate change may have traits that are better adapted to future climatic conditions than those species found in less-stressful environments. Plants that occur in stressful habitats often have a suite of traits, known as the stress-resistance syndrome (SRS), which are thought to reduce the negative effects of

89

heat and drought stress (Chapin et al., 1993). These species may therefore be lessresponsive to changes in climatic conditions than species without SRS traits, such as widespread species with broader habitat tolerances.

Moreover, species that specialize on stressful habitats may also have lower phenotypic plasticity (Ellison and Parker, 2002; Pohlman et al., 2005), either in their morphological traits or in their phenological patterns. Strong selection for local adaptation can lead to reduced phenotypic plasticity (Ellison and Parker, 2002; Pohlman et al., 2005), and many rare and endemic plants are thought to be habitat specialists (Rabinowitz et al., 1981; Izco, 1998, Espeland and Emam, 2011) that are restricted in their ranges due to the lack of suitable habitat. Therefore, stress-adapted specialist plant species should be less responsive to climate change in comparison to species that occur in multiple habitat types. Alternatively, local adaptation to a stressful environment may include the ability to track short periods of favorable abiotic conditions, such as cooler temperatures or rain events (Aronson, 1992; Angert et al., 2010). Stress-adapted species would then be more responsive to climate change in order to avoid higher-stress conditions later in the season.

In addition to intrinsic factors, extrinsic mechanism, such as local abiotic conditions, may affect the impact of regional climate change on individuals (Kudo and Hirao, 2006) and the evolution of populations in unpredicted ways. Many local factors, such as plant cover, soil composition, and exposed bedrock, may also influence microclimate conditions, such as the retention and distribution of heat and water throughout a habitat. Evaporative cooling of plants via transpiration can buffer temperatures in the surrounding habitat (Georgescu et al., 2011), vegetation structure can

reduce the wind exposure experienced by other plants near them (Lortiek et al. 2002), and the latent heat of exposed bedrock may increase soil and air temperatures close to the ground. These local factors can indirectly influence the degree to which an individual is impacted by regional climate change by either mitigating or intensifying climate conditions. For instance, plants that occur in soils with low water-holding capacity may be more responsive to increases temperature, which should reduce plant-available water.

In this study, I address three hypotheses that potentially influence differential responses of endemic species and their closely-related congeners to regional warming based on two principles, pre-adaptation and local habitat effects. The first predicts that plants that are endemic to high-stress habitats are less responsive to regional climate change than closely-related, widespread species. Alternatively, the second hypothesis predicts that stress-adapted species are more responsive to changes in abiotic conditions via greater ability to track suitable habitat in time. The third predicts that all plants in exposed, xeric habitats have greater response to regional warming, regardless of geographic range size. I test these predictions using a novel, continuous descriptor of development stage that was calculated for each herbarium specimen and a categorical variable describing the habitat in which the individual was collected. Using data collected from 21 weather stations over the 110 years throughout the region to which these species are endemic (Missouri and Arkansas), I determined which factors (range, habitat, weather or change in climate through time) are the primary drivers of phenology in all species.

METHODS

Study System—I selected two species restricted to glades in the Ozark Region of Missouri and Arkansas, *Delphinium treleasei* (Ranunculaceae) and *Scutellaria bushii*

(Lamiaceae); each of which has at least one widespread congener (*D. carolinianum ssp. carolinianum*, *S. ovata*, and *S. parvula*) that grows on glades but is not restricted to them. Glades are hot, dry habitats with exposed bedrock, thin soils, and limited herbaceous plant cover that occur within a matrix of more mesic oak-hickory woodlands. They are relatively stressful habitats, which are hotter than the surrounding woodland and prairie habitats during the spring and summer months (data not shown) when these plants are photosynthetically active and blooming. Comparing these congeneric pairs provides insight into the factors that restrict one species to glades and not the other. Moreover, comparing restricted species to their widespread congeners mitigates the potential influence of evolutionary history on the biological traits correlated with endemism. Multiple congeneric comparisons also make the results of this study more broadly applicable (Bevill and Louda, 1999).

Climatic data—In order to document potential changes in climate, I acquired climate data from fourteen weather stations throughout Missouri (Figure 5-1), six weather station in Arkansas, and one in Kansas, for a total of 22 weather stations. Stations were chosen based on the duration of available data and location, in order to accurately describe the climate variables throughout the range of our target species. Climate data were available for at least five, spatially distributed weather stations between 1897 and 1905, between 1905 and 1931 roughly half of the weather stations had available data, and after 1931 all weather stations contributed data for most years. The data from all weather stations was checked for missing values. If any weather station did not have data for every month in a given year, the data from that weather station for that year were excluded from the analyses. The climatic data were reduced to annual averages across all

sites in order to control for spatial variation in climate. I conducted a principle

components analysis (PCA) to reduce the multiple abiotic variables (i.e., average

temperature, average high temperature, average minimum temperature, and yearly

precipitation) into 2 principle components describing the climate in multivariate space.

Since average minimum and maximum temperatures are inherently correlated with

average annual temperature, I first regressed minimum and maximum temperatures

against average annual temperature, and the residuals from this analysis were used in the

PCA. In this way, I was able to more accurately describe climate space by isolating the

effects of maximum and minimum temperatures on phenology, independent of annual

average temperature. This is particularly important if the range of temperatures has an

influence on phenology. The summary of these data represented by the first two principle

components allowed me to explore the response of each species to a more meaningful

variable that incorporated multiple interacting factors contributing to climate.

Plant phenology—All available herbarium specimens for our target species were

acquired from six herbaria that were considered to have the largest holdings of these

species, the Missouri Botanical Garden, University of Arkansas Herbarium, Illinois

Natural History Museum, United States National Herbarium, New York Botanical

Gardens, and Field Museum. The development stage of each specimen was calculated

using a continuous variable that standardized for floral output. All floral buds, open

flowers, and fruits were counted, and I ranked each unit (i.e., bud as zero, flower as 1,

and fruit as 2), summed them, and divided by the total floral output. Development stage,

was calculated as,

$$DS = \frac{b*0 + f*1 + r*2}{2(b+f+r)} \qquad\qquad \text{Equation 1.0}$$

93

where b is the number of buds, f is the number of flowers and r is the number of fruits. Therefore, phenological stage ranges between zero and one, where zero denotes a plant that is in full bud, 0.5 denotes a plant in full bloom, and one reflects an individual in full fruit. Development stage of all *Scutellaria* taxa was biased by development stage; many more individuals were collected at the developmental limits (i.e., zero and one). Individuals at these limits were excluded from the analyses, in order to remove this bias and acquire a normal distribution. All collection dates were converted into a continuous Julian date.

In order to control for latitudinal variation in climatic conditions, I used latitude as a covariate for all analyses in order to account for this variation. Many modern specimens were georeferenced and for other specimens with sufficient descriptive locality information on the herbarium labels, I estimated latitude via satellite imagery in Google Earth (Google, 2011). Specimens that could not be georeferenced were excluded from the analyses. Using the descriptors on the herbarium labels, habitat was categorized into four main types, Glade, Prairie, Woodland and Disturbed, representing the major habitat types in which these species were found. The designation "Glade" included all specimens with habitat described as upland prairies, rocky prairies, bald knobs, etc. "Woodland" refers to specimens collected from habitats characterized as rocky woodlands, wet woodland, upland woodland, forests, etc. "Prairie" included specimens collected from meadows, prairies and wet prairies, and "Disturbed" denotes habitats along roadsides, railroads, and power-lines, unless otherwise specified on the herbarium specimen. There were only 3 specimens in woodland habitats for *S. parvula*; therefore I pooled all non-glade habitats in order to increase replication.

Statistical Analyses—Regression analyses were conducted to determine whether and which abiotic factors and their composite climate variables (PC 1 and 2) were changing through time. Multiple regression was used to test which variable and their interactions explained the most variation in phenological stage for each species. Specifically, I tested for the effects of climate in a given year (defined as principle component 1(PC 1)), change in climate through time (represented by time in years), habitat (the common species only), and the interactions of these factors on the relationship between development stage and collection date. The interaction between climate (PC 1) and collection date tested the prediction that the phenology of these species is controlled by climate and not (for instance if the species is photoperiodic). Change in phenology due to climate change was documented as the interaction between collection date and year. Latitude was included as a covariate in order to account for spatial variation in phenology. Model reduction was used (*step* in the R Statistical Package (R Development Core Team 2008), in order to refine the statistical result. The *step* function systematically removes higher-order terms from the model structure, compares the Akaike information criterion (AIC; a measure of relative goodness of fit that penalizes model complexity) between models, and reduces the model to that with the best fit (i.e., lowest AIC).

All variables were transformed for normality, and if normality could not be achieved, the statistical test was repeated with summarized data or via nonparametric tests. Time in years could be not normalized for either *Delphinium* taxa or for *S. ovata*, but the results were consistent when I conducted the analyses when time in years was categorized into pre- and post-1960 time periods. I report the results of the analysis with

time as a categorical variable for the *Delphinium* taxa for ease of interpretation. For the

Scutellaria species, I report the results of the analysis with time as a continuous variable,

because the development stage of *S. bushii* was biased by time (all pre-1960 individuals

were in full fruit). Analyses could only be conducted for the latter part of the century.

Latitude was non-normal for *D. treleasei*, and analyses were conducted with and without

the covariate. There was no difference in the analyses for *D. treleasei*, so I report the

analyses conducted on the categorical time variable here.

RESULTS

Regional climate change—The first principle component of climate (PC 1) in

Missouri and Arkansas explained 69.46% variation, and the second explained an

additional 27.21%, for a total of 96.66% of variation explained by the first two principle

components. The first principle component of climate has changed over the last 117 years

($DF = 109$, $R^2 = 0.179$, $F = 23.73$, $P < 0.0001$; Figure 5-2A) but the second has not ($DF = 109$, $R^2 = 0.0131$, $F = 1.44$, $P > 0.05$; data not shown). Therefore, I focus on the effect of

changes in PC1 on phenology as the climate change factor. When tested independently,

minimum temperatures ($DF = 109$, $R^2 = 0.079$, $F = 9.31$, $P = 0.029$; Figure 5-2B) and

precipitation ($DF = 109$, $R^2 = 0.0939$, $F = 11.29$, $P = 0.0011$; Figure 5-2C) were

positively correlated with time and are driving the changes in climate (PC 1) toward a

warmer and wetter climate. There was no change in average ($DF = 109$, $R^2 = 0.019$, $F = 2.13$, $P > 0.05$) or maximum daily temperatures ($DF = 109$, $R^2 = 0.0002$, $F = 0.023$, $P > 0.05$) through time.

Phenological responses to climate change— the development stage of *D.*

treleasei, the rare species, was significantly affected by collection date (N = 20, $F =$

26.37, $P < 0.0001$) and climate (N = 20, $F = 8.04$, $P = 0.0114$), and had a marginal

response to climate change (N = 20, $F = 3.68$, $P = 0.0725$; Figure 5-3A), in congruence

with my hypotheses. Latitude also explained a marginally significant amount of variation

in the development stage of *D. treleasei* (N = 20, $F = 4.34$, $P = 0.0535$) but did not

improve the performance of the model; therefore, it was removed from the final model.

Not surprisingly, collection date explained a significant amount of the variation in the

development stage of *D. carolinianum* ($N = 79$; $F = 11.95$; $P = 0.0010$). The variation in

development stage of *D. carolinianum* was not significantly described by latitude or any

interaction term. For instance, there was no effect of habitat on response to climate

change ($N = 79$, $F = 0.07$; $P > 0.05$). Neither latitude nor climate was a significant factor

($N = 79$, $F = 0.03$, $P > 0.05$; $N = 79$, $F = 0.61$, $P > 0.05$; respectively) in explaining the

development stage of *D. carolinianum*.

Due to bias in the age of individuals of *Scutellaria bushii* throughout the century

(all individuals from the early-20th century were in fruit), only analyses based on modern

collections (1960 to present) could be analyzed, and the replication was reduced to 11.

During this period, variation in the phenophase of *S. bushii* was explained by collection

date (N = 11, $F = 20.17$, $P = 0.0020$) and marginally by time (N = 11, $F = 3.69$, $P =$

0.0912; Figure 5-4A), my measure of climate change. Latitude (N = 11, $F = 0.189$, $P >$

0.05; removed from the final model via *step*) and climate (N = 11, $F = 2.45$, $P > 0.05$) did

not explain a significant amount of variation in the phenophase of *S. bushii*, but low

replication and the lack of early-century data make these analyses inconclusive.

Variation in the development stage of *S. ovata* was explained by latitude (N = 55,

$F = 9.64$, $P = 0.0031$) and marginally by collection date (N = 55, $F = 3.64$, $P = 0.0620$).

There was no significant interaction between habitat and collection date (N = 55, F = 1.81, $P > 0.05$) or between habitat and response to climate change (i.e., Habitat by Time by Julian date; N = 55, F = 0.41, $P > 0.05$). Neither climate (N = 55, F = 1.96, $P > 0.05$) nor time (N = 55, F = 1.93, $P > 0.05$; Figure 5-4B; removed from the final model via *step*) explained a significant amount of the variation in phenology for *S. ovata*. Therefore, spatial variation in day length or temperature appears to account for the phenology of *S. ovata*.

Scutellaria parvula's development stage was correlated only with collection date (N = 22, F = 5.54, P = 0.0289). There was no interaction between climate and collection date (N = 22, F = 0.18, $P > 0.05$; removed from final model via *step*) or between collection date and change through time (N = 22, F = 1.16, $P > 0.05$; Figure 5-4C; removed from final model via *step*), suggesting that other factors are stabilizing the phenological response of *S. parvula* to climate change, such as day length, genetic constraints, or interspecific interactions. There was no effect habitat (N = 22, F = 0.79, $P > 0.05$; removed from final model via *step*) or its interaction with time (N = 22, F = 0.072, $P > 0.05$) on the phenology of *S. parvula*. Latitude also did not explain a significant amount of variation seen in the development stage of *S. parvula* (N = 22, F = 0.11, $P > 0.05$; removed from final model via *step*).

DISCUSSION

In this study, I find that related pairs of widespread and rare endemic species respond differently to regional climate change, and intrinsic factors appear to drive their phenological patterns. The climate of the Ozark region has seen an increase in minimum temperatures and precipitation over the past century, and one of the rare plants,

Delphinium treleasei, is responding with phenological change. I predicted that species that are endemic to high-stress environments would be less responsive to climatic change, assuming local adaptation to abiotic stress. However, the results indicate the opposite. The endemic *D. treleasei* is blooming earlier but the phenology of the widespread species, *D. carolinianum*, has not changed within any habitat, including glades. Therefore, the phenological change documented in *D. treleasei* is not driven by local habitat or stress tolerance, contrary to my predictions. While stress tolerance *per se* is not driving the phenology of *D. treleasei*, local adaptation may still be playing an important role through phenotypic plasticity. Greater plasticity in phenology would allow individuals to track temporal variation in climate and either avoid more stressful conditions or capitalize on favorable conditions. For instance, if a species specializes on hot, dry environments, it may be more responsive to a reduction in stress, such as an increase in precipitation (Aaronson, 1992; Angert et al., 2010). The differential responses of sympatric congeners may induce changes in their interspecific interactions. On average, *D. carolinianum* blooms roughly 2.8 days earlier than *D. treleasei*. Therefore within native habitats, the phenologies of the *Delphinium* species are converging. These species of *Delphinium* can hybridize (Warnock, 1987), and they share some pollinators (Miller-Struttmann, data not published). Therefore, hybridization rates and competition for shared pollinators could increase in the future if the phenology of *D. treleasei* continues to advance.

None of the *Scutellaria* species responded to changes in climate with shifts in phenology. Variation in the phenology of one common congener, *S. ovata*, was influenced by spatial variation and marginally by collection date, but neither climate nor

climate change affected phenology. *Scutellaria ovata* may be responding to day length or to climatic factors that are not changing in this region, such as high temperatures. The developmental stage of another widespread species, *S. parvula*, was correlated with only collection date, and there was no effect of spatial variation or climate. Similarly, the climatic factors to which *S. parvula* is responsive may not be changing in this region of the degree of change in key climatic factors may not be sufficient to affect the phenology of this species. On the other hand, additional factors such as interspecific interactions could be stabilizing the response of *S. parvula* to climate change if they are exerting selective pressure on phenology in opposing directions (Elzinga et al., 2007; Strauss and Irwin, 2004). The phenology of the endemic *S. bushii* was marginally described by climate change, as indicated by difference in phenology since 1970. Interestingly, the direction of the relationship between climate change and phenology is counter-intuitive. *Scutellaria bushii* is blooming marginally later through time, not earlier as I predicted and as seen in *D. treleasei*. Climate change in Missouri and Arkansas is driven by two main factors, minimum temperature and precipitation. Unlike many species, the phenology of *S. bushii* may not be responding to temperature, since minimum temperatures are increasing and should result in the advancement of flowering. Precipitation has increased by roughly 20 cm (20%) over the last century, may be driving the phenology of *S. bushii*. An increase in precipitation could lead to later onset of flowering or to a longer flowering period. However, the low replication of scorable specimens reduced my ability to rigorously test the prediction that intrinsic factors drive the phenological response of *S. bushii* to climate change, and this pattern should be interpreted with caution. Detailed

field experiments should be conducted to establish if this pattern is real and to explicitly test the mechanisms potentially leading to the phenological shifts seen in *S bushii*.

This is the first study of which I am aware that documents the phenological shifts of plant species using a continuous phenophase measure. A continuous measure allowed me to disentangle the effect of collection date from phenological stage, which is a confounding factor in most phenological studies using herbarium specimens. Most studies rely on more general categories, such as in fruit or in flower, and could be misleading if collection date and phenophase are correlated through time. Herbarium records are an abundant and important resource for understanding the effects of climate change on phenology. However, data collected from these records should be analyzed carefully, and the results interpreted with caution. Incorporating vital biological information, such as the developmental stage of the individual, will improve our understanding and prediction of species responses to climate change. Understanding the determinants of plant phenology allows scientists to summarize and categorize the types of species that will respond to climate change and in which direction they will respond. This broader scale understanding will improve our ability to predict which species should be of future conservation concern and those that may have lower risk of decline.

Figure 5-1. A map of the 21 weather stations (●) in Missouri and Arkansas from which data were summarized in order to investigate changes in climate through time (1987-2009). Stations were chosen based on duration of data and location in order to account for spatial variation in climate throughout the region in which the herbarium specimens were collected.
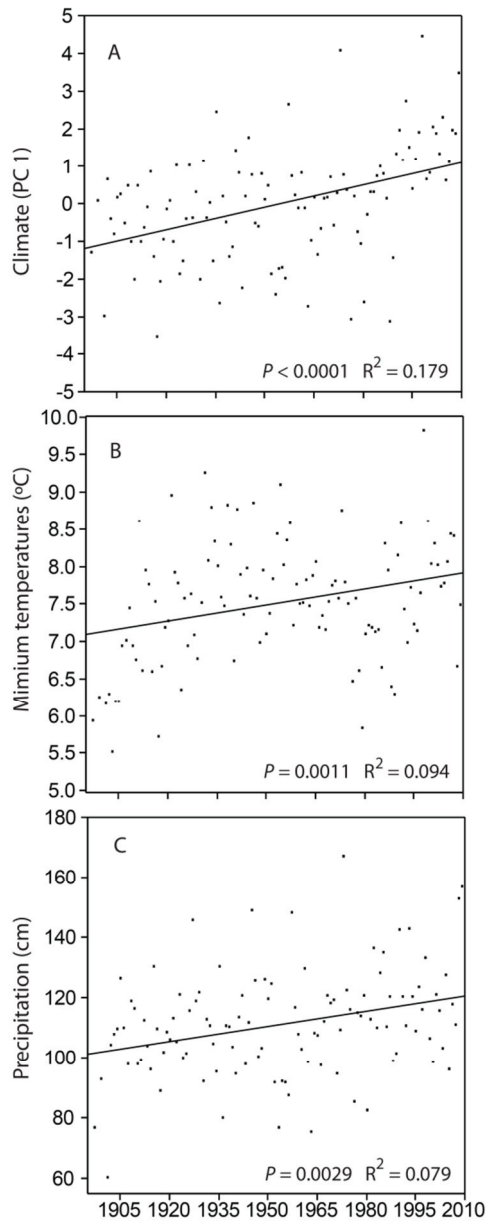
Figure 5-2. Changes in climatic variables between 1897 and 2009 in Missouri and Arkansas as documented by 21 weather stations, including (A) the composite variable Climate, defined as the first principle component (PC 1) from a principle components analysis of four abiotic variables (i.e., mean temperature, maximum temperature, minimum temperature, and precipitation), (B) average monthly minimum temperature (ºC), and (C) total annual precipitation (cm).
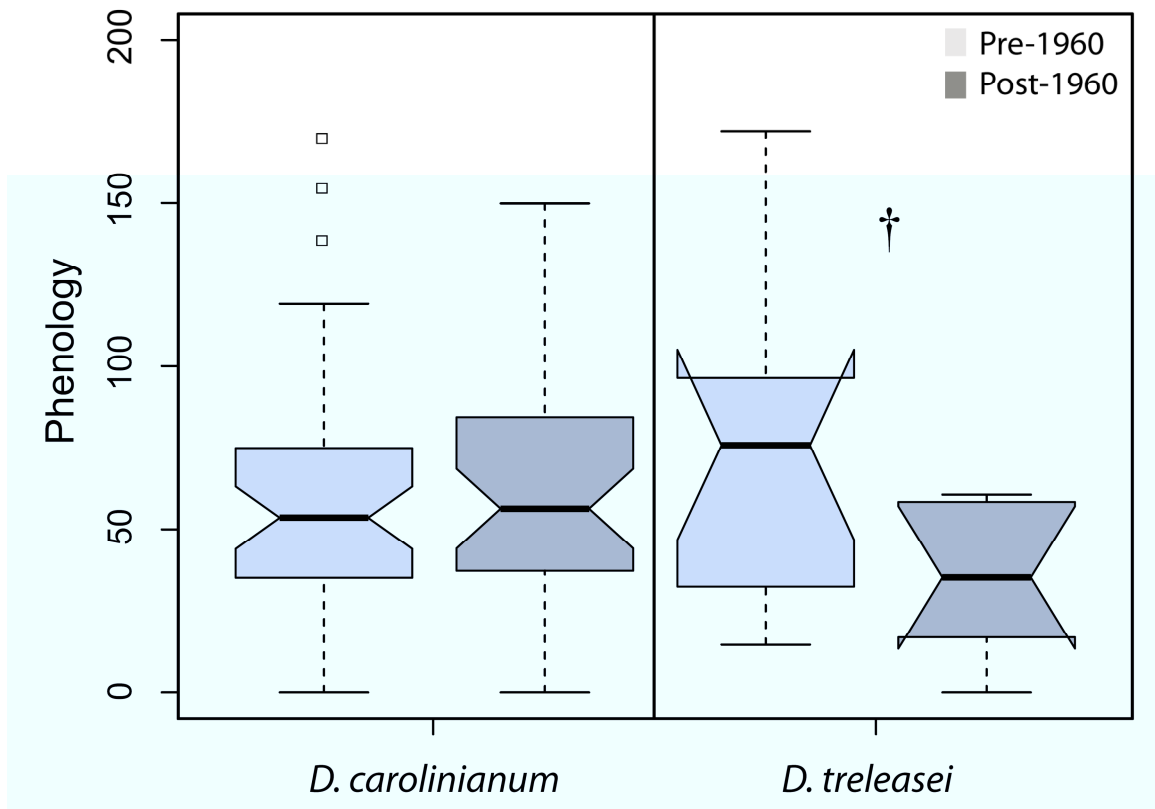
Figure 5-3. Phenology of an endemic plant, *Delphinium treleasei*, and its widespread

congener, *D. carolinianum*, during two period of time, before and after 1960 (pre- and

post-1960, respectively). Phenology is defined as the product of collection date and

phenophase, which is a continuous variable that describes the developmental stage of an

individual. Statistical significance is denoted by a dagger (†) for results with *P* < 0.075.

Figure 5-4. Phenology of an endemic plant, *Scutellaria bushii* (A), and two widespread

congeners, *S. ovata* (B) and *S. parvula* (C) during the past 40 to 105 years in Missouri

and Arkansas. Phenology is defined as the product of phenophase and collection date,

and only *S. bushii* had a marginally significant response to climate change through time.

CHAPTER SIX

Pages 106 to 111

CONCLUSION

The goal of this dissertation was to determine the relationship between reproduction and rarity in three glade-endemic plants based on predictions developed from two prominent ecological principles, reproductive assurance and stress-adaptation. I confirm the predicted that locally-abundant, regionally-rare species (LARR) are more resistant to stressful abiotic conditions and have more specialized reproductive systems than the CC species. Moreover, the LARR and CC species responded differently to regional climate change, which could lead to a 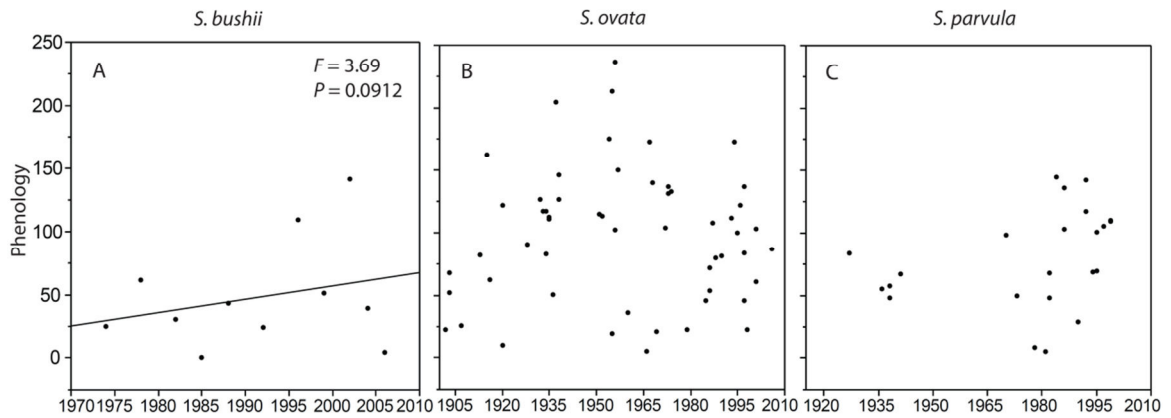shift in their interspecific interactions. By coalescing abiotic and biotic mechanisms into a suite of predictions that could be tested simultaneously, this research is more biologically realistic that previous studies of rarity and reproduction and corroborates the necessity for a more nuanced theory relating the two.

Contrary to the traditional prediction that rare species have more generalized reproductive systems, two of the three LARR species studied in this dissertation had larger flowers and more specialized pollination systems in comparison to their CC species. This pattern confirms my predictions that optimal resource allocation differed between congeners and that the LARR taxa had traits that are associated with higher outcrossing rates. Therefore, factors other than reproductive assurance via low pollen availability may be influencing the pollination biology and reproductive success of these species, such as offspring quality and local-adaptation. The LARR species did not differ consistently from the CC species in vegetative traits that are associated with stress-adaptation and do not appear to be more stress tolerant than the CC species. While some traits indicate that the LARR species are not locally adapted to the glade environment, I

could not measure certain key traits (i.e., biomass and root to shoot ratios) that may affect their ability to tolerate stressful conditions.

Experimental manipulations of abiotic conditions (i.e., water availability and temperature) confirm that the glade endemic plants are more resistant to high stress conditions. The CC species had reductions in biomass and growth parameters with an increase in stress, and the LARR species did not. Moreover, allocation to reproductive output differed between two of the three pairs of congeners. The LARR species had fewer, higher-quality offspring than their CC species, and stress level did not affect offspring quality or quantity in the LARR species. Offspring quality may increase survival rates and population viability in more stressful habitats. When under low stress conditions, the CC species produced many more seeds than the LARR species, but seedset was equivalent to their LARR congeners when stress was high. Differences in reproductive allocation between congeners corroborate my prediction that production of high quality offspring is adaptive in stressful environments. Greater offspring abundance in the CC species may allow them to invade novel habitats with a greater range of abiotic conditions, and therefore may contribute to their larger bigeographic ranges. The CC species also had traits associated with greater competitive ability for pollinators, such as being taller and producing more flowers. However, total display size did not differ, since the LARR species had fewer but marginally larger flowers.

Field competition experiments determined that the LARR and CC species did not differ in their ability to compete for pollinators and that pollinator behavior (visit duration) also did not differ either between congeners or treatments. Landscape-scale spatial variation in floral traits was mirrored by pollinator behavior; plants at sites with

shower plants were visited more frequently than those at sites with less attractive individuals. Interestingly, one LARR species, *D. treleasei*, had greater reproductive success (more pollen tubes germinating down the style) than its CC species at the site with higher visitation rates, despite the fact that visitation rate and visit duration were not different between congeners. More germinating pollen tubes without a difference in pollinator effort suggests that the LARR species has greater proportional pollination success (e.g., higher fertilization rates per unit of pollination effort). This pattern supports my prediction that specialized pollination systems are more efficient and result in the transfer of high quality pollen. Similarly, the *Echinacea* species differed in pollen deposition rates at one site. *Echinacea paradoxa* (LARR) had more pollen on their stigmas, and neither visitation rate nor visit duration differed between species. However, this difference did not result in greater reproductive success of the LARR species. Pollen tube growth was low for both species, which could reflect low pollen quality or a reduction of pollen tube growth in cut inflorescences. These results indicate an important role for large-scale mechanisms and pollination specialization in determining local reproductive success of LARR species and their CC species.

Finally, this work documents the differential responses of LARR and CC species in their responses to climate change. The CC species, which I predicted would be more responsive to regional climate change, have not changed in their phenological patterns. The LARR species, on the other hand, are responding to climate change, but their phenological shifts are in opposing directions. One species, *D. treleasei*, is advancing in flowering date and the other, *S. bushii*, is delaying. The variation in responses between the LARR species suggests that they are responding to different climatic variables.

Delphinium treleasei is blooming earlier, which is consistent with higher minimum temperatures. *Scutellaria bushii*, which is blooming later, may be responding to an increase in precipitation that has occurred in this region. Few studies have looked at the role of precipitation on floral phenology, and this study indicates that it warrants more exploration. The differences between congeners in response to climate change imply that interspecific interactions may also shift with the new abiotic conditions. The *Delphinium* species are converging in their phenologies, which could result in competition for shared pollinators or greater hybridization rates.

The research in this dissertation advances our understanding of how locally-abundant, regionally-rare species differ in their intrinsic traits from and in their interactions with their common congeners. I demonstrate that both biotic and abiotic factors, specifically pollination biology and stressful abiotic conditions, may be contributing synergistically to the range restriction of three glade endemic plant species. Determining how biotic and abiotic factors interact to affect species traits, and potentially their distributions, is particularly important for rare and endemic species, many of which are at increasing risk of extinction. Differences in the responses of LARR and CC species to regional climate change further support the need for a more nuanced understanding of the seven types of rarity. In light of the current rate of climate change, a more informative framework with which to predict which rare species are most vulnerable to changes in climate is imperative.

Future work

In this dissertation, I document the unique attributes of three species that fall into one of the seven types of rarity, locally-abundant and regionally rare taxa. Hitherto, the

focus of most research into rarity has focused on those species that are locally-rare, and a robust body of theory and empirical studies explores the ecological and evolutionary causes and consequences of this type of rarity. However, the theory is less developed for the other six types of rarity, which warrant closer examination. In future work I will expand this theory in order to better understand the ecological and evolutionary mechanisms that drive variation in species distributions. Specifically, future work should explore the mechanisms behind the disparity in offspring quality and quantity between congeners by documenting the relationship between mating system and offspring survival in endemic and widespread species.

My research shows that LARR and CC species differ in resource allocation to reproduction, which indicates that offspring quality may be adaptive in stressful habitats. In order to more fully understand what excludes the LARR species from non-glade habitats, I advise that future research explore the role of propagule pressure and offspring quality on the invasion success of the LARR and CC species into novel habitats and on survival in high stress environments. While greenhouse studies can inform how species respond to stress under controlled conditions, the abiotic conditions on the glades cannot be reproduced. Field studies should be conducted in order to confirm that these patterns are consistent in more realistic conditions and in the context of natural ecological communities.

Research into additional pairs of related species, which vary in their distributions and in the habits on which they specialize, will determine how generalizable the results from this research are. If rare species that are habitat specialists are more dependent on their coevolved pollinators for persistence, they may be more susceptible to pollinator

111

declines. The concomitant decline of oligolectic (specialist), pollinating insects and pollinator-dependent plants in Europe (Biesmeijer et al., 2006) illustrates the need for a more holistic understanding of the mechanisms determining species biogeographical ranges, particularly for rare and endemic species, many of which are at increasing risk of extinction. Coalescing abiotic and biotic mechanisms into a suite of predictions that can be tested simultaneously will not only make the results more biologically realistic, but it will also make them more relevant. With a greater understanding of the factors that determine variation in species distributions, we will be better equipped to protect, conserve and restore Earth's declining biodiversity in a rapidly changing environment.

LITERATURE CITED

Abrams, P.A., Rueffler, C. & Kim, G. (2008) Determinants of the strength of disruptive and/or divergent selection arising from resource competition. *Evolution*, **62**, 1571-1586.

Anderson, B. & Johnson, S.D. (2008) The geographical mosaic of coevolution in a plant-pollinator mutualism. *Evolution; international journal of organic evolution*, **62**, 220-225.

Anderson, I.A. & Busch, J.W. (2006) Pollinator-mediated selection weakens floral integration in self-compatible taxa of *Leavenworthia* (Brassicaceae). *American Journal of Botany*, **93**, 860-867.

Angert, A.L. (2009) The niche, limits to species distributions, and spatiotemporal variation in demography across the elevation ranges of two monkeyflowers. *Proceedings of the National Academy of Sciences of the United States of America*, **106 Suppl**, 19693-19698.

Angert, A.L., Horst, J., Huxman, T.E., and Venable, D.L. (2010) Phenotypic plasticity and precipitation response in Sonoran desert winter annuals. *American Journal of Botany*, **97**, 405-411.

Aragón, C.F., Escudero, A. & Valladares, F. (2008) Stress-induced dynamic adjustments of reproduction differentially affect fitness components of a semi-arid plant. *Journal of Ecology*, **96**, 222-229.

Aronson, J., Kigel, A., Shmida, A., & Klein, J. (1992) Adaptive phenology of desert and Mediterranean populations of annual plants grown with and without water stress. *Oecologia*, **89**, 17-26.

Armbruster, P. & Reed, D.H. (2005) Inbreeding depression in benign and stressful environments. *Heredity*, **95**, 235-242.

Badeck, F.W., Bondeau, A., Bottcher, K., Doktor, D., Lucht, W., Schaber, J. & Sitch, S. (2004) Responses of spring phenology to climate change. *New Phytologist*, **162**, 295-309.

Baker, H.G. (1955) Self-compatibility and establishment after "long-distance" dispersal. *Evolution,* **9**, 347-349

Baskin, J.M. & Baskin, C.C. (1974) Some aspects of the autecology of prairie larkspur (*Delphinium virescens*) in Tennessee cedar glades. *American Midland Naturalist*, **92**, 58-71.

Baskin, J.M. & Baskin, C.C. (1982) Ecological life cycle and temperature relations of seed germination and bud growth of *Scutellaria parvula*. *Bulletin of the Torrey Botanical Club*, **109**, 1-6.

Baskin, J.M. & Baskin, C.C. (1985) Photosynthetic pathway in 14 southeastern cedar glade endemics, as revealed by leaf anatomy. *American Midland Naturalist*, **114**, 205-208.

Baskin, J.M. & Baskin, C.C. (1988) Endemism in rock outcrop plant communities of unglaciated eastern United States: An evaluation of the roles of the edaphic, genetic and light factors. *Journal of Biogeography*, **15**, 829-840.

Baskin, C.C., Baskin, J.M. & Quarterman, E. (1972) Observations on the Ecology of *Astragalus tennesseensis*. *American Midland Naturalist*, **88**, 167-182.

Bell, J.M., Karron, J.D. & Mitchell, R.J. (2005) Interspecific competition for pollination lowers seed production and outcrossing in *Mimulus ringens*. *Ecology*, **86**, 762-771.

Bevill, R.L. & Louda, S.M. (1999) Comparisons of related rare and common species in the study of plant rarity. *Conservation Biology*, **13**, 493-498.

Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemuller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J., and Kunin, W.E. (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, **313**, 351-354

Brisson, J.A, Strasburg, J.L. & Templeton, A.R. (2003) Impact of fire management on the ecology of collared lizard (*Crotaphytus collaris*) populations living on the Ozark Plateau. *Animal Conservation*, **6**, 247-254.

Brown, B.J., Mitchell, R.J. & Graham, S.A. (2002) Competition for pollination between an invasive species (purple loosestrife) and a native congener. *Ecology*, **83**, 2328-2336.

Brown, B. & Mitchell, R. (2001) Competition for pollination: Effects of pollen of an invasive plant on seed set of a native congener. *Oecologia*, **129**, 43-49.

Bryant, J.P., Chapin, F.S. & Klein, D.R. (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, **40**, 357-368.

Chapin, F. S., I., Autumn, K. & Pugnaire, F. (1993) Evolution of suites of traits in response to environmental stress. *The American Naturalist*, **142**, S78-S92.

Chase, J. & Leibold, M. (2003) *Ecological niches: linking classical and contemporary approaches (interspecific interactions)*. University of Chicago Press, Chicago, IL.

Chase, J.M., Abrams, P.A., Grover, J.P., Diehl, S., Chesson, P., Holt, R.D., Richards, S.A., Nisbet, R.M. & Case, T.J. (2002) The interaction between predation and competition: A review and synthesis. *Ecology Letters*, **5**, 302-315.

Cheptou, P.O., Berger, A., Blanchard, A., Collin, C. & Escarre, J. (2000) The effect of drought stress on inbreeding depression in four populations of the Mediterranean outcrossing plant *Crepis sancta* (Asteraceae). *Heredity*, **85 Pt 3**, 294-302.

Cleland, E.E., Chiariello, N.R., Loarie, S.R., Mooney, H.A. & Field, C.B. (2006) Diverse responses of phenology to global changes in a grassland ecosystem. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 13740-13744.

Clinebell, R.R.I. & Bernhardt, P. (1998) The pollination ecology of five species of *Penstemon* (Scrophulariaceae) in the tallgrass prairie. *Annals of the Missouri Botanical Garden*, **85**, 126-136.

Dafni, A. (1993) *Pollination ecology: A practical approach.* Oxford University Press, Oxford, U. K.

Darwin, C. (1877) *On the various contrivances by which British and foreign orchids are fertilized* (ed. D. Appleton). New York.

Del Castillo, R.F. & Trujillo, S. (2008) Effect of inbreeding depression on outcrossing rates among populations of a tropical pine. *The New Phytologist*, **177**, 517-24.

Devaux, C. & Lande, R. (2009) Displacement of flowering phenologies among plant species by competition for generalist pollinators. *Journal of evolutionary biology*, **22**, 1460-1470.

Dickson, C.E. & Petit, S. (2006) Effect of individual height and labellum colour on the pollination of *Caladenia* (syn. Arachnorchis) *behrii* (Orchidaceae) in the northern Adelaide region, South Austrailia. *Plant Systematics and Evolution*, **262**, 65-74.

Domínguez Lozano, F. & Schwartz, M.W. (2005) Patterns of rarity and taxonomic group size in plants. *Biological Conservation*, **126**, 146-154.

Dose, V. & Menzel, A. (2004) Bayesian analysis of climate change impacts in phenology. *Global Change Biology*, **10**, 259-272.

Eckert, C.G., Ozimec, B., Herlihy, C.R., Griffin, C.A., & Routley, M.B. (2009) Floral morphology mediates temporal variation in the mating system of a self-compatible plant. *Ecology*, **90**, 1540-1548.

Elliott, S.E. (2009) Subalpine bumble bee foraging distances and densities in relation to flower availability. *Environmental Entomology*, **38**, 748-756.

Ellison, A.M. & Parker, J.N. (2002) Seed dispersal and seedling establishment of *Sarracenia purpurea* (Sarraceniaceae). *American Journal of Botany*, **89**, 1024-1026.

Elzinga, J.A., Atlan, A., Biere, A., Gigord, L., Weis, A.E. & Bernasconi, G. (2007) Time after time: Flowering phenology and biotic interactions. *Trends in Ecology & Evolution*, **22**, 432-439.

Erhardt, A. & Rusterholz, H.-P. (1998) Do Peacock butterflies (Inachis io L.) detect and prefer nectar amino acids and other nitrogenous compounds? *Oecologia*, **117**, 536-542.

Espeland, E.K. & Emam, T.M. (2011) The value of structuring rarity: The seven types and links to reproductive ecology. *Biodiversity and Conservation*, 963-985.

Fargione, J., Brown, C.S. & Tilman, D. (2003) Community assembly and invasion: An experimental test of neutral versus niche processes. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 8916-8920.

Fausto , Jr ., J.A., Eckhart, V.M. & Geber, M.A. (2001) Reproductive assurance and the evolutionary ecology of self-pollination in *Clarkia xantiana*. *America Journal of Botany*, **88**, 1794-1800.

Fenster, C.B., Armbruster, W.S., Wilson, P., Dudash, M.R. & Thomson, J.D. (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics*, **35**, 375-403.

Firman, R.C. & Simmons, L.W. (2008) Polyandry facilitates postcopulatory inbreeding avoidance in house mice. *Evolution*, **62**, 603-611.

Fishman, L. & Willis, J.H. (2006) Pollen limitation and natural selection on floral characters in the yellow monkeyflower, Mimulus guttatus. *New Phytologist*, 1-9.

Flanagan, R.J., Mitchell, R.J. & Karron, J.D. (2010) Increased relative abundance of an invasive competitor for pollination, *Lythrum salicaria*, reduces seed number in *Mimulus ringens*. *Oecologia*, **164**, 445-454.

Forrest, J., Inouye, D.W. & Thomson, J.D. (2010) Flowering phenology in subalpine meadows: does climate variation influence community co-flowering patterns? *Ecology*, **91**, 431-440.

Fox, C.W. & Reed, D.H. (2010) Inbreeding depression increases with environmental stress: An experimental study and meta-analysis. *Evolution*, 246-258.

Foxe, J.P., Slotte, T., Stahl, E.A., Neuffer, B., Hurka, H. & Wright, S.I. (2009) Recent speciation associated with the evolution of selfing in *Capsella*. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 5241-5245.

Galloni, M. (2008) Visitor diversity and pollinator specialization in Mediterranean legumes. *Flora*, **203**, 94-102.

Georgescu, M., Lobell, D.B. & Field, C.B. (2011) Direct climate effects of perennial bioenergy crops in the United States. *Proceedings of the National Academy of Sciences*, **108**, 4307-4312.

Gregory, R.D. & Gaston, K.J. (2000) Explanations of commonness and rarity in British breeding birds: Separating resource use and resource availability. *Oikos*, **88**, 515-526.

Grime, J.P. (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist*, **111**, 1169–1194.

Halpern, S.L., Adler, L.S. & Wink, M. (2010) Leaf herbivory and drought stress affect floral attractive and defensive traits in *Nicotiana quadrivalvis*. *Oecologia*, **163**, 961-971.

Harder, L.D., Richards, S.A. & Routley, M.B. (2008) Effects of reproductive compensation, gamete discounting and reproductive assurance on mating-system diversity in hermaphrodites. *Evolution*, **62**, 157-172.

Hardin, G. (1960) The competitive exclusion principle. *Science,* **131**, 1292-1297.

Hauser, T.P. & Loeschcke, V. (1996) Drought Stress and Inbreeding Depression in *Lychnis flos-cuculi* (Caryophyllaceae). *Evolution*, **50**, 1119-1126.

Heschel, M.S., Hausmann, N. & Schmitt, J. (2005) Testing for stress-dependent inbreeding depression in *Impatiens capensis* (Balsaminaceae). *American Journal of Botany*, **92**, 1322-1329.

Ibanez, I., Primack, R.B., Miller-Rushing, A.J., Ellwood, E., Higuchi, H., Lee, S.D., Kobori, H. & Silander, J.A. (2010) Forecasting phenology under global warming.

Philosophical Transactions of the Royal Society B: Biological Sciences, **365**, 3247-3260.

Rosenzweig, C., Casassa, G., Karoly, D.J., Imeson, A., Liu, C., Menzel, A., Rawlings, S., Root, T.L., Seguin, B. & Tryjanowiski, P. (2007) Assessment of observed changes and responses in natural and managed systems. *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contributions of Working Group II to the Fourth Assessment Report of te Intergovernmental Panel on Climate Change.* (eds. M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J. van der Linden, & C.E. Hanson) Cambridge University Press, Cambridge, U.K., 79-131.

Internicola, A.I., Page, P.A., Bernasconi, G. & Gigord, L.D.B. (2007) Competition for pollinator visitation between deceptive and rewarding artificial inflorescences: an experimental test of the effects of floral colour similarity and spatial mingling. *Functional Ecology*, **21**, 864-872.

Ishii, H.S., Hirabayashi, Y. & Kudo, G. (2008) Combined effects of inflorescence architecture, display size, plant density and empty flowers on bumble bee behaviour: Experimental study with artificial inflorescences. *Oecologia*, **156**, 341-350.

Izco, J. (1998) Types of rarity of plant communities. *Journal of Vegetation Science*, **9**, 641-646.

Jacquemyn, H. (2005) Does nectar reward affect rarity and extinction probabilities of orchid species? An assessment using historical records from Belgium and the Netherlands. *Biological Conservation*, **121**, 257-263.

Jacquemyn, H. & Brys, R. (2008) Density-dependent mating and reproductive assurance in the temperate forest herb *Paris quadriolia* (Trilliaceae). *American Journal of Botany*, **95**, 294-298.

Jenkins, S.E. & Jenkins, M.A. (2006) Effects of Prescribed Fire on the Vegetation of a Savanna-Glade Complex in Northern Arkansas. *Southeastern Naturalist*, **5**, 113-126.

Hegland, S.J. & Totland, Ø. (2008) Is the magnitude of pollen limitation in a plant community affected by pollinator visitation and plant species specialisation levels? *Oikos*, **117**, 883-891.

Karron, J.D., Mitchell, R.J., Holmquist, K.G., Bell, J.M. & Funk, B. (2004) The influence of floral display size on selfing rates in *Mimulus ringens*. *Heredity*, **92**, 242-248.

Karron, J.D., Holmquist, K.G., Flanagan, R.J. & Mitchell, R.J. (2009) Pollinator visitation patterns strongly influence among-flower variation in selfing rate. *Annals of Botany*, **103**, 1379-1383.

Kelly, C.K., Woodward, F.I. & Crawley, M.J. (1996) Ecological correlates of plant range size: Taxonomies and phylogenies in the study of plant commonness and rarity in Great Britain. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **351**, 1261-1269.

Kennedy, B.F. & Elle, E. (2008) The inbreeding depression cost of selfing: Importance of flower size and population size in *Collinsia parviflora* (Veronicaceae). *American Journal of Botany*, **95**, 1596-1605.

Knight, T.M., Steets, J.A., Vamosi, J.C., Mazer, S.J., Burd, M., Campbell, D.R., Dudash, M.R., Johnston, M.O., Mitchell, R.J. & Ashman, T.L. (2005) Pollen limitation of

plant reproduction: Pattern and process. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 467-497.

Kremen, C., Williams, N.M. & Thorp, R.W. (2002) Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 16812-16816.

Kruckeberg, A.R. & Rabinowitz, D. (1985) Biological aspects of endemism in higher plants. *Annual Review of Ecology and Systematics*, **16**, 447-479.

Kucera, C.L. & Martin, S.C. (1957) Vegetation and soil relationships in the glade region of the southwestern Missouri Ozarks. *Ecology*, **38**, 285-291.

Kudo, G. & Harder, L.D. (2005) Floral and inflorescence effects on variation in pollen removal and seed production among six legume species. *Functional Ecology*, **19**, 245-254.

Kudo, G. & Hirao, A.S. (2005) Habitat-specific responses in the flowering phenology and seed set of alpine plants to climate variation: Implications for global-change impacts. *Population Ecology*, **48**, 49-58.

Kudo, G., Ida, T.Y. & Tani, T. (2008) Linkages between phenology, pollination, photosynthesis, and reproduction in deciduous forest understory plants. *Ecology*, **89**, 321-331.

Kunin, W.E. & Gaston, K.J. (1997) Rare-common differences: An overview. *The biology of rarity: causes and consequences of rare-common differences*. (eds. K.J. Kunin, W.E. & Gaston), pp. 12-29. Chapman & Hall, London, UK.

Lambert, A.M., Miller-Rushing, A.J. & Inouye, D.W. (2010) Changes in snowmelt date and summer precipitation affect the flowering phenology of *Erythronium grandiflorum* (glacier lily; Liliaceae). *American Journal of Botany*, **97**, 1431-1437.

Lau, J.A., McCall, A.C., Davies, K.F., McKay, J.K. & Wright, J.W. (2008) Herbivores and edaphic factors constrain the realized niche of a native plant. *Ecology*, **89**, 754-762.

Lavergne, S., Thompson, J.D., Garnier, E. & Debussche, M. (2004) The biology and ecology of narrow endemic and widespread plants: A comparative study of trait variation in 20 congeneric pairs. *Oikos*, **107**, 505-518.

Learn, G.H.J., & Schaal, B.A. (1987) Population subdivision for ribosomal DNA repeat variants in *Clematis fremontii*. *Evolution*, **41**, 433-438.

Lipow, S., Bernhardt, P. & Vance, N. (2002) Comparative rates of pollination and fruit set in widely separated populations of a rare orchid (*Cypripedium fasciculatum*). *International Journal of Plant Sciences*, **163**, 775-782.

Lloyd, K.M., Lee, W.G. & Wilson, J.B. (2002) Competitive abilities of rare and common plants: Comparisons using *Acaena* (Rosaceae) and *Chionochloa* (Poaceae) from New Zealand. *Conservation Biology*, **16**, 975-985.

Lortiek, C.J., Michalet, R., Paolini, L., Pugnaireq, F.I., Newingham, B., Aschehoug, E.T., Armasq, C., Kikodze, D. & Cook, B.J. (2002) Positive interactions among alpine plants increase with stress. *Zoologica*, **417**, 844-848.

MacDougall, A.S., Boucher, J., Turkington, R. & Bradfield, G.E. (2006) Patterns of plant invasion along an environmental stress gradient. *Journal of Vegetation Science*, **17**, 47-56.

Maestre, F.T., Callaway, R.M., Valladares, F. & Lortie, C.J. (2009) Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology*, **97**, 199-205.

Martén-Rodríguez, S. & Fenster, C.B. (2010) Pollen limitation and reproductive assurance in Antillean Gesnerieae: A specialists vs. generalist comparison. *Ecology*, **91**, 155-165.

McLernon, S.M., Murphy, S.D. & Aarssen, L.W. (1996) Heterospecific pollen transfer between sympatric species in a midsuccssional old-field community. *American Journal of Botany*, **83**, 1168-1174.

Mevi-Schutz, J. & Erhardt, A. (2005) Amino acids in nectar enhance butterfly fecundity: a long-awaited link. *The American naturalist*, **165**, 411-419.

Miller-Rushing, a J. & Inouye, D.W. (2009) Variation in the impact of climate change on flowering phenology and abundance: An examination of two pairs of closely related wildflower species. *American Journal of Botany*, **96**, 1821-1829.

Miller-Rushing, A.J., Katsuki, T., Primack, R.B., Ishii, Y., Lee, S.D. & Higuchi, H. (2007) Impact of global warming on a group of related species and their hybrids: Cherry tree (Rosaceae) flowering at Mt. Takao, Japan. *American Journal of Botany*, **94**, 1470-1478.

Miller-Rushing, A.J. & Primack, R.B. (2008) Global warming and flowering times in Thoreau′s Concord: A community perspective. *Ecology*, **89**, 332-341.

Mitchell, R.J., Karron, J.D., Holmquist, K.G. & Bell, J.M. (2004) The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. *Functional Ecology*, **18**, 116-124.

Mitchell, R.J., Flanagan, R.J., Brown, B.J., Waser, N.M. & Karron, J.D. (2009) New frontiers in competition for pollination. *Annals of Botany*, **103**, 1403-1413.

Mitchell, R.J., Irwin, R.E., Flanagan, R.J. & Karron, J.D. (2009) Ecology and evolution of plant-pollinator interactions. *Annals of Botany*, **103**, 1355-1363.

Moeller, D.A. & Geber, M.A. (2005) Ecological context of the evolution of self-pollination in *Clarkia xantiana*: population size, plant communities, and reproductive assurance. *Evolution*, **59**, 786-799.

Muneepeerakul, R., Bertuzzo, E., Lynch, H.J., Fagan, W.F., Rinaldo, A. & Rodriguez-Iturbe, I. (2008) Neutral metacommunity models predict fish diversity patterns in Mississippi-Missouri basin. *Nature*, **453**, 220-222.

Murray, B.R., Thrall, P.H., Gill, A.M. & Nicotra, A.B. (2002) How plant life-history and ecological traits relate to species rarity and commonness at varying spatial scales. *Austral Ecology*, **27**, 291-310.

Naug, D. & Arathi, H.S. (2007) Receiver bias for exaggerated signals in honeybees and its implications for the evolution of floral displays. *Biology Letters*, **3**, 635-637.

Neiland, M.R.M. & Wilcock, C.C. (1998) Fruit set, nectar reward, and rarity in the Orchidaceae. *American Journal of Botany*, **85**, 1657-1671.

Nelson, P. & Ladd, D. (1982) Preliminatry report on the identification, distribution and classification of Missouri glades. *Proceedings of the Seventh North American Prairie Conference*. (ed. C. Kucera), pp. 59-76. Southwestern Missouri State University, Springfield, MO.

Nieuwstadt, M.G.L. van & Ruano Iraheta, C.E. (1996) Relation between size and foraging range in stingless bees (Apidae, Meliponinae). *Apidologie*, **27**, 219-228.

Ogden, E.C., Raynor, G.S., Hayes, J., DM, L. & Haines, J. (1974) *Manual for sampling air-borne pollen*. Hafner Press, New York.

Osborne, J.L., Martin, A.P., Carreck, N.L., Swain, J.L., Knight, M.E., Goulson, D., Hale, R.J. & Sanderson, R. a. (2008) Bumblebee flight distances in relation to the forage landscape. *The Journal of animal ecology*, **77**, 406-415.

Parachnowitsch, A.L. & Kessler, A. (2010) Pollinators exert natural selection on flower size and floral display in *Penstemon digitalis*. *New Phytologist*, **188**, 393-402.

Pasquet, R.S., Peltier, A., Hufford, M.B., Oudin, E., Saulnier, J., Paul, L., Knudsen, J.T., Herren, H.R. & Gepts, P. (2008) Long-distance pollen flow assessment through evaluation of pollinator foraging range suggests transgene escape distances. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 13456-13461.

Pohlman, C.L., Nicotra, A.B. & Murray, B.R. (2005) Geographic range size, seedling ecophysiology and phenotypic plasticity in Australian *Acacia* species. *Journal of Biogeography*, **32**, 341-351.

Quarterman, E. (2007) Ecology of cedar glades. I. Distribution of glade flora in Tennessee. *Bulletin of the Torrey Botanical Club*, **77**, 1-9.

R Development Core Team. (2008) R: A language and environment for statistical computing.

Rabinowitz, D., Rapp, J.K., Sork, V.L., Rathcke, B.J., Reese, G.A. & Weaver, C, J. (1981) Phenological properties of wind- and insect-pollinated prairie plants. *Ecology*, **62**, 49-56.

Rader, R., Edwards, W., Westcott, D.A., Cunningham, S.A. & Howlett, B.G. (2011) Pollen transport differs among bees and flies in a human-modified landscape. *Diversity and Distributions*, **17**, 519-529.

Rafferty, N.E., and Ives, A.R. (2011) Effects of experimental shifts in flowering phenology on plant-pollinator interactions. *Ecology Letters*, **14**, 69-74.

Reynolds, R.J. & Fenster, C.B. (2008) Point and interval estimation of pollinator importance: a study using pollination data of *Silene caroliniana*. *Oecologia*, **156**, 325-332.

Ricketts, T.H., Regetz, J., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Bogdanski, A., Gemmill-Herren, B., Greenleaf, S.S., Klein, A.M., Mayfield, M.M., Morandin, L.A., Ochieng, A., Potts, S.G. & Viana, B.F. (2008) Landscape effects on crop pollination services: Are there general patterns? *Ecology Letters*, **11**, 499-515.

Riginos, C., Heschel, M.S. & Schmitt, J. (2007) Maternal effects of drought stress and inbreeding in *Impatiens capensis* (Balsaminaceae). *American Journal of Botany*, **94**, 1984-1991.

Rodríguez-Gironés, M.A. & Santamaría, L. (2007) Resource competition, character displacement, and the evolution of deep corolla tubes. *The American naturalist*, **170**, 455-464.

Rosenzweig, C., Casassa, G., Karoly, D.J., Imeson, A., Liu, C. Menzel, A., Rawlins, S., Root, T.L., Seguin, B., & Tryjanowski, P. (2007) Assessment of observed changes and responses in natural and managed systems. *Climate Change 2007: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds. M.L.

Parry, O.F. Canziani, J.P. Palutikof, P.J. van der Linden, & C.E. Hanson) Cambridge University Press, Cambridge, U.K. 79-131.

Ryberg, W.A. & Chase, J.M. (2007) Predator-dependent species-area relationships. *American Naturalist*, **170**, 636-642.

Rymer, P., Whelan, R., Ayre, D., Weston, P. & Russell, K. (2005) Reproductive success and pollinator effectiveness differ in common and rare species (Proteaceae). *Biological Conservation*, **123**, 521-532.

SAS Institute, Inc. (2009) JMP Statistical Discover Software 8.

Sherry, R.A., Zhou, X., Gu, S., Arnone, J.A., Schimel, D.S., Verburg, P.S., Wallace, L.L. & Luo, Y. (2007) Divergence of reproductive phenology under climate warming. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 198-202.

SigmaScan Pro 5.0. (2002) Systat Software Inc.

Silvertown, J.; Franco M., Pisanty I., and Mendoza, A.M. (1993) Comparative plant demography – relative importance of life-cycle components to the finite rate of increase in woody and herbaceous perennials. *Journal Ecology*, **81**, 465-476.

Soberón, J. (2007) Grinnellian and Eltonian niches and geographic distributions of species. *Ecology Letters*, **10**, 1115-1123.

Sprigler,R. & Chang, S. (2009) Pollen limitation and reproduction varies with population size in experimental populations of *Sabatia angularis* (Gentianacea). *Botany*, **87**, 330-338

Strauss, S.Y. & Irwin, R.E. (2004) Ecological and evolutionary consequences of multispecies plant-animal interactions. *Annual Review of Ecology, Evolution, and Systematics*, **35**, 435-466.

Templeton, A.R., Robertson, R.J., Brisson, J. & Strasburg, J. (2001) Disrupting evolutionary processes: The effect of habitat fragmentation on collared lizards in the Missouri Ozarks. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 5426-5432.

Tilman, D. (1976) Ecological competition between algae: Experimental confirmation of resource-based competition theory. *Science*, **192**, 463-465.

Tilman, D. (2004) Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 10854-10861.

Totland, Ø. and Eide, W. (1999) Environmentally-dependent pollen limitation on seed production in alpine *Ranunculus acris*. *Ecoscience*, **6**, 173-179.

Van Zandt, P.A. (2007) Plant defense, growth, and habitat: A comparative assessment of constitutive and induced resistance. *Ecology*, **88**, 1984-1993.

Van Zandt, P.A., Collins, E., Losos, J.B. & Chase, J.M. (2005) Implications of food web interactions for restoration of Missouri Ozark glade habitats. *Restoration Ecology*, **13**, 312-317.

Volkov, I., Banavar, J.R., Hubbell, S.P. & Maritan, A. (2003) Neutral theory and relative species abundance in ecology. *Nature*, **424**, 1035-1037.

Wagenius, S. & Lyon, S. (2010) Reproduction of *Echinacea angustifolia* in fragmented prairie is pollen-limited but not pollinator-limted. *Ecology*, **91**, 733-742.

Walck, J.L., Baskin, J.M. & Baskin, C.C. (1999) Ecology of the endangered species *Solidago shortii*. VII. Survivorship and flowering, and comparison with common, geographically-widespread *Solidago* Species. *Journal of the Torrey Botanical Society*, **126**, 124-132.

Walck, J.L., Baskin, J.M. & Baskin, C.C. (2002) Why is *Solidago shortii* narrowly endemic and *S. altissima* geographically widespread? A comprehensive comparative study of biological traits. *Journal of Biogeography*, **28**, 1221-1237.

Waller, D.M., Dole, J. & Bersch, A.J. (2008) Effects of stress and phenotypic variation on inbreeding depression in *Brassica rapa*. *Evolution*, **62**, 917-931.

Warnock, M.J. (1987) Vicariant distribution of two *Delphinium* species in southeastern United States. *Botanical Gazette*, **148**, 90-95.

Whittall, J.B. & Hodges, S. a. (2007) Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature*, **447**, 706-709.

Wikelski, M., Moxley, J., Eaton-Mordas, A., López-Uribe, M.M., Holland, R., Moskowitz, D., Roubik, D.W. & Kays, R. (2010) Large-range movements of neotropical orchid bees observed via radio telemetry. *PLoS ONE*, **5**, e10738.

Williams, S.E., Williams, Y.M., VanDerWal, J., Isaac, J.L., Shoo, L.P. & Johnson, C.N. (2009) Ecological specialization and population size in a biodiversity hotspot: how rare species avoid extinction. *Proceedings of the National Academy of Sciences of the United States of America*, **106 Suppl**, 19737-19741.

Wilson, P., Castellanos, M.C., Wolfe, A.D. & Thomson, J.D. (2006) Shifts between bee and bird pollination in *Penstemons*. *Plant–Pollinator Interactions: From Specialization to Generalization*. (eds N.M. Waser & J. Ollerton), p. 47–68. The University of Chicago Press, Chicago, IL.

Wright, S. (1922) Coefficients of inbreeding and relationship. *The American Naturalist*, **56**, 330-338.

Yatskievych, G. (1999) *Steyermark's Flora of Missouri, 2nd ed., Vol. 1*. Missouri Department of Conservation, Jefferson City, Missouri.

WASHINGTON UNIVERSITY IN ST. LOUIS

Division of Biology and Biomedical Sciences
Program in Ecology, Evolution, and Population Biology

Dissertation Committee:

Barbara Schaal, Chairperson
Peter Hoch, Advisor
Jim Cheverud
Kenneth M. Olsen
Kevin G. Smith
Alan R. Templeton

CAUSES OF RARITY IN GLADE-ENDEMIC PLANTS: IMPLICATIONS FOR

RESPONSES TO CLIMATE CHANGE

by

Nicole Miller-Struttmann

A dissertation presented to the
Graduate School of Arts and Sciences
of Washington University in
partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

August 2011
Saint Louis, MO

ABSTRACT OF THE DISSERTATION

CAUSES OF RARITY IN GLADE-ENDEMIC PLANTS: IMPLICATIONS FOR

RESPONSES TO CLIMATE CHANGE

by

Nicole Miller-Struttmann

Doctor of Philosophy

Division of Biology and Biology

Program in Ecology, Evolution, and Population Biology

Washington University

St. Louis, MO 2011

This dissertation documents the relationship between stress-adaptation and reproductive specialization in three endemic plant species (*Delphinium treleasei*, *Echinacea paradoxa*, and *Scutellaria bushii*) that are locally abundant but restricted to stressful habitats and their closely-related congeners (*D. carolinianum*, *E. pallida*, *S. ovata*, and *S. parvula*) that have broader habitat use, including hot, xeric habitats. In Chapter Two, I test two hypotheses concerning reproductive specialization in endemic plants and their common congeners (CC). I quantify morphological traits that are associated with stress-adaptation and reproductive specialization, pollinator behavior, and reproductive specialization. The locally abundant, habitat specialist (LAHS) species were significantly different from their CCs in vegetative traits but the direction of the differences was not consistent among genera. On the other hand, two of the three LAHS taxa had larger flowers and fewer dominant pollinators than their CCs.

In Chapter 3, I examine the responses of the LAHS and CC plants to drought and high heat conditions in order to explicitly test the prediction that the LAHS species are more resistant to high-stress environments. The LAHS species were more resistant to stress, and the CC species were not. Moreover, the LAHS species had fewer, higher-quality offspring, whereas the CC species have more seeds of lower quality. In Chapter 4, I test the hypothesis that two LAHS species are poorer competitors for pollinators, as predicted by traditional theory of reproduction in rare species. The congeneric pairs did not compete for pollinators but varied in their morphological traits and reproductive success across sites.

Finally, the research in Chapter 5 explores the response of two LAHS species and their CCs to regional climate change. Using herbarium specimens, I test the prediction that LAHS species that specialize on stressful habitats are less responsive to climate change in comparison to their CC. The LAHS species are responding to climate change by flowering earlier, but none of the widespread species exhibited a change in phenology with climate change. This dissertation supports a more nuanced theory of the relationship between reproductive specialization and rarity that addresses additional factors influencing rare taxa, such as stress-adaptation, and that has important implications for species' responses to climate change.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my husband, Justin (Benny) Struttmann, for all of his support, patience, and humor. I would not have been able to finish this dissertation without his encouragement, logistical support, and ability to make me laugh, which never failed to give me perspective. You inspire me to be the best scientist and individual that I can, and I am forever thankful for your love. I would also like to thank both of my families and my friends for all of their support and for accompanying me on this crazy journey that is graduate school.

I would like to thank my committee for their enormous gift of time and knowledge. To Barbara Schaal, I cannot thank you enough for supporting and believing in me throughout my graduate career. Your advice has always been insightful and much appreciated. I thank Alan Templeton for bestowing as much of his vast knowledge of the glade system, evolutionary ecology, and statistics as I was able to absorb. To Jim Cheverud, I thank you for your statistical insight and generous gift of time over which we discussed it. I thank Ken Olsen for his insight into the evolutionary underpinnings of my research. To Kevin Smith, I thank you for your in-depth analysis of my work. Your advice and feedback have been irreplaceable; they have improved my understanding of the results that I present here and how it pertains to the field of ecology as a whole. Finally, I would like to thank my primary advisor, Peter Hoch. I am truly grateful to you for all of your sage advice and patience. You are a true mentor, and I am here today because of your support, guidance, and balance of honest evaluation and encouragement. Your enthusiasm for science is infectious, and I have greatly enjoyed working with you.

TABLE OF CONTENTS

List of Tables

List of Figures

CHAPTER ONE

Pages 1 to 4

INTRODUCTION

A fundamental goal in ecology and evolutionary biology is to determine the mechanisms that influence species distributions, particularly among rare and widespread species. Both biotic and abiotic factors are thought to contribute to species distributions, particularly in light of their reproductive biology. There are several competing theories contributing to our understanding of the relationship between reproduction and rarity. The first is that of reproductive assurance, that rare species should maintain the ability to self-fertilize in order to buffer reproductive success from variability in pollination success. Rare species are predicted to have higher selfing rates and to be self-compatible (Baker, 1955; Kelly et al., 1996; Neiland and Wilcock, 1998; Izco, 1998; Fausto et al., 2001; Murray et al., 2002; Domínguez Lozano and Schwartz, 2005; Jacquemyn, 2005; Moeller and Geber, 2005). This theory is realistic for locally rare species that may be infrequently visited by pollinators. However, species that are locally abundant but regionally rare may not be limited by pollen availability, and other factors may play a more prominent role in determining their reproductive biology, such as stressful abiotic conditions (Armbruster and Reed, 2005; Fox and Reed, 2011). Rare species may have greater outcrossing rates in order to avoid negative genetic effects of inbreeding or genetic drift common to small population sizes (Wright 1922; Del Castillo and Trujillo, 2008; Firman and Simmons, 2008; Kennedy and Elle, 2008; Espeland and Emam, 2011).

My objective was to examine the relationship between rarity and reproduction in three locally abundant, regionally rare glade plant species that are endemic to stressful habitats, in comparison to widespread, closely-related species that occur on glades but are not restricted to them. Glades are south to southwest facing, rocky outcroppings found throughout the Ozark region of Missouri and Arkansas. They are characterized by high

quantity of exposed bedrock, low herbaceous cover, limited soil structure, and hot and xeric conditions (Baskin and Baskin, 1982; Nelson and Ladd, 1982; Yatskievych, 1999; Templeton et al., 2001). Glades have been of interest to ecologists and evolutionary biologists for decades due to their assemblage of endemic species, many with restricted ranges (Kucera and Martin, 1957; Baskin et al., 1974, 1982; Baskin and Baskin, 1985; Learn and Schaal, 1987; Jenkins and Jenkins, 2006) and their spatial structure, which makes them ideal for studies of dispersal (Brisson et al., 2003), spatial genetic structure (Learn and Schaal, 1987; Templeton et al., 2001; Brisson et al., 2003), metapopulation dynamics (Ryberg and Chase, 2007), and many other ecological and evolutionary topics (e.g., Van Zandt et al., 2005; Van Zandt, 2007).

Glades are also ideal for the study of rarity and reproductive theory, since they serve as habitat for several endemic plant species occurring in sympatry with widespread, related congeners. Therefore, the mechanisms that restrict one species, and not the other, to glades can be explored while controlling for the potential effect of evolutionary history on species traits (Bevill and Louda, 1999). In this dissertation, I test hypotheses concerning the relationship between stress-adaptation and reproductive biology in three endemic glade species (*Delphinium treleasei*, *Echinacea paradoxa*, and *Scutellaria bushii*) in comparison to their widespread, closely-related species (*D. carolinianum*, *E. pallida*, *S. ovata*, and *S. parvula*). Each of the following four chapters has an introduction to the principles behind the specific hypotheses to be tested, new data and analyses, and separate tables and figures.

In the first chapter, I document differences in the morphological traits, pollination biology, and breeding systems of three congeneric species pairs through an *in situ* field,

3

observational study. In the second chapter, I explicitly test three hypotheses: (1) glade endemic species are more resistant to experimental stress, (2) they have floral traits that are associated with decreased attractiveness to pollinators but increased outcrossing rates, and (3) reproductive output is allocated to fewer, higher quality offspring in comparison to their widespread congeners. The research presented in Chapter 4 tests the prediction that the floral traits of two stress-adapted endemic plants confer lower competitive ability for pollination services in comparison to their common congeners. The research in Chapter 5 documents the phenological responses of two of the congeneric species pairs to regional climate change. The final chapter concludes the dissertation with a discussion of the major results and their conservation implications, particularly in light of recent global changes.

CHAPTER TWO

Pages 5 to 31

REPRODUCTIVE BIOLOGY OF HABITAT-SPECIALIST TAXA REVEALS A COMOPLEX RELATIONSHIP BETWEEN RARITY AND REPRODUCTIVE SPECIALIZATION

INTRODUCTION

The relative importance of biotic and abiotic mechanisms in determining species'

ranges is an fundamental debate in ecology and evolutionary biology. Competitive

exclusion (Hardin, 1960; Kunin and Gaston, 1997; Lloyd et al., 2002) and specialization

to abiotic or biotic conditions may restrict the biogeographical range of a species

(Rabinowitz et al., 1981; Kruckeberg and Rabinowitz, 1985; Kunin and Gaston, 1997;

Gregory and Gaston, 2000). Historically, the relationship between reproductive biology

and rarity has focused on rare species with small populations sizes. Rare plants are

predicted to have self-compatible breeding systems and less specialized pollination

systems in order to maintain reproduction in response to low pollen availability (Baker,

1955; Kelly et al., 1996; Izco, 1998; Neiland and Wilcock, 1998; Fausto et al., 2001;

Murray et al., 2002; Domínguez Lozano and Schwartz, 2005; Jacquemyn, 2005; Moeller

and Geber, 2005). While this prediction of reproductive assurance may be relevant for

those species that are locally rare, habitat specialists, many of which are locally abundant,

should be less restricted by pollen receipt, allowing for factors other than pollen

availability to influence their reproductive biology (Rabinowitz et al., 1981; Kruckeberg

and Rabinowitz, 1985; Fenster et al., 2004; Williams et al., 2009), such as resource

limitation or specialization on stressful abiotic conditions. Rabinowitz et al. (1981)

categorized species distributions into seven forms of rarity based on three factors:

population size, geographic extent, and habitat specificity (Table 2-1). Locally-abundant,

habitat specialist (LAHS) plants may experience very different selective pressures, such

as for increased outcrossing rates, enhanced offspring quality, or decreased resource-loss

in stressful environments.

For instance, rare species that specialize on a particular set of abiotic conditions may have mechanisms that increase genetic variation, which may buffer the population against inbreeding depression or genetic drift (Espeland and Emam, 2011). If resources are limiting, investment in fewer, larger flowers that restrict the number and type of effective pollinators should increase outcrossing rates (Karron et al., 2004; Mitchell et al., 2004; Bell et al., 2005; Karron et al., 2009; Mitchell et al., 2009) and potentially enhance offspring quality. Plants with fewer flowers open concurrently are visited less frequently and for shorter periods of time, resulting in the increased deposition of outcross pollen. By limiting access to floral rewards (e.g., by increased spur or tube length), species may restrict the number and type of visitors that can access the reward, resulting in more specialized pollination systems (Anderson and Johnson, 2008), which should also increase offspring vigor and decrease resource losses through investment in lower-quality, self-fertilized offspring (Darwin, 1877; Whittall and Hodges, 2007).

On the other hand, plants that are adapted to stressful (i.e., hot and dry) conditions often have similar suites of traits that are thought to be adaptive (Bryant, J.P., Chapin, F.S., & Klein, D. R., 1983; Chapin, F.S. 1980; Chapin, F. S., Autumn, K., & Pugnaire, F., 1993; Grime, 1977). While advantageous in more extreme abiotic habitats (Bryant et al., 1983; Chapin et al., 1993; Grime, 1977), stress adaptations often come at a cost, such as decreased competitive ability in the absence of stress (Baskin, J. M. & C. C. Baskin, 1988; Grime, 1977) and potentially in reduced attractiveness to pollinators in more productive habitats. For instance, stress-adapted species are often shorter, with overall reduced floral output, and lower seed production (Aragón et al., 2008; Kudo et al., 2008). Plants that are taller (Dickson and Petit, 2006) and have larger floral displays are more

attractive to pollinators and could be competitive dominants for pollinators (Erhardt and Rusterholz, 1998; Naug and Arathi, 2007; Aragón et al., 2008), which may select for more generalized pollination systems (Muchhala et al., 2010). Moreover, nectar production, an important floral attraction trait, is reduced in order to conserve water in xeric environments (Halpern et al., 2010) and therefore may result in reduced attractiveness to pollinators, if sugar quantity is similarly reduced. As survival becomes increasingly important for population stability, resources allocated to reproduction (e.g., floral attraction traits) may be reduced (Silvertown et al., 1993), potentially resulting in lower floral output and competitive ability for pollinators.

I conducted observational and experimental studies to test six hypotheses regarding the relative importance of habitat specialization via stress-adaptation versus reproductive specialization in three locally abundant, habitat specialist (LAHS) plants that are endemic to the Ozark glades (i.e., geographically and edaphically restricted habitats characterized by hot and dry conditions) and congeners of these three species that can occur on glades but are not restricted to them. First, I test the hypotheses that LAHS plants have traits associated with stress-resistance and/or pollination specialization, such as smaller leaves and fewer, smaller flowers. Next, I test the predictions that LAHS plants have lower rates of autogamy and are more dependent on their pollinators for their reproductive success (i.e., more pollen-limited) in comparison to closely related species with broader habitat use. Finally, I test the hypotheses that visitation rates are lower and pollination specialization scores are higher in comparison to their common congeners (CCs). In order to determine rates of autogamy and pollen limitation, I conducted breeding system and pollen-supplementation experiments. The

hypotheses regarding pollination specialization and pollinator movement were examined through a three-year observational study of pollinator behavior. I show that two of the three LAHS taxa are have more specialized reproductive biology (morphology and pollination), but do not have traits associated with increased stress-resistance. I then discuss the implications of these findings for species responses to climate change and conservation efforts in stressful environments.

MATERIALS AND METHODS

Study System—The glades of the Ozark Mountain Region are rocky, arid outcroppings that are spatially restricted to south or southwest facing ridge tops within a matrix of more mesic oak-hickory forest matrix (Learn and Schaal, 1987; Baskin and Baskin, 1988; Templeton et al., 2001). They are dominated by grasses and forbs and contain several endemic plant species (Nelson and Ladd, 1982; Yatskievych, 1999), many with restricted ranges. Temperature data recorded at three replicate glade, forest, and prairie sites between December 2007 and August 2008, indicate that glades are significantly hotter than the surrounding habitats during the spring and summer months ($DF = 3688$, $F = 2.36$, $p = 0.0017$; data not shown), when many LAHS glade plant species are photosynthetically active and blooming.

I chose three LAHS glade species, *Delphinium treleasei* (Ranunculaceae), *Echinacea paradoxa* (Asteraceae - Heliantheae), and *Scutellaria bushii* (Lamiaceae) that are restricted to glades in the Ozark Region in Missouri and Arkansas but locally abundant (i.e., hundreds of individuals blooming concurrently per glade). Each has a common congener (respectively, *D. carolinianum*, *E. pallida*, *S. ovata* and *S. parvula*, hereafter common congeners (CC)) that may be locally abundant on glades but is not

9

restricted to them. I use two species of *Scutellaria* because neither matches all of my criteria. *Scutellaria ovata* tends to occur at the edges of glades where there is greater soil depth and canopy cover, and it is less often found in the center of glades; *S. parvula* occurs in the center of glades, but these glade specialists may represent a subspecies within the broader species (George Yatskievych, pers. com.). Comparing congeneric pairs provides insight into the factors that restrict one species to glades but not the other. Moreover, comparing several unrelated LAHS species to their common congeners mitigates the potential influence of evolutionary history on the biological traits correlated with endemism, such as specialized pollination. This provides a phylogenetically controlled study of the mechanisms contributing to the restricted ranges of these species (Kruckeberg and Rabinowitz, 1985; Bevill and Louda, 1999). Multiple congeneric comparisons allow for broader interpretation of the results from this study (Bevill and Louda, 1999) and a greater understanding of the relationship between stress-adaptation and reproductive biology, which has hitherto been not been explicitly explored.

Vegetative traits—I quantified selected vegetative that I hypothesized would differ between the stress-adapted and non-stress-adapted species, measured for 10-15 individuals per site for multiple sites per species (average = 4.82 sites and 144.5 individuals per species; Table 2-2; Figure 2-1). Over three field seasons (2007 – 2009), morphological traits were measured at no fewer than four glade sites for each LAHS species and no fewer than one non-glade (i.e., prairie and woodland) and two glade sites for the widespread taxa (Table 2-2). I was not able to obtain data on above- or belowground biomass due to restrictions on the collection of the LAHS plants and the required sampling techniques (which would include substantial destruction of glade

habitat). Therefore, biomass was estimated as the total number of leaves and total stem length (i.e., the product of the number of stems and stem length). In 2009, one medium-sized leaf per individual was measured for leaf thickness, pressed and digitized. The leaf area of each leaf was quantified in Sigma Scan (SYSTAT Software Inc., 2002), and total leaf area was calculated (i.e., average leaf area multiplied by the total number of leaves).

Floral traits—I measured floral attraction traits (i.e., display size, nectar volume) and floral morphological traits that I predicted could affect pollinator behavior and pollinator effectiveness (i.e., amount of pollen carried and pollinator fidelity). Due to morphological differences, some floral attraction traits were quantified differently for each of the genera. For the *Delphinium* species, corolla area was calculated as the product of corolla width and corolla height; distance to the nectar reward refers to the length of the floral spur. I measured corolla area of the *Scutellaria* taxa as the square of the lower corolla, often called the landing pad, and distance to nectar as floral tube depth. For both *Scutellaria* and *Delphinium*, I documented the number of flowers produced per individual and the number of flowers open concurrently. The proportion of flowers open was calculated as the number of flowers open concurrently divided by the total number of flowers produced per individual, and total display size was calculated as the product of corolla area, as defined above, times number of flowers open. In order to estimate nectar production, plants were bagged prior to anthesis, left undisturbed for several hours (4-6 hours for *Delphinium* species and 5-6 hours for *Scutellaria* species, except *S. parvula* at one site where individuals were bagged for 29 hours, because nectar levels were too low to quantify after shorter time periods), and nectar was collected via micro-capillary tubes. Nectar production was then quantified as nectar volume produced per hour.

For the *Echincaea* species, corolla area was calculated as the product of the width and length of an average ray petal, and total display size is corolla area multiplied by the total number of ray petals. The number of flowers open per inflorescence was the sum of all florets that were shedding pollen or had receptive stigmas, and the proportion of florets open was quantified as the total number of florets open divided by the total number of florets produced. For all species, relative floral output was standardized by total stem length, in order to account for variation in individual plant size. Nectar was not collected from *Echinacea* due to low nectar production and small floret size.

Reproductive success and breeding systems—In order to estimate reproductive success in a common habitat, fruits were collected from individuals at the glade sites, and seed set was quantified for up to three fruits per individual. For *Delphinium* and *Scutellaria* species, total reproductive success is defined as the average number of seeds per fruit multiplied by the total floral output. For *Echinacea* taxa, total reproductive success is defined as the proportion of achenes (florets) with a fertilized seed multiplied by the total number of achenes per capitulum (inflorescence). Since *Echinacea* species are known to produce unfertilized capsules that collapse when pressure is applied, thirty seeds per capitulum were gently pressed with forceps to confirm fertility and ensure accurate estimates of reproductive success. Seeds that did not collapse under the small amount of applied pressure were assumed to be fertile. For all species, relative reproductive success was standardized by total stem length.

I determined the degree to which each species is self-compatible by breeding system studies conducted under controlled greenhouse conditions or in natural populations when flowering could not be induced in the greenhouse. Wild-collected seed

of the *Delphinium* and *Echinacea* taxa were germinated, transplanted and grown to flower in the greenhouse. I documented the phenology of reward presentation (e.g., corolla expansion, first nectar production, and stigma receptivity) under greenhouse conditions and in the field. I was unable to induce flowering of *Scutellaria bushii* in the greenhouse and conducted breeding system experiments for all *Scutellaria* taxa in the field. Thirty individuals per species were bagged before anthesis to exclude pollinators, pollinated according to one of three treatments, and kept bagged until fruits developed. To demonstrate the role of pollinators in fertilization, the capacity of each species to self-pollinate, and the degree to which each species is or is not self-incompatible, I applied self-pollen, outcross pollen, and no pollen to 10 individuals of each species, respectively. Following maturation, I collected the fruit and quantified reproductive success as noted above. Degree of autogamy was calculated as the ratio of reproductive success in the bagged and in the pollen supplement treatments. Dependence on pollinators for self-pollination was calculated as the ratio of bagged and self-pollinated treatments.

 Pollination biology— I conducted a pollen supplementation experiment at each field site to quantify ambient limitation of reproductive success via inadequate pollination. I applied outcross pollen to all receptive stigmas of 10 individuals, excluded pollinators from 10 individuals to estimate autogamous fertilization, and tagged 10 additional individuals which remained open to pollinators and acted as the control. Following maturation, all marked flowers or achenes were collected, and the reproductive success quantified. Seed fertility of the *Echinacea* taxa was estimated as noted above. Pollen limitation was calculated as supplemented reproductive success minus ambient reproductive success.

In order to document the pollination specialization of these target plant species, I recorded the number, type, and behavior (e.g., visit duration (s), anther and stigma contact) of all floral visitors through 30 minute observations during peak periods of activity and collected a representative number of each visitor for pollen counts and identification. Due to the spatial and temporal variation inherent in plant-pollinator interactions, pollinator observations and insect collections were conducted at each site over two years, 2008 and 2009. In 2008, ten individual plants were observed for each species per site, and in 2009, fifteen individuals were observed. Replication was increased in 2009 in order to compensate for low visitation rates. Pollinator observations and collections were conducted at both glade and non-glade (i.e., prairies and woodlands) sites for all CC species, in order to control for potential differences in pollinator assemblages and behavior across sites. One inflorescence per individual was observed for 30 minutes, two to three times during the blooming period (10-15 individuals x 2 observation days x 3 sites per species = 60-90 individuals per species in 2008 and 2009, respectively; Table 2-2) in order to account for individual and temporal variation in insect activity. Since insect identification is often impossible during field observations, visitors were categorized into functional groups during field observations. Visitation rate of each species was calculated as the product of the visitation rate of the functional group per 30 minute observation and the proportion of individuals of that functional group represented by the given species.

Following collection in the field, visitors in Hymenoptera and Lepidoptera were identified by experts, where possible, to the species level (86.5%), and those in Coleoptera, Diptera, and Hemiptera were identified to family. Pollen was washed from

insects with ethanol, mounted on slides and stained with Calberla's solution (Ogden et al., 1974; Dafni, 1993; Clinebell and Bernhardt, 1998). Pollen loads were determined for each insect species as the average number of pollen grains of the focal plant species found on the insect. Pollen was identified with the aid of a pollen reference library of all co-blooming species, and total pollen flow (L_j) of the focal plant species was calculated,

$$L_j = \sum_{i=1}^{S_j} (p_i v_i)$$
<div align="right">Eq. 1</div>

where S_j is the total number of insect species visiting plant species j, p_i is number of species j pollen grains carried by visiting species i, and v_i is visitation rate of insect species i to plant species j. I measured the specialization of each plant species via the Generalization Index (i.e., the number of insect species that account for 95% of pollen flow), and the relative importance of all pollinators was quantified as the Dominance Index of Pollinator Importance (D_{PIj}, modified from Galloni, 2008). The Dominance Index is a modification of the Simpson's diversity index that accounts for both insect pollen load and visitation rate,

$$D_{PIj} = \sum_{i=1}^{S_j} [(p_i v_i / L_j)^2]$$
<div align="right">Eq. 2</div>

(symbols are the same as in Eq. 1). The Dominance Index ranges from zero (i.e., multiple pollinators each account for an equal proportion of pollen flow) to one (i.e., one pollinator accounts for all of the pollen flow) and is a measure of the pollinator importance that accounts for pollinator richness within a defined pollinator community (Galloni, 2008).

Statistics—All morphological, reproductive, and pollination traits were normalized where necessary and tested for significant differences between congeners via one-tailed t-test in R Statistical Software (R Development Core Team 2008). The direction of each t-test (i.e., greater than or less than; indicated in the results table) was based on the following hypotheses: (1) the LAHS species have traits associated with the xeric SRS and their CC species do not, (2) the LAHS species have floral traits that are more attractive to pollinators than are those of the CC species, (3) the CC species have more generalist pollination systems than their LAHS congeners, and (4) the CC species have higher rates of autogamy than their LAHS congeners. In order to control for multiple tests of each of the above hypotheses via multiple traits, the significance values of each set of traits was modified using the Šidak Correction for multiple comparisons (Šidak, 1967). Variables with low replication (i.e., $N < 6$) that either had either marginally significant *t*-values or had variables that could not be normalized were analyzed via permutation ANOVA (aovp in lmperm package; R Development Core Team 2008). All permANOVA results of non-normalizable data did not differ from the results of the *t*-tests; therefore I report only the *t*-test results.

RESULTS

Vegetative traits—Vegetative traits differed significantly between congeners but were not consistent among genera. Generally, there were no differences between results from analyses that included and those that excluded non-glade habitats, but for clarity, I discuss the differences within glades only, unless otherwise specified (all results are recorded in Table 2-3). *Delphinium treleasei*, the LAHS taxa, did have thicker leaves than its CC as I predicted, but there were no differences in total stem length or in total

16

leaf area, two estimates of biomass. Contrary to my predictions, the LAHS *D. treleasei* had greater average leaf area (Table 2-3, Figure 2-2a) than its CC *D. carolinianum* and was not significantly shorter. In congruence with my hypotheses, the LAHS *Echinacea paradoxa* was marginally shorter than its CC, *E. paradoxa*, but there were no differences in any other vegetative trait measured, including average leaf area (Figure 2-2b), leaf thickness, or total leaf area. The LAHS *S. bushii* was significantly different from its CC, *S. ovata*, in congruence with my hypotheses for vegetative traits, but not from *S. parvula*. *Scutellaria bushii* was shorter than *S. ovata* but not than *S. parvula*, and *S. bushii* had marginally significantly lower leaf area than *S. ovata* but not its other CC, *S. parvula* (Table 2-3, Figure 2-2c). Contrary to my hypotheses, *S. bushii* was significantly taller in comparison to both *Scutellaria* CCs (*S. ovata* and *S. parvula*). There were no differences in leaf thickness or total leaf area between *S. bushii* (LAHS) and *S. ovata* or *S. parvula*.

  Floral traits—As predicted, *Delphinium treleasei* (LAHS) had significantly larger flowers than *D. carolianum* (Table 2-3, Figure 2-2d). On the other hand, distance to nectar, total floral output, relative floral output, and the number of flowers open concurrently did not differ between *Delphinium* taxa (Table 2-3). There were no differences between *Echinacea* species, except in ray petal size. The LAHS *E. paradoxa* did not differ from *E. pallida* in any floral trait, including corolla area (Table 2-3; Figure 2-2e), in congruence with my predictions. *Scutellaria bushii* (LAHS) had significantly larger flowers (Table 2-3; Figure 2-2f) and greater distance to the nectar reward than both CCs, as predicted. In comparison to *S. ovata*, *S. bushii* (LAHS) also had lower relative floral output across all habitats (Table 2-3). There were no differences in relative floral output (i.e., the number of flowers per cm total stem length) or nectar volume (glades

only) between *S. bushii* and *S. parvula*. Total display size (total corolla area) was marginally larger for *S. bushii* in comparison to *S. parvula*, as predicted. Contrary to my predictions, *Scutellaria bushii* did not have fewer flowers open concurrently or lower proportion of flowers open in comparison to *S. parvula*. When compared to *S. ovata*, *S. bushii* had significantly lower relative floral output, but there were no differences in the number or proportion of flowers open.

Reproductive success and Breeding system experiments— Relative reproductive success (total seed set per unit total stem length) was significantly lower for the *E. paradoxa* (LAHS) but not for the other two LAHS taxa (Table 2-4) in comparison to their common congeners. There were no differences in total reproductive success between any congeneric pair or in rates of autogamy, geitonogamy and outcrossing (Table 2-4).

Pollination biology— *Delphinium treleasei* (LAHS) had a lower generalization score than *D. carolinianum* (Figure 2-2g) but was not less pollen limited when non-glade habitats were included in the analysis (Table 2-3). Pollinators did not probe fewer flowers consecutively or visit for shorter durations when visiting *D. treleasei* in comparison to *D. carolinianum*, and there was no difference between species in pollinator dominance or fidelity (Table 2-3). *Echinacea paradoxa* (LAHS) was marginally more pollen limited than its CC, as predicted, but pollinator behavior and pollination generalization (i.e., the number of pollinators to account for 95% of pollen flow; Figure 2-2h) did not differ between species. *Scutellaria bushii* (LAHS) did not differ from *S. parvula* in pollinator behavior or generalization score (Table 2-3; Figure 2-2i). The number of flowers visited consecutively did not differ between *S. ovata* and *S. bushii* on the glades, but when non-glade habitats were included, visitors probed marginally fewer flowers of and visited

marginally less frequently to *S. bushii* than *S. ovata*, as predicted (Table 2-3). Due to low insect abundance, pollinator collections were insufficient at all but one of the glades where *S. ovata* was studied; therefore, I was unable to conduct the analysis with glade sites only. However when non-glade habitats were included, *S. bushii* had a significantly lower generalization score than *S. ovata* (Table 2-3; Figure 2-2i) but not in dominance or pollinator fidelity.

DISCUSSION

Species of each of the seven forms of rarity (Rabinowitz et al., 1981; Kunin and Gaston, 1997; Gregory and Gaston, 2000) may experience different selective pressures resulting in a wide range of pollination systems, given the appropriate abiotic or biotic environment. Here I explore the reproductive biology of three locally abundant and regionally-rare (LAHS) plant species that are endemic to stressful environments. The floral traits and pollinator behavior documented in two of the three species in this study support the hypothesis that glade LAHS species have more specialized reproductive systems. While these results generally support my prediction that persistence in stressful environments may result in pollination specialization, there is little evidence for stress-adaptation *per se*.

Glade endemics did not consistently share morphological traits associated with stress-adaptation, and differences in a few traits, such as leaf area (Figure 2-2b), had conflicting patterns across genera. For instance, leaves of *S. bushii* (Figure 2-2c) were marginally smaller than *S. ovata* but not than the other CC, *S. parvula.* Neither *D. treleasei* (Figure 2-2a) nor *E. paradoxa* (Figure 2-2b) had smaller leaves than their respective CCs. Height has a similar, contradictory pattern. *S. bushii* is shorter than *S.*

19

ovata but not than *S. bushii*; *Echinacea paradoxa* is marginally shorter than its CC, and there is no difference between *Delphinium* species. One key trait that I was not able to assess due to collection restrictions was below-ground growth, and it should be explored in order to have a more complete understanding of the vegetative traits associated with stress-adaptation in plants that are endemic to stressful environments, such as glades. However, the lack of consistent differences between congeners in the selected traits measured here suggests either that other features that were not measured here contribute to adaptation to the hot, dry conditions on glades or that there is a lack of adaptation to xeric environments entirely.

On the other hand, two of the three LAHS species had larger flowers (i.e., corolla area) than their common congeners (Figure 2-2 d–f), with the exception of *Echinacea paradoxa* (Figure 2-2e; Table 2-3). Moreover, *S. bushii* (LAHS) species had longer floral tubes than both of its common congeners (CC). I predicted that the LAHS species would have fewer pollinators that account for the majority of pollen flow. In congruence with this hypothesis, *Delphinium treleasei* (LAHS) also had a more specialized pollination system than its congener (Figure 2-2g), and *S. bushii* had a more specialized pollination system than *S. ovata* (Figure 2-2i), though specialization could only be tested when all habitats were included due to low pollinator abundances. On the other hand, there was no difference in the dominance index, which controls for the richness of visitors, between any congeners. This finding suggests that while the number of species that account for the majority of the pollen flow (i.e., the generalization score) was significantly lower, the dominance of any individual pollinator species was not different between LAHS taxa and their congeners. Visitation rates and total seed production also did not differ between

congeners. However, higher specialization in some of the LAHS taxa could lead to greater outcrossing and presumably higher offspring quality. Offspring quality (e.g., germination rate) was not assessed in this study. Therefore, further study is required to explore the effects of these mechanisms on overall reproductive success.

Unlike *Scutellaria* and *Delphinium*, the LAHS *Echinacea* paradoxa did not differ from *E. pallida* in pollination generalization, and it had lower relative reproductive success and was marginally more pollen limited than its CC. This disparity in reproductive success could lead to the exclusion of the LAHS *E. paradoxa* from habitats where competition for resources is more intense or where total reproductive output is more instrumental in population establishment (e.g., via founder effects). I have defined reproductive success as total seed set, but there are other potentially important traits that could affect fitness, specifically offspring quality (as measured by germination rate and establishment). While not significant, there is also a trend toward more generalized pollination in *E. paradoxa* (LAHS; Figure 2-2h), a finding that warrants greater exploration to offset low replication ($N = 4$) at the site level caused by low pollinator abundances in 2009.

In order to achieve reproductive assurance, rare plants are predicted to be self-compatible, assuming either low pollen availability due to local rarity or unpredictable pollination. However, reproductive assurance may not be optimal for locally abundant or stress-adapted taxa, which may be more limited by other resources than by pollen. Rates of autogamy of the LAHS plants in this study reflect neither selection for reproductive assurance nor reduction of inbreeding. Rates of autogamy appear to be conserved across taxa and may reflect the importance of evolutionary history, not habitat specialization, in

determining rates of autogamy. While there is mixed support for pollination specialization, two of the three LAHS plants have larger floral displays (i.e., corolla area) than their congeners, which is congruent with our predictions for optimal resource allocation in LAHS taxa to increase attractiveness and potentially outcrossing rates. More specialized pollination systems, as seen in *D. treleasei*, may result in higher pollinator efficiency and lower stigmatic occlusion, which in turn may increase outcrossing rates and offspring quality. If there is greater variation in floral traits, selection may act on those traits associated with pollination specialization, thereby increasing outcrossing rates while conserving the ability to self-pollinate. Additionally, inbreeding depression is predicted to be exacerbated in stressful environments (Wright, 1922; Hauser and Loeschcke, 1996; Cheptou et al., 2000; Waller et al., 2008; but see Armbruster and Reed, 2005) and could result in selection for increased outcrossing rates as well. The documentation of the mating systems and the strength of selection for traits conferring increased outcrossing rates are required to corroborate the hypothesis of higher inbreeding depression in stressful environments.

Despite the lack of support for a stress-reproduction tradeoff, the results of this study support a more nuanced approach to the relationship between rarity and reproductive specialization. The *Delphinium* and *Scutellaria* LAHS species have traits in congruence with my predictions of increased specialization and outcrossing rates, but the *Echinacea* species does not, suggesting that both mechanisms (i.e., increased selection for outcrossing and reduced allocation to reproduction) may be acting concurrently in different LAHS taxa. Further study of additional LAHS species and larger sample sizes at the site level will be needed to determine the relative prominence of each mechanism.

These endemic glade plants may not be more stress-resistant or adapted to local abiotic conditions, but they may be restricted from more productive habitats via competitive exclusion. It is unclear whether LAHS glade plants are specifically adapted to glades or are simply restricted to glades due to low competitive ability in more productive habitats, and the relative importance of these mechanisms should be explored more in future research on the range restriction of species to stressful habitats.

The conventional paradigm of reproductive assurance in pollen-limited plants, such as locally rare plants, is an important theoretical prediction that has been supported by some studies (Fausto et al., 2001; Moeller and Geber, 2005). However, reproductive theory should be expanded to include more explicit predictions for each of the seven forms of rarity. The explicit integration of non-biogeographic factors, such as stress-adaptation and competition for pollinator services, into this paradigm could greatly enhance our understanding of the factors that determine and are affected by species eco-geographical distributions. This study provides insight into stress-adaptation and pollination specialization as two potential factors restricting the ranges of three glade-endemic LAHS species. The pattern of more specialized pollination systems (reflected in the LAHS *D. treleasei*) and lower relative reproductive success (as in the LAHS *E. paradoxa*) suggest a tradeoff between allocation to offspring quality and quantity that could affect the ability of rare plants to invade less-stressful habitats. Reduced reproductive success could inhibit the ability of LAHS species to colonize new habitats and increase their risk of extinction. Moreover, specialization on insect pollinators found on or near glades could further restrict the ecogeographical range of these species.

In response to climatic change, many species are predicted to shift their ranges, adapt to a new environment, or go extinct. Low colonization potential and high habitat specialization could inhibit the ability of rare species to track their optimal climatic conditions or adapt to novel habitats and environmental conditions, resulting in an even greater restriction of their ranges and an increase in their risk of extinction. Insight into the relative importance of potential confounding factors related to each of the seven forms of rarity is imperative for our understanding of the mechanisms determining the biogeographical ranges of all species and for the creation of effective conservation and management plans for rare and endemic species. This study contributes to the development of a more nuanced theory regarding the interaction between rarity and reproductive specialization, which will inform our understanding and protection of rare and endemic plant species.

Table 2-1. Description of Rabinowitz's Seven Forms of Rarity based on geographic extent, population size and habitat specificity (modified from Rabinowitz et al., 1981). Bold font represents the locally abundant, habitat specialist (LAHS) taxa and the underlined font represents the common congeners in this study.

| | *Geographic Extent (GE)* | | | |
	Large		Small	
Population Size (PS)				
Somewhere large	<u>Common</u>	Large PS Large GE Narrow HS	Large PS Small GE Generalist HS	**Large PS (LA)** **Small GE** **Narrow HS (HS)**
Somewhere small	Small PS Large GE Broad HS	Small PS Large GE Narrow HS	Small PS Small GE Broad HS	Small PS Small GE Narrow HS
	Broad	Narrow	Broad	Narrow
	Habitat Specificity (HS)			

Table 2-2. Replication for each species by habitat for the number of field sites and the number of plants for which I documented morphological traits (Morph) and observed pollinator behavior, and the number of insects collected for pollen load analysis (Insects). * Field sites were not mutually exclusive; therefore, field sites per species do not sum to total number of field sites.

Plant species	Habitat	Field Sites*			Plants (no.)		Insects (no.)
		Morph	Observ	Insect	Morph	Observ	
Delphinium	Glade	5	4	3	90	75	137
carolinianum	Prairie	3	1	1	40	10	21
Delphinium treleasei	Glade	8	7	3	193	128	157
Echinacea pallida	Glade	4	4	2	90	72	40
	Prairie	2	2	2	73	20	213
Echinacea paradoxa	Glade	5	5	2	110	62	60
Scutellaria bushii	Glade	7	4	3	244	123	31
Scutellaria ovata	Glade	3	3	1	72	48	86
	Woodland	9	5	2	133	71	46
Scutellaria parvula	Glade	5	4	3	96	88	34
	Woodland	2	0	0	7	0	0
Total		31	23	18	1148	697	825

Table 2-3. Results (i.e., *t*-values) from analyses of morphological traits and pollination biology of three locally abundant, habitat specialist (LAHS) taxa and their common congeners (CC) in glades and across all sites (including non-glade habitats, such as prairies and glades). Numbers in parentheses denote significant *P*-values from permutation ANOVAs that were conducted for marginally significant *t*-test results and N < 6. Letters refers to traits that were analyzed within a single site (a; *Echinacea* corolla area and total display size only) and to traits for which only comparisons included all habitats could be conducted (b; *Scutellaria bushii* vs. *S. ovata* pollination biology only). Symbols represent significance levels based on the restricted α via the Šidak Correction for multiple comparisons (α_{SC}).

Vegetative traits (α_{SC} = 0.010): † *P* < 0.05, *P < 0.01, ** *P* < 0.001, *** *P* < 0.0001

Floral traits (α_{SC} = 0.006): † *P* < 0.03, *P < 0.006, ** *P* < 0.001, *** *P* < 0.0001

Pollination biology (α_{SC} = 0.017): † *P* < 0.085, *P < 0.017, ** *P* < 0.001, *** *P* < 0.0001

	Prediction CC vs. LAHS (sign t-value)	Delphinium		Echinacea		Scutellaria			
						S. ovata		S. parvula	
		Glades	All sites	Glades	All sites	Glade	All sites	Glade	All sites
Vegetative traits									
Height	> (+)	0.93	1.19	**2.84**[†]	**2.04**[†]	**8.31*****	**6.49*****	-4.27	-3.31
Leaf area	> (+)	-10.4	--	1.62	--	**12.48**[†]	--	-2.66	--
Leaf thickness	< (-)	**-4.82***	--	1.64	--	2.28	--	-2.12	--
Total stem length	> (+)	-0.53	-0.38	-0.79	-0.21	-2.76	-3.15	-4.58	-3.15
Total leaf area	> (+)	-4.09	--	0.77	--	-0.79	--	-3.05	--
Floral traits									
Corolla area	< (-)	**-3.13***	**-3.36***	-1.79[a]	--	**-4.82***	**-4.83****	**-14.7*****	--
Distance to nectar	< (-)	-1.64	**-2.07**	--	--	**-5.88***	**-5.49***	**-24.6*****	--
Nectar volume	< (-)	1.58	--	--	--	--	--	-1.11	--
No. flowers open	> (+)	1.19	0.95	-0.38	0.01	1.26	**2.16**	-3.94	--
Proportion of flowers open	> (+)	**2.25**[†]	1.67	-2.08	--	1.35	1.08	**2.12**	--
Relative floral output	> (+)	0.28	-0.29	0.52	--	**3.80***	**3.85***	-0.44	-0.37
Total display size	< (-)	-0.25	-0.06	-0.48[a]	--	-1.43	-1.11	**-4.81**[†]	--
Total floral output	< (-)	0.29	0.79	0.39	--	1.40	1.98	**-4.26***	**-3.26***
Pollination biology									
Visitation rate	> (+)	1.00	0.63	0.85	0.47	0.64	**1.99**[†]	1.84	--
Visit duration	> (+)	-0.23	-0.48	0.08	0.24	-0.50	0.56	0.14	--
Flowers visited consecutively	> (+)	0.75	0.51	--	--	1.66	**2.64**[†]	-0.26	--
Pollen limitation	> (+)	0.07	0.57	**6.14**[†]	--	-0.39	-0.77	-0.26	--
Generalization score	> (+)	**2.24**[†] **(0.014)**	**2.27**[†] **(0.0002)**	-1.18	-0.46	--	**2.68**[†b] **(0.001)**	1.00	--
Dominance	< (-)	-1.90	-1.44	1.45	0.59	--	-2.06[b]	-1.67	--
Pollinator fidelity	< (-)	-0.15	0.65	-1.40	-0.39	--	-1.97[b]	-2.38	--

Table 2-4. Comparison of reproductive success and breeding systems between three locally-rare, habitat specialist (LAHS) taxa and their common congeners (CC). Results shown are *t-values*, and significance levels are denoted with symbols († $P < 0.10$, $*P < 0.05$, $** P < 0.01$, $*** P < 0.0001$). Breeding system studies of the *Delphinium* and *Echinacea* taxa were conducted in the greenhouse. *Scutellaria bushii* could not be induced to flower in the greenhouse; therefore, breeding system studies were conducted in the field. Numbers in parentheses denote significant *P*-values from permutation ANOVAs that were conducted for marginally significant *t*-test results and N < 6).

	Delphinium	*Echinacea*	*Scutellaria*	
			S. ovata	S. parvula
Relative reproductive success	0.34	**2.33†(0.004)**	2.45	0.58
Total reproductive success	0.43	1.95	1.70	-0.04
Autogamous seed set	0.93	0.40	-0.22	-0.68
Geitonogamy seed set	1.65	0.60	--	0.001
Outcross seed set	-1.63	0.05	0.25	-0.40

Figure 2-1. A map of 31 glade and non-glade (woodland and prairies) field sites (○) in Missouri at which I quantified morphological traits and observed pollinators for three locally abundant, habitat specialist species (*Delphinium treleasei*, *Echinacea paradoxa*, and *Scutellaria bushii*) in comparison to their common congeners (*D. carolinianum*, *E. pallida*, *S. ovata*, and *S. parvula*). Glade density was coded in to a heat-density map from Nelson and Ladd (1982). The original data represented a range in glade density per 7.5 degree minute quadrats (approximately 144 km^2 in Missouri); therefore the heat map reflects a rough estimate of glade density per 144 km^2 as of 1982.
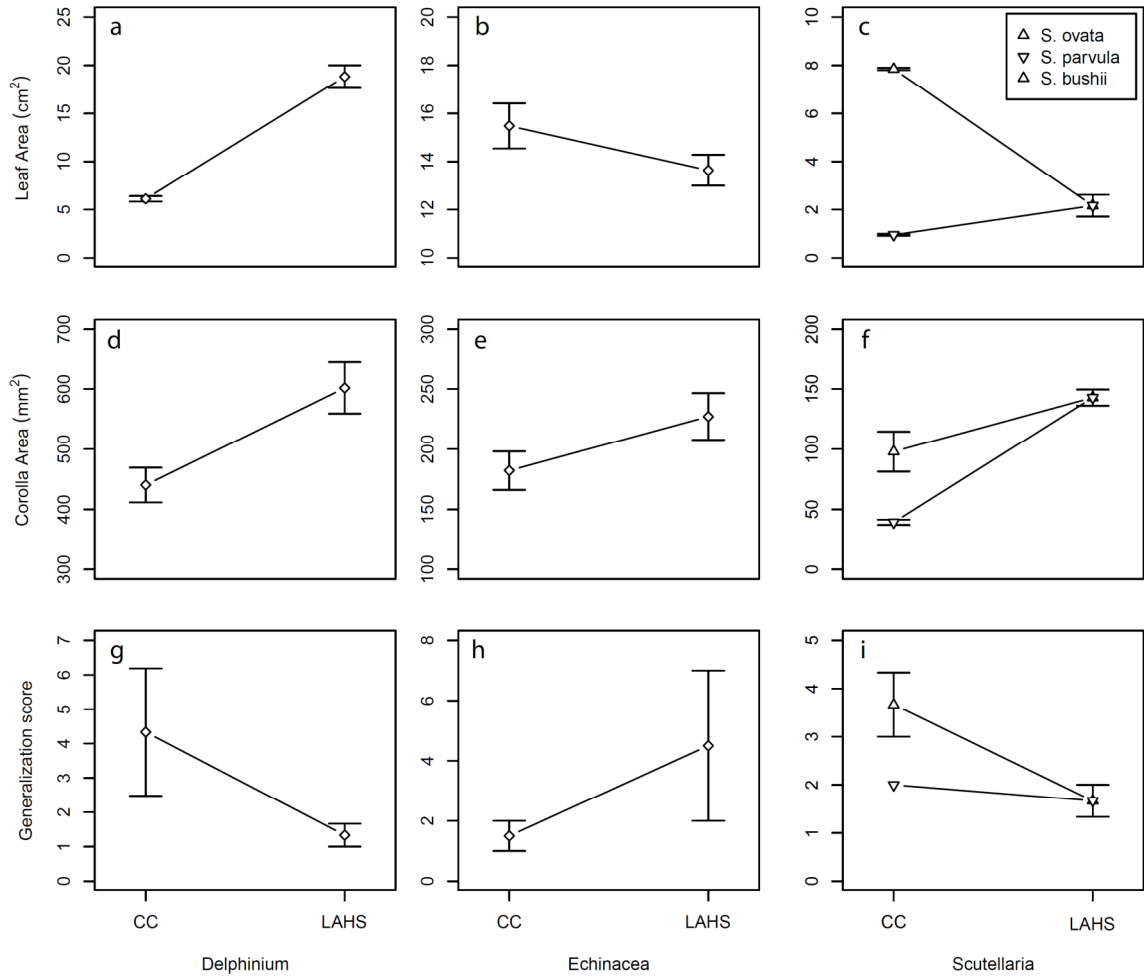
Figure 2-2. Morphological traits and pollination biology of three locally abundant, regional-rare plant species (LAHS; *Delphinium treleasei* (◇)*, Echinacea paradoxa* (◇)*,* and *Scutellaria bushii* (⬚)) in comparison to their common congeners (CC; respectively, *D. carolinianum* (◇), *E. pallida* (◇), *S. ovata* (△) and *S. parvula* (▽)) as quantified in the field and graphed by distribution and genus; Leaf area (cm^2; a – c), Corolla area (mm^2; d – f), and Generalization score (g – i; no. species to account for 95% of pollen flow; the comparison between *S. bushii* and *S. ovata* was calculated with data from glade and non-glade habitats due to low pollinator densities on glades; *S. parvula* does not have standard error bars, because it's generalization score was identical across all sites). All other results shown were calculated from within-glade comparisons.

CHAPTER THREE

Pages 32 to 64

RESOURCE ALLOCATION DIFFERS BETWEEN RARE AND WIDESPREAD

PLANT SPECIES IN RESPONSE TO EXPERIMENTAL STRESS

INTRODUCTION

In 1981, Rabinowitz and colleagues developed a conceptual matrix describing seven types of rarity based on geographical range, habitat specificity, and local population size. A recent review of studies that reference this framework indicates that species in each category of rarity have some similar traits (Espeland and Emam, 2011). For instance, species that are habitat specialists and have geographically restricted ranges are more likely to have outcrossing mating systems, which are thought to maintain genetic diversity and reduce the potential for genetic drift (Espeland and Emam, 2011). In order to maintain high outcrossing rates, species that specialize on restricted habitats should have mechanisms that restrict the movement of self-pollen and enhance the transfer of outcross pollen, including herkogamy (the separation of reproductive parts in space; Lavergne et al., 2004), more specialized pollination systems (Darwin, 1877; Rymer et al., 2005; Galloni, 2008), or a reduction in the number of co-blooming flowers (Karron et al., 2004; Mitchell et al., 2004). The reduction in co-blooming flowers, for instance, alters pollinator behavior, resulting in fewer within-plant movements and the deposition of more outcross pollen (Karron et al., 2004; Mitchell et al., 2004). Specialized pollination systems have fewer pollinating species that collect and transfer pollen between plants, which can increase outcrossing rates and decrease the deposition of heterospecific pollen. Heterospecific pollen deposition interferes with the establishment and growth of conspecific pollen on the stigma and can lead to a reduction in seed set (McLernon et al., 1996).

In addition to buffering populations against genetic drift (Espeland and Emam, 2011), higher outcrossing rates could also facilitate population viability if offspring

quality is important, for instance if inbreeding depression is prevalent (Cheptou et al., 2000; Fox and Reed, 2011). Specifically, local adaptation and offspring quality (e.g., higher germination rates and survivorship) should be particularly important for the maintenance of populations in stressful (defined here as high temperatures and water stress) environments where the potential for mortality and negative genetic effects may be exacerbated (Hauser and Loeschcke, 1996; Heschel et al., 2005; Armbruster and Reed, 2005; Riginos et al., 2007). Many plants that specialize on stressful environments have a suite of traits that are thought to be adaptive (Grime, 1977; Chapin et al., 1993; Walck et al., 1999, 2002; Van Zandt, 2007), for instance by increasing water-use efficiency. The presence of these convergent traits across a broad taxonomic range implies that specialization on habitats with stressful conditions may require a relatively narrow set of traits that should be conserved across generations. Moreover, recruitment from more suitable habitats cannot buffer populations in the stressful environment via source-sink dynamics, since few if any other populations exist in more benign habitats. Therefore, species that specialize on xeric habitats should have less plasticity in their traits and be more tightly restricted to the local environment (Ellison and Parker, 2002; Pohlman et al., 2005).

Traits that are adaptive in xeric conditions may also reduce the attractiveness of xeric-adapted plant species and their ability to compete for pollinator services. For instance, plants that are shorter and have lower reproductive output may be able to tolerate high-stress environments, but they are less attractive to pollinating insects (Dickson and Petit, 2006; Mevi-Schutz and Erhardt, 2005; respectively), which could lead to a reduction in pollen receipt. While a small floral display (fewer flowers open

34

concurrently) may reduce within-plant movements and increase outcrossing rates, it may also reduce visitation rate, pollination success, and total seed set. In order to optimize their investment in reproduction under stressful conditions, specialist plants should exhibit traits that will reduce resource loss while increasing offspring quality. When offspring quality comes at a cost to total reproductive output, lower seed set in a stress-adapted plant may reduce its ability to colonize novel sites (Kelly et al., 1996b; MacDougall et al., 2006; Soberón, 2007), and therefore may contribute to range restriction of stress-adapted specialists. By identifying the mechanisms that restrict species ranges and contribute to their extinction risk, we will be better able to predict which species are affected by future climate changes and focus limited resources toward conservation of the species most vulnerable to extinction.

In this study, I manipulate temperature and plant-available water in order to test three hypotheses concerning the relationship between xeric-adaptive traits, pollinator attraction traits, and reproductive success in three locally abundant, habitat specialist species (LAHS) in comparison to a common congeneric species (CC). The first hypothesis states that LAHS species are better adapted to the glade environment in comparison to related species with more generalist habitat use. Specifically, the LAHS species should have traits associated with adaptation to hot, xeric habitats (via differential resource allocation) and therefore be resistant to abiotic stress (defined here as drought and high temperature). I predict that the CC species will have a reduction in key physiological traits, such as relative growth rate and specific leaf area, with an increase in stress, and that the LAHS species will not. The second hypothesis predicts that LAHS species have floral traits that are associated with decreased attractiveness to pollinators

but increased outcrossing rates, and the widespread species will have traits that are more attractive to a variety of pollinators. Finally, I test the prediction that offspring quality will be higher in the stress-adapted LAHS species than their CC species, which may facilitate offspring recruitment in stressful environments.

MATERIALS AND METHODS

Study Species

In order to assess the prediction that locally abundant, habitat specialist (LAHS) species are adapted to hot, xeric conditions, I will compare the response of three LAHS plants (*Delphinium treleasei, Echinacea paradoxa*, and *Scutellaria bushii*) and their common congeneric species (CC; *D. carolinianum*, *E. pallida*, and *S. ovata*, respectively) to experimental manipulations of heat and water availability that are characteristic of the habitats to which they are endemic. These three LAHS species are all endemic to the Ozark glades in Missouri and Arkansas, which are hot, xeric environments with thin soils and exclusively herbaceous cover, located within a matrix of more mesic woodland habitat (Nelson and Ladd, 1982; Baskin and Baskin, 1988; Templeton et al., 2001; Yatskievych, 1999). All three CC species also occur on glades but are not restricted to them, which will offer insight into the mechanisms that restrict the LAHS species and not the CC species to glades. Comparing closely-related species also controls for the potential effect of evolutionary history on the vegetative and floral traits of LAHS species, and the multiple comparisons across a range of evolutionary history make the results of this study more broadly applicable.

Experimental manipulations

The stress resistance of three LAHS species and their CC species to drought and high heat conditions was estimated via greenhouse and growth chamber experiments. Individuals of each congeneric pair were exposed to a series of manipulated abiotic conditions, in accordance with *in situ* field observations and optimized in the greenhouse, and their fitness responses were compared. Seeds collected in the field during the summer of 2008 were cold stratified at 4°C for 90 days and allowed to germinate. Forty-four to 50 seedlings of each species were planted in a 3:2:1 ratio of Metromix360:Turface athletics:New Plant Life Topsoil mix, to approximate the relatively rapid draining soils associated with glades. Individual plants were allowed to establish for one week and randomly assigned to one of two treatments within two separate experiments, one that manipulated water availability (low and moderate water) and temperature regime (high and moderate temperature), with 22 to 45 replicates per treatment depending on the species pair (Table 3-1). Due to low growth rates of the *Delphinium* taxa, both the drought and temperature experiment were conducted twice in order to increase replication (from 64 to 133 individuals for the temperature experiment and 91 to 193 replicates for the drought experiment). For the second experiment, established individuals were first cold stratified, then removed from the coldroom, and exposed to the same treatments as above. Individuals were randomly arranged in order to reduce potential spatial variation in abiotic conditions, and the temperature regimes were rotated weekly between two environmental chambers to diminish a potential chamber effect.

Drought experiment—Watering regimes were designed to reach soil water-holding capacity and were applied three times per week. The *Delphinium* and *Echinacea* species were first planted in small (7.6 cm diameter) pots, in order to maintain

appropriate soil-moisture levels and where transplanted into large pots (11.4 cm diameter) after the first 49 days of treatment. The amount of water administered per treatment scaled with pot size (i.e., 50 mL for small pots and 100 mL for large pots) in order to account for the increased soil volume. The *Scutellaria* species grew faster and were planted directly into the larger pots. The *Delphinium* and *Echinacea* species require vernalization, which simulate winter conditions, before they will bolt and flower. Following 49 days of treatment, all individuals were placed in an environmentally controlled coldroom (4ºC) and remained un-manipulated for 49 days. The individuals were then removed from the coldroom and allowed 10 days to acclimate. Cold stratification was repeated until the individual began flowering or until they had experienced four cold treatments, after which all individuals were harvested. During the fall of 2010, the water treatments were increased to 100 mL and 150 mL, respectively, for the *Echinacea* taxa due to low relative humidity in the greenhouse. Plants were fertilized once a week with Peters Professional 15-16-17 Peat-Lite Special at 150 ppm nitrogen dissolved in water to reduce the effect of resource limitation on plant traits. All individuals were given equal amounts of fertilizer (50 and 100 mL for small and large pots, respectively) and the additional water required for the control treatment was added before the individuals were watered with fertilizer water, in order to reduce loss of nutrients via leaching.

Temperature experiment—The temperature regime for each species pair was determined via field observations of temperatures on and off glades during peak flowering time of each genus (Table 3-1) and incorporated diurnal temperature change. The temperature regime for the *Echinacea* species was reduced from 30/38 ºC after the

first round of treatment, because growth was minimal and mortality was relatively high. For clarity, I focus on the response of these species following the first round of treatment. Temperature regimes commenced after an acclimation period during which the air temperature was incrementally increased (two or three degrees Celsius every 3 or 4 days, for a total of 12 days). Since the *Delphinium* and *Echinacea* taxa require vernalization before flowering, all individuals were placed in a coldroom for 49 days as above. Before cold stratification, the high temperature treatment was incrementally decreased and was subsequently increased before re-administering the experimental treatments upon removal from the coldroom. Cycles between treatment administration and cold stratification were repeated until flowering commenced or for up to four cold treatments, after which all individuals were harvested. Plants were watered with fertilizer once a week in order to reduce the potential effect of resource limitation on plant traits.

Morphological measurements

Vegetative traits—In order to assess the response of the LAHS and CC species pairs to the above treatments, vegetative and reproductive traits were quantified. Plant height, number of stems, and vegetative output (i.e., leaf size and number) were noted weekly. Three leaves per individual were tagged upon emergence, and leaf death noted in order to calculate leaf turnover. Leaf thickness was measured for up to five leaves per individual and averaged. In the drought experiment with the *Delphinium* species, many of the leaves were relatively small and fragile, and leaf thickness could not be measured without damaging the leaves; therefore, I could not test for differences in leaf thickness for this experiment.

Following fruit maturation, final morphological measurements were taken (e.g., final height, number of leaves, leaf thickness and trichome density), and plants where harvested, separated into vegetative, reproductive, and below ground biomass. One leaf per plant was removed, digitized for leaf area quantification in SigmaScan Pro 5.0 (SigmaScan Pro 5.0, 2002). The roots were washed thoroughly to remove soil particles, and all plant material was dried at 40-50ºC for at least 48 hours and weighed. Total biomass, relative growth rate (total biomass divided by the age of the plant in days), leaf longevity, shoot to root ratio, and specific leaf area (SLA; the ratio of leaf area and dry mass) were quantified. Specific leaf area is a measure of leaf density; lower SLA indicates a more dense leaf, which is associated with lower evapotranspiration and photosynthetic rates. Relative reproductive output was calculated as the mass of reproductive material divided by total biomass (the sum of reproductive, shoot, and root biomass).

Reproductive traits—In order to simulate pollination and ensure seed set, I applied outcross pollen to three flowers per individual per week during blooming for the *Delphinium* and *Scutellaria* taxa and up to twenty florets for *Echinacea* species. Following maturation, the fruits were harvested. Due to morphological differences among genera, some traits, such as reproductive success, were quantified differently for each congeneric pair (see below).

Delphinium *congeners*

Vegetative traits—The *Delphinium* species have roughly round leaves that vary in the degree to which they are dissected. Therefore, I visually estimated the proportion of a circle filled by the leaf (i.e., 0.1, 0.25, 0.33, 0.5, 0.67, 0.75, and 1), and estimated the

radius of the circle as the length of the leaf from the center (where the petiole and leaf blade merge) to the edge. Leaf area was then calculated as π times the radius squared (π x (length of leaf blade)^2 x proportion of circle). Throughout the experiment, biomass was estimated in a non-destructive manner as total leaf area (leaf number multiplied by estimated leaf area).

Reproductive traits—Individuals were checked three times a week for initiation of flowering, and reproductive phenology was defined as the age of the individual at the time of inflorescence initiation. Three flowers per plant were tagged while still in bud and monitored three times per week until flower senescence in order to determine flower longevity. At peak flowering, I measured the floral traits (i.e., sepal width, sepal height, floral spur length, and anther-stigma separation) of three flowers for each individual. Corolla area was calculated as the product of sepal area and height, and anther-stigma separation was measured from the top of stamen to the stigma of the lowest ovary. Nectar was collected from up to nine flowers per individual, and nectar volume was calculated. Nectar sugar content was determined using a Brix refractometer, and floral output was quantified as the number of flowers produced.

Reproductive success—Following the collection of fruits, the number of fruits that developed seeds and the number seeds produced per pollinated flower were quantified. Total seed set was calculated as the product of average seed set per pollinated fruit and total floral output. In order to estimate seed quality, up to thirty seeds per individual were weighed, and mass per seed was calculated.

Echinacea *congeners*

41

Vegetative traits—Throughout the experiment, biomass was estimated in a non-destructive manner as total leaf area (the product of leaf number and estimated leaf area) for the *Echinacea* species. Leaf area was calculated as the width times the length of an average leaf blade. Two individuals were subject to an additional cold treatment and growth period after the inflorescence senesced due to mislabeling; none of the data collected after the inflorescences senesced (e.g., biomass measures) were used in the analyses.

Reproductive traits—Individuals were checked three times a week for initiation of inflorescence, and reproductive phenology was defined as the age (days) of the individual at the time of initiation. Corolla area was calculated as the number of ligules multiplied by the area of an average ligule size (i.e., the product of ligule width and length). The number disk florets were counted, and nectar volume and sugar content were quantified for three florets per sexual stage (i.e., male and female), since *Echinacea* species are protandrous. Few individuals of *E. paradoxa* bloomed in the temperature experiment; therefore, nectar production and quality could not be compared.

Reproductive success—Since *Echinacea* species are known to produce unfertilized capsules that collapse when pressure is applied, the pollinated capitula were gently pressed with forceps to confirm fertility. Seeds that did not collapse under the small amount of applied pressure were assumed to be fertile. Total reproductive success was quantified as the proportion of capsules that were fertile times the total number of florets produced. A virus infected some individuals resulting in the senescence of their inflorescences, and these individuals were excluded from the analyses of floral output

42

and reproductive success. However, they were used in analyses of initiation of inflorescence, relative reproductive output, and total biomass.

Scutellaria *congeners*

Vegetative traits—Due to a large number of leaves and high variability of leaf size, the non-destructive estimate of biomass for the *Scutellaria* taxa was quantified as total stem length. One leaf per individual was collected, and the number of trichome was counted in each of three, three by three millimeter squares haphazardly placed in upper third, middle third, and lower third of the leaf. Trichome density was quantified as the total number of trichomes per 27 mm^2.

Reproductive traits— Individuals were checked three times a week for initiation of flowering, and reproductive phenology was defined as the age of the individuals in days at the time flowering commenced. Three flowers per plant were tagged while still in bud and monitored three times per week until flower senescence in order to determine flower longevity. At peak flowering, I measured the floral traits (i.e., petal width, petal depth height, floral tube length) of three flowers for each individual. Floral output was quantified as the number of flowers produced. The LAHS *S. bushii* produced only cleistogamous flowers, which do not open, and all fruits were fertilized internally. Therefore, I did not pollinate any flowers of either species in order to maintain a balanced comparison. All measures of reproductive success are based on self-pollinated fruits and should be interpreted with caution.

Reproductive success—During the breakdown process, the number of seeds per fruit was counted for ten fruits and averaged. Total seed set was calculated as the average number of seeds per fruit multiplied by total floral output, and offspring quality was

determined via germination trails. Up to twenty and ten seeds for the drought and temperature experiments, respectively, were cold stratified for 90 days, placed in an environmentally-controlled chamber (21°C), and allowed to germinate. Replication was different between the two experiments due to differences in total seed set, and replication of germination rates for the temperature experiment was biased (44 to 4, control to high temperature respectively) due to low fruit set in the high stress treatment. Since total seed set could not be accurately quantified, I estimated total reproductive success as the proportion of fruits to set seed.

Statistical analyses

In order to control for multiple comparisons among congeners and treatments, vegetative traits of the *Delphinium* species were analyzed via two-way, nested MANOVA with Species and Treatment as factors and treatment nested within Experiment in order to account for replicate experiments. Only one individual of *D. trealeasi* (LAHS) bolted in the first experiment (i.e., the one without a cold treatment before manipulations commenced); therefore, the floral traits of the *Delphinium* taxa were analyzed for the second experiment only, and the experiment effect was removed. The morphological traits of *Echinacea* and *Scutellaria* taxa were tested via two-way MANOVA with Species and Treatment as factors. Since not all individuals flowered, analyses of vegetative and reproductive traits were conducted independently in order to account for the loss in replication that occurred. Some traits were only quantified for a subset of individuals, which would have decreased the replication of the MANOVA substantially and were analyzed separately. Those traits that were analyzed independently of the others, and therefore do not account for multiple comparisons, are indicated in the

44

results section and corresponding results table (Table 3-2). Two traits of the *Delphinium* species had an exponential distribution in the temperature experiment, could not be normalized, and were analyzed via general linear model using the reciprocal link function in JMP Statistical Software (SAS Institute 2009).

Individuals were also ranked by whether or not they initiated inflorescences, and differences among congeners and treatments were tested via two-way logistic regression with Species and Treatment as factors using JMP Statistical Software (SAS, 2009). Analysis of the *Delphinium* species accounted for replicate experiments as above. The nectar sugar content of the *Delphinium* species often reached the maximum of the Brix refractometer, which truncated the continuous variable at 50%. Therefore, I ranked nectar sugar content based on concentration (i.e., if the sugar concentration was greater than or equal to 50 percent then it was coded as one and if it was less than 50 percent it was coded as 0), and logistic regression was used to test for differences among treatments and congeners as above.

All data were transformed for normality (except where noted above), and if they could not be transformed, the analyses were repeated using permutation ANOVA (*aovp* in the R Statistical Package (R Development Core Team 2008)). I used permutation ANOVA in order to incorporate the nested experimental design of the *Delphinium* species into a nonparametric framework. Permutation ANOVA is a Monte Carlo resampling technique (without replacement) that estimates a null expectation based on a random resampling of the available data and compares each of 5000 runs to the null. Statistical significance is quantified as the number of runs for which the F-statistic is greater than or equal to the observed value.

RESULTS

Delphinium *congeners*

Drought experiment—Only three *D. carolinianum* and no *D. treleasei* individuals bolted and flowered in the precipitation experiments combined, despite the large number of individuals (193) and duration of the experiment (175 treatment days, 322 days including cold stratification events). Watering treatments had been optimized in the greenhouse using the *Delphinium* taxa, and growth was not biased by treatment, which suggests the stress treatment *per se* did not cause the low rate of flowering. Therefore, only vegetative traits could be analyzed. Relative growth rate, total biomass, and shoot to root ratio were lower in the LAHS *D. treleasei* in comparison to its CC congener (Table 3-2), as I predicted. However, there was no effect of treatment or an interaction between treatment and species in any trait measured, which suggests that there is no adaptive benefit to these traits in response to drought. No leaf traits (i.e., leaf area, specific leaf area, total leaf area, and leaf longevity; Table 3-2) differed between species or treatments.

Temperature experiment—The *Delphinium* congeners differed in their responses to stress, and the LAHS, *D. treleasei*, was less negatively affected than its congener, in congruence with my hypothesis that the LAHS species are more stress resistant. *Delphinium carolinianum* (CC) had lower relative growth rate and total seed set in the high stress treatment, but *D. treleasei* did not differ between treatments (Table 3-2; Figure 2-1A and C, respectively). Temperature regime had a significant effect on some traits in both *Delphinium* species, suggesting that they are either conserved, linked to evolutionarily stable traits, or do not represent an adaptive benefit in these plants. Height, total biomass, and shoot to root ratio were significantly lower in the high temperature

treatment than the control treatment for both species (Table 3-2). In addition, the number of flowers open and floral display size decreased with an increase in temperature. Leaf longevity, on the other hand, was higher (Table 3-2), and relative reproductive output was lower in the high temperature treatment, as predicted by stress theory.

The *Delphinium* congeners differed in some vegetative and reproductive traits, regardless of temperature treatment. As I predicted, *D. treleasei* (LAHS) was shorter, had thicker leaves, and produced fewer flowers in total (Table 3-2). *Delphinium treleasei* also had fewer flowers open concurrently and higher nectar sugar content, which may affect outcrossing rates and offspring quality if pollinator behavior is affected. The difference in the number of flowers open concurrently is not driven by reduced floral output *per se*, since the proportion of flowers open was marginally lower for *D. treleasei* than *D. carolinianum*. On the other hand, anther-stigma separation was lower in the LAHS *D. treleasei* (Table 3-2), which I predicted to be higher in order to increase the potential for outcross pollination. Moreover, the longevity of *D. treleasei*'s leaves and flowers was shorter in comparison to *D. carolinianum*. I predicted that leaf and flower longevity would be higher in the LAHS species in order to reduce loss of resources and increase the potential for outcross pollination, respectively. There were no or marginal differences in some leaf traits (leaf area, specific leaf area, and leaf longevity; Table 3-2) and some floral traits (inflorescence initiation, corolla area, and floral spur length; Table 3-2).

Echinacea *congeners*

Drought experiment—The *Echinacea* species did not differ in their responses to watering regime, contrary to my predictions. Vegetative and floral traits did not differ between species and treatments indicating the *E. paradoxa* is not more locally-adapted or

specialized than its CC congener with respect to the traits measured here. Both species had shorter stems, lower relative growth rates, reduced total biomass, and fewer florets in the drought treatment than the control treatment (Table 3-2), indicating that the drought treatment was inducing a stress response.

Echinacea paradoxa (LAHS) had thinner leaves and greater reproductive output (number of disk florets) than its CC, *E. pallida*, contrary to my prediction that LAHS species would have thicker leaves and fewer florets. Moreover, *E. paradoxa* (LAHS) bloomed later than *E. pallida* (CC). There were no differences in several traits (i.e., leaf area, leaf longevity, shoot to root ratio, corolla area, relative reproductive output, reproductive phenology, total seed set, and the proportion of pollinated florets that were viable; Table 3-2), which suggests that these traits are either evolutionary conserved or both species are adapted to local conditions.

Temperature experiment—One trait, specific leaf area (Table 3-2), differed between species in response to the temperature treatments. *Echinacea paradoxa* (LAHS) had higher specific leaf area (SLA) than *E. pallida* in the control treatment, but there was no difference in the high temperature treatment (Table 3-2; Figure 3-2A). This difference in SLA suggests that *E. paradoxa* responds to stress by reducing water-loss and photosynthetic rates, and *E. pallida* (CC) has an increase in evapotranspirative cooling, which results in similar SLA in the high stress environment.

Temperature treatment had a significant effect on leaf area, relative growth rate, and total biomass (Table 3-2), which indicates that the treatments were effective and that evolutionary history may be influencing the response of these species to an increase in temperature. Probability of flowering was the only trait that differed between species

(Table 3-2), with *E. pallida* (CC) being more likely to flower than *E. paradoxa* (Table 3-2; Figure 3-2B). However, it is important to note that few individuals bolted and flowered, and the majority of them were *E. pallida* (CC; 17 of 20), which decreased the power with which to test for differences among species and treatments.

Scutellaria *congeners*

Drought experiment—*Scutellaria bushii* (LAHS) and *S. ovata* responded differently to the watering regimes, and *S. bushii* was more resistant to drought stress, confirming my hypothesis of greater resistance in the LAHS taxa. *Scutellaria ovata* (CC) had a reduction in height (Figure 3-3A), relative growth rate, and total seed set (Figure 3-3B), but *S. bushii* did not (Table 3-2), which resulted in statistically significant differences between taxa in the control treatment only. In other words, the *Scutellaria* congeners did not differ in these traits under drought conditions. Reproductive phenology was only different between the congeners in the control treatment; *S. bushii* bloomed significantly later and was more variable than *S. ovata*. There was no difference between species across the drought treatments.

Total biomass and total floral output decreased for both species with a decrease in water availability (Table 3-2), although the response was more substantial in *S. ovata* (CC) than *S. bushii* (LAHS). Both responded similarly to a decrease in water availability with a shift in resource allocation, specifically shoot to root ratio was lower for both species in the drought treatment in comparison to the control treatment. Leaf thickness (Table 3-2) was significantly lower and relative reproductive output (Table 3-2; Figure 3-3C) was higher in *S. bushii* than *S. ovata* (Table 3-2), contrary to my predictions. On the other hand, trichome density (Table 3-2) and germination rate (Table 3-2; Figure 3-3D)

49

of *S. bushii* were higher than its common congener, *S. ovata*, corroborating my hypothesis of greater stress-adaptation and offspring quality in the LAHS species.

Temperature experiment—The *Scutellaria* congeners responded differently to the temperature treatments, but the direction of those differences varies by trait. In congruence with my hypothesis, trichome density of *S. bushii* was higher than *S. ovata* and did not vary with temperature treatment. *Scutellaria ovata*, on the other hand, had an increase in trichome density with an increase in stress. Total floral output was lower for both species in the high temperature treatment, but the reduction was much larger in *S. ovata* (CC) than *S. bushii*. On average, *S. ovata* had a reduction of 388 (72.9%) flowers, and *S. bushii* had 58 (59.2%) fewer flowers in the high stress treatment. Both species also had a decrease in height with an increase in temperature, resulting in the height of *S. ovata* in the high temperature treatment equaling that of *S. bushii* in the control treatment. Contrary to my predictions, specific leaf area, relative growth rate, and total biomass were not different between temperature treatments for *S. ovata* but were for *S. bushii* (LAHS), which had a reduction in all three traits with an increase in temperature.

In response to an increase in temperature, both species had lower leaf area, were less likely to bloom, produced fewer seeds, and had lower relative reproductive output, which suggests that more resources are being allocated to survival rather than reproduction under high stress conditions. Some vegetative and floral traits differed between the taxa, which supports a role for trait differences in the maintenance of species range size. Leaves of *S. bushii* (LAHS) were smaller (had lower leaf area) and thinner than *S. ovata*, offering contradictory evidence for stress adaptation in the LAHS species. Relative reproductive output and germination rate was greater for *S. bushii* than *S. ovata*,

50

implying that offspring quality may be important in order to specialize on these restricted habitats. *Scutellaria bushii* bloomed significantly earlier than *S. ovata* (Table 3-2).

DISCUSSION

The relative importance of abiotic and biotic mechanisms in determining species ecogeographical distributions is a central and critical topic in ecology. Here, I document the potential for both factors to act synergistically in three locally abundant, regionally-rare (LAHS) species. The LAHS species were less responsive to experimental stress in key physiological traits, and two of the three differed from their common congeneric (CC) species in floral traits and offspring quality, which confirms my hypotheses that the LAHS species are locally adapted to the xeric glad environment and that the trade-off between resource allocation to vegetative and floral traits differs in LAHS and CC species.

Each of the LAHS species was more resistant to high stress environments in comparison to their widespread congeners, despite a stress response in both species, and only one CC species (*S. ovata* in the temperature experiment) had any traits that were less responsive than the LAHS species. While all species were negatively affected by drought or high temperature conditions, the LAHS species had fewer traits negatively affected by an increase in stress, particularly those important for water conservation. For instance, the relative growth rate (RGR) of *Delphinium treleasei* (LAHS) did not differ between temperature treatments, but *D. carolinianum* had a significant reduction in RGR with an increase in stress (Figure 3-1A). Relative growth rate (RGR) is thought to be adaptive in stressful environments as it is associated with low photosynthetic rates and high water-

use efficiency. Relative growth rate of *D. treleasei* was also lower than *D. carolinianum* in the precipitation experiment, which corroborates my prediction of stress-adaptation.

Similarly, the impact of stress on specific leaf area differed between the *Echinacea* species and potentially reflects two strategies for responding to abiotic stress. *Echinacea paradoxa* (LAHS) produced more dense leaves (lower SLA) in the high stress environment, which is associated with reduced water loss, lower RGR, and lower resource turnover. *Echinacea pallida*, on the other hand, produces leaves with higher SLA in the high temperature conditions, which may reflect an increase in evaporative cooling. Contrary to my predictions, *E. pallida* was more likely to bolt and had fewer florets than *E. paradoxa* (Figure 3-3 B and C, respectively) in the precipitation experiment, which suggests that *E. pallida* has an accelerated life cycle and lower total reproductive output in comparison to *E. paradoxa*. However, these *Echinacea* species are perennial and live longer than the duration of this study. Therefore, I could not test for this mechanism.

The *Scutellaria* species also differed in their response to an increase in stress, and *S. bushii* was generally more resistant to stress. For instance, *Scutellaria ovata* (CC) was shorter in the drought treatment in comparison to the control treatment, and *S. bushii* had no change in height (Figure 3-3A), which is associated with stress-adaptation. This pattern was consistent in several other traits (e.g., total biomass, relative growth rate, etc.), further corroborating my hypothesis that LAHS species are more resistant to stress. Height is also an important attraction trait for many insect species and may result in higher competitive ability of *S. ovata* for pollinator services in less stressful habitats. In the high stress environment, there was no height difference between *S. ovata* and *S.*

bushii, indicating that the competitive advantage of height may be reduced. The results

from the temperature experiment with the *Scutellaria* species are less clear, with some

traits differing in the direction I predicted and others in the opposite direction,

particularly the vegetative traits. This variation in response to stress between temperature

and drought stress may reflect the different physiological mechanisms that underlie

resistance to these traits, or it may reflect the simplicity of the experimental design. In

order to pin-point which abiotic stressor dominated species responses given limited

space, I conducted independent experiments and did not cross the water and temperature

treatments. However, a combination of abiotic factors is likely contributing to the unique

conditions on glades in concert. The rocky substrate and extreme abiotic conditions on

glades make *in situ*, population-level, field manipulations difficult, and the establishment

of individuals from the species of interest is unreliable. Therefore, future studies should

combine manipulations of abiotic factors in common garden experiments with *in situ*

observational studies that document both biotic and abiotic factors at the individual level.

The combination of common garden experiments and observational studies will

contribute to a more biologically realistic understanding of abiotic and biotic factors in

determining the reproductive success and population growth of LAHS and CC species.

In addition to absolute differences in the degree of response, the greater variation

in response of the CC species to the high and moderate stress conditions suggests that

they have greater phenotypic plasticity in the measured traits. While I did not explicitly

test for differences in phenotypic plasticity, which requires genetically identical

individuals, the trend of greater phenotypic change in the CC species vs. the LAHS

species is consistent across the genera. Greater phenotypic variation in the traits of the

CC species in comparison to their LAHS congeners could result several potential processes, including genetic assimilation and/or genetic homeostasis. Genetic assimilation is the process by which phenotypic variation in response to an environmental condition becomes canalized and no longer requires the environmental signal to be expressed (Waddington 1953a, b; Pigliucci et al., 2006). Under consistent selective pressure for the xeric-adaptive traits, the genetic reaction norm of a given trait should vary less between environments, as documented by the reduced response of the LAHS species to changes in stress. From the perspective of the CC species which have broader habitat use, the differences in phenotypic variation between CC and LAHS species suggest the mechanism of genetic homeostasis, or the maintenance of genetic variability through adaptation to a diverse set of environmental conditions (Waddington, 1953b, Lerner, 1954), for instance as would be experienced across multiple habitat types. Future studies should explicitly test the relative importance of these mechanisms in determining the differences in phenotypic variation between CC and LAHS species.

Key floral attraction traits also differed between congeners in these experiments, as I predicted if resources for reproduction are allocated differently in LAHS and CC species. *Delphinium carolinianum* was significantly taller than its LAHS congener and could be a better competitor for pollinator services, if height dominates attractiveness. *Delphinium treleasei* (LAHS) had fewer flowers open concurrently, which is less attractive to pollinators but often increases outcrossing rates via a reduction in within plant movement by the pollinator. While only marginally significant, the flowers of *D. treleasei* were larger than *D. carolinianum*, suggesting that the resources committed to reproduction are allocated to fewer, larger flowers. Previous research indicates that *D.*

treleasei has fewer, larger flowers in the field and that they attract more specialized pollinators (Miller-Struttmann, Chapter 1). Nectar sugar concentration was also higher in *D. treleasei* than *D. carolinianum*, but there was no difference in nectar volume, which indicates that more concentrated nectar reward may be an important attraction trait for the species that specialize on water-limited habitats. Many nectar-collecting insects can detect nectar quality and choose flowers accordingly (Wilson et al., 2006), resulting in preferential visitation to those species with the greater reward. Moreover, higher sugar concentration is advantageous in stressful environments where water is limiting and may compensate for the reduction in floral display. On the other hand, greater viscosity may make the nectar of the LAHS species more difficult to remove from the flower and may reduce the number of species able to extract the reward. Choice trials should be conducted with known pollinators of these species to determine whether nectar sugar concentrations consistent with field measurements affect rate of nectar removal and the number of species able to capitalize on the resource. The LAHS species, *S. bushii*, did not produce chasmogamous flowers, and I could not quantify floral attraction traits for this species. However, relative reproductive output was higher in *S. bushii* (LAHS), contrary to the prediction of stress-resistance, but in congruence with my prediction that higher-quality offspring, which require greater resource investment, are required in order to species to survive and specialize on stressful habitats.

The measures of reproductive success in *D. treleasei* and *S. bushii* (LAHS) documented here confirm my prediction that LAHS species have fewer, higher-quality offspring, which may increase survival rates and population viability in more stressful habitats. Both LAHS species had significantly higher offspring quality (i.e., seed mass

and germination rate, respectively). Conversely, total seed set of both CC species (*D. carolinianum* and *S. ovata*) was significantly higher in the control treatments but was equivalent to their LAHS congeners in the high stress treatments. The difference in total seed set between LAHS and CC species in the control treatment may reflect an important role for propagule pressure in determining species ranges. High propagule pressure is thought to contribute to range expansion in some species by reducing recruitment limitation and increasing the number of individuals and genotypes with the potential to establish in a new habitat (Kelly et al., 1996; MacDougall et al., 2006; Soberón, 2007). In less stressful habitats, the higher seed set of the CC species may allow it to establish more quickly than the LAHS species, increase in population size, and/or outcompete it for abundant resources. However, in stressful habitats, high offspring quality may enhance survival and allow adaptation to local conditions, thus reinforcing the pattern of range restriction to specific, stressful abiotic conditions seen in these LAHS species.

Given the rate of current climatic change and the prevalence of entomophilous pollination, understanding the relative importance of biotic and abiotic mechanisms in determining the biogeographical ranges of species is imperative. Few studies consider biotic and abiotic mechanisms in concert, despite a long history of inquiry into each individually. Future studies should work to understand how these mechanisms interact and in which cases they will predominate, rather than regard them as mutually exclusive. While biological information is lost by reducing continuous traits into categories, the seven forms of rarity offer a framework through which to make and test predictions concerning which mechanisms are most relevant for species with similar distributions. In this study, I explored the potential interaction between abiotic stress and reproductive

allocation in three regionally-rare, habitat-specialist species, which are characterized by a type of rarity that is often ignored in the discussion of rarity and found that both may contribute to the restriction of their ranges to glade habitats. These results imply an important role for pollination biology in mediating the interaction between the stress-adaptation and reproductive success in stressful environments. In an era of increasing anthropogenic impacts on natural habitats, a more in-depth and nuanced understanding of the determinants of species biogeographical ranges will create more efficient and hopefully more effective conservation strategies.

Table 3-1. A description of the temperature regimes, number of cold stratifications required to induce flowering, and the floral traits that were quantified differently for each congeneric pair. Temperature regimes are based on field collected data, and reflect the night and day time temperatures, respectively. Temperatures were gradually changed manner over two hours in order to reduce heat shock. The *Echinacea* species had a temperature regime of 28/30ºC for the first application of the treatment. However due to slow growth, the temperature regime was changed to 27/35ºC, and analyses did not include data recorded from the first treatment application.

	Temperature Regime (low/high)	*Replication (Drought/Temp)*		*No. cold treatments*	*Corolla area (CA)*	*Display size*	*Floral output*	*Reproductive success*
		Total	*Bolted*					
Delphinium	21/29 ºC	193/133	0/62	0 - 2	Sepal width x height	CA x no. flowers open	No. flowers	Mass per seed
Echinacea	27/35 ºC	89/94	36/20	3 - 4	Ray petal width x length	CA x no. ray petals	No. florets	Fertilized florets: Pollinated florets
Scutellaria	28/38 ºC	90/103	87/70	0	Petal width x height	CA x no. flowers open	No. flowers	Germination rate

Table 3-2. Statistical results from analyses of vegetative traits, floral traits, and reproductive success between three congeneric species pairs and in response to experimental manipulations of water availability and air temperature. Analyses of the experiments with the *Delphinium* species have an additional term (Treatment nested within Experiment), since each experiment (drought and temperature) was repeated in order to increase replication. Few individuals (3) bolted in the drought experiment with the *Delphinium* species, and only vegetative traits could be analyzed. Significance values are indicated by symbols († denotes $P < 0.1$, * indicates $P < 0.05$, ** signifies $P < 0.01$, and *** symbolizes $P < 0.001$) and indicated in bold font. Some traits did not meet the assumption of normality for parametric tests, and a non-parametric resampling procedure (permutation ANOVA) was used to assess differences between species and treatments. When results of the parametric and non-parametric analyses differed, the significance levels from the non-parametric tests are indicated in parentheses, and those that are significant are indicated in bold font.

ø denotes that analyses were run independent of the other traits (i.e., not corrected for multiple comparisons) due to low replication of that trait.

‡ signifies analyses that were conducted using data from one of the two experimental replications (*Delphinium* species height and reproductive traits only)

• indicates that the analyses were conducted with data from both replicate experiments for the *Delphinium* temperature experiment.

^ signifies that the data have an exponential distribution and were analyzed independent of the other variables via general linear model using a reciprocal link function.

º denotes that analyses were run independent of the other traits using logistic regression.

	Drought			Temperature		
	Species	*Treatment x Experiment*	*Species x Treatment*	*Species*	*Treatment x Experiment*	*Species x Treatment*
Delphinium						
Vegetative traits						
Height[‡]	--	--	--	**21.52*****	**10.53****	0.006
Leaf thickness	--	--	--	**13.43*****	2.88[†]	1.69
Leaf area[ø]	2.18	0.11	0.34	3.43[†]	2.86[†]	0.98
Specific leaf area	0.39	0.11	0.49	0.09	1.12	1.25
Total leaf area	0.30	1.23	0.62	--	--	--
Leaf longevity	1.48	0.092	0.031	(0.27)	(0.0627)	(0.84)
Relative growth rate[^]	**(0.0014)**	(0.41)	(0.92)	1.05	**6.53***	**5.24***
Shoot:Root ratio	**(<0.0001)**	(0.12)	(0.65)	**(0.0196)**	(0.094)	(0.34)
Total biomass[^]	**(0.0008)**	(0.87)	(0.96)	0.057	**11.41*****	1.04
Reproductive traits[‡]						
Inflorescence initiation[ø•]	--	--	--	0.89	2.96	1.30
Anther-stigma separation	--	--	--	**8.91****	0.005	1.03
Corolla area	--	--	--	3.04[†]	3.45[†]	1.11
Display size	--	--	--	3.09[†]	**6.62***	0.70
Floral output	--	--	--	**5.75***	**9.21***	0.29
Floral spur length	--	--	--	0.03	0.44	0.66
Flower longevity	--	--	--	**13.01****	2.24	1.17
Nectar volume	--	--	--	0.06	1.60	1.94
Nectar sugar content[ø]	--	--	--	**5.15***	**5.21***	0.66
No. flowers open	--	--	--	**11.88****	**4.70***	0.68
Proportion of flowers open	--	--	--	3.26	0.25	0.04
Relative reproductive output	--	--	--	0.58	**5.60***	0.63
Reproductive phenology	--	--	--	(0.90)	(0.10)	(0.077)
Reproductive success[‡]						
Seed quality	--	--	--	**13.80*****	**9.24****	1.49
Total seed set	--	--	--	(0.11)	(0.093)	**(0.051)**
	Species	*Treatment*	*Species x Treatment*	*Species*	*Treatment*	*Species x Treatment*
Echinacea						
Vegetative traits						
Height	3.09[†]	**17.05****	0.16	0.59	2.90	0.46
Leaf area	0.07	2.31	1.70	0.14	0.35	0.43
Leaf thickness	**12.42****	0.85	0.19	0.00	**5.44***	0.61
Leaf longevity	1.58	1.79	0.11	2.05	3.72[†]	1.80
Specific leaf area	1.39	0.06	0.22	3.83[†]	0.70	**6.83***
Relative growth rate	**6.69***	**13.85****	0.05	(0.18)	**(<0.0001)**	(0.67)

Total biomass	2.03	**16.61****	0.18	(0.43)	**(<0.0001)**	(0.42)
Shoot:Root ratio	(1.00)	(0.12)	(0.58)	(0.50)	(1.00)	(0.57)
Reproductive traits						
Inflorescence initiation°	1.00	1.91	0.61	**15.06****	0.01	0.96
Corolla area	0.16	1.63	0.02	0.00	2.75	0.07
Floral output	**19.15****	**12.69****	0.09	0.03	0.01	0.34
Nectar sugar content	0.03	0.40	0.01	--	--	--
Nectar volume	1.69	0.80	0.07	--	--	--
Relative reproductive output	(0.11)	(0.066)	(1.00)	0.21	1.16	1.35
Reproductive phenology	**(0.0261)**	(0.18)	(0.94)	0.45	1.87	4.51[†]
Reproductive success						
Proportion fertile capitula	0.58	1.63	0.58	0.60	0.10	0.44
Total seed set	0.60	3.16[†]	0.12	1.34	0.06	0.79
Scutellaria						
Vegetative traits						
Height	**23.61*****	**22.52*****	**4.33***	**198.2*****	**202.0*****	**29.03*****
Leaf area	**1158*****	**13.5****	1.97	**146.3*****	**26.47*****	2.11
Leaf longevity°	--	--	--	2.28	1.0	0.01
Leaf thickness	**308.0*****	0.01	0.07	**80.75*****	0.11	1.90
Shoot:Root ratio	0.61	**4.92***	0.75	0.04	0.33	2.18
Specific leaf area	0.20	1.88	0.24	0.42	3.02[†]	**5.24***
Trichome density	**12.17****	0.37	1.36	**27.71*****	1.83	**11.00****
Relative growth rate	**239.2*****	**35.51*****	**14.94****	**(<0.0001)**	**(<0.0001)**	**(0.0136)**
Total biomass	**(<0.0001)**	**(<0.0001)**	**(<0.0001)**	**(<0.0001)**	**(<0.0001)**	**(<0.0001)**
Reproductive traits						
Inflorescence initiation°	--	--	--	1.71	**33.77*****	0.02
Floral output	**101.6*****	**148.9*****	**54.97*****	(0.53)	**(<0.0001)**	**(<0.0001)**
Relative reproductive output	**7.94****	2.28	0.69	(0.058)	**(<0.0001)**	(0.34)
Reproductive phenology	(0.14)	(0.96)	**(0.0233)**	**5.13***	1.32	2.55
Reproductive success						
Total seed set	**79.25*****	**49.91*****	**6.15***	--	--	--
Total fruit set	--	--	--	2.10	**3.20****	0.07
Germination rate	**31.31*****	0.96	0.03	**(0.0286)**	(0.093)	(0.57)

Figure 3-1. Vegetative and reproductive traits of a locally abundant regionally rare species (LAHS), *Delphinium treleasei,* in comparison to its common congener, *D. carolinianum* and in response to experimental temperature treatments, (A) relative growth rate (mg/day; square root transformed), (B) total potential seed set (average number of seeds per fruit times the total number of flowers; square root transformed), (C) floral output (number of flowers; $\log_e$ transformed), and (D) average seed mass (mg). Statistically significant differences between treatments and species in panels (A) and (C) are designated by having different letters (those that share letters are not significantly different). In panels (B) and (D), statistical significance is indicated by asterisks (* designates $P < 0.05$, ** indicates $P < 0.01$, and *** represents $P < 0.001$).
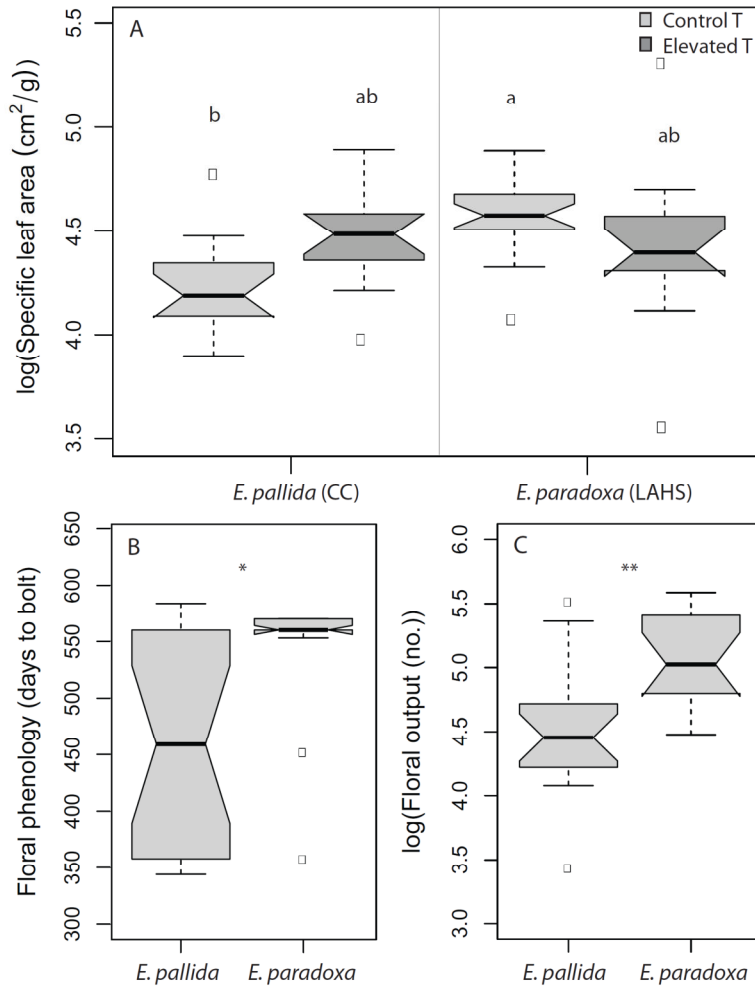
Figure 3-2. Vegetative and reproductive traits of *Echinacea paradoxa* (LAHS) and *E. pallida* (CC) in response to temperature treatments and compared to each other (data represented in panels (B) and (C) are from the precipitation experiment), (A) specific leaf area (cm^2 per unit biomass; log$_e$ transformed), (B) floral phenology (age in days at date of inflorescence initiation), and (C) floral output (number of florets; log transformed). Statistically significant differences between treatments and species in panels (A) and (C) are designated by having different letters (those that share letters are not significantly different). In panels (B) and (D), statistical significance is indicated by asterisks (* designates $P < 0.05$, ** indicates $P < 0.01$, and *** represents $P < 0.001$).

Figure 3-3. Vegetative and reproductive traits of *Scutellaria bushii* (LAHS) in comparison to *S. ovata* (CC) and in response to watering treatments, (A) height (cm), (B) relative reproductive output (square root transformed), (C) total potential seed set (average number of seeds per fruit times total floral output), and (D) germination rate. Statistically significant differences between treatments and species in panels (A) and (C) are designated by having different letters (those that share letters are not significantly different). In panels (B) and (D), statistical significance is indicated by asterisks (* designates $P < 0.05$, ** indicates $P < 0.01$, and *** represents $P < 0.001$).

CHAPTER FOUR

Pages 65 to 89

SITE VARIATION, NOT COMPETITION, AFFECTS POLLINATION SUCCESS IN

TWO ENDEMIC GLADE SPECIES

INTRODUCTION

A primary tenet of ecology is the role of competitive exclusion in determining species ranges (Tilman, 1976; Chase et al., 2002; Chase and Leibold, 2003; Fargione et al., 2003; Lau et al., 2008; Angert, 2009), although some studies challenge this tenet (Volkov et al., 2003; Muneepeerakul et al., 2008). Historically, the theory of competitive-exclusion has been conceptualized in terms of abiotic resources (Hardin, 1960; Tilman, 1976; Chase and Leibold, 2003; Rodríguez-Gironés and Santamaría, 2007; Abrams et al., 2008). Biotic resources, such as pollination services, are also limiting in many habitats (Totland and Eide, 1999; Knight et al., 2005; Fishman and Willis, 2006; Spigler and Chang, 2009; Martén-Rodríguez and Fenster, 2010; Wagenius and Lyon, 2010) and may lead to the extinction of local populations via reduced reproductive success. Many plants compete for pollinators (Brown et al., 2002; Bell et al., 2005; Devaux and Lande, 2009; Mitchell et al., 2009), and species with low relative abundance are often poorer competitors (Flanagan et al., 2010). Biotic resources could, therefore, contribute to the restriction of some rare species' ranges. An increase in competition for shared pollinators reduces the seed set of poorer competitors (Brown and Mitchell, 2001; Bell et al., 2005), and should restrict species from establishing populations in habitats characterized by competition for pollinators. Moreover, species that occur on stressful habitats often have a similar suite of traits that are adaptive in stressful abiotic conditions (Grime, 1977; Chapin et al., 1993), specifically in hot and xeric environments, but may lead to reduced competitive ability for pollinators in more productive habitats, as is seen with competition for other resources (Maestre et al., 2009).

Rare taxa are thought to be less reliant on their pollinators by adopting self-compatibility (Darwin, 1877; Fausto et al., 2001; Moeller and Geber, 2005; Harder et al., 2008; Jacquemyn and Brys, 2008; Martén-Rodríguez and Fenster, 2010), which ensures reproductive success despite low pollination success. Plants that are self-compatible often have lower investment in floral traits to attract pollinators and often have smaller and less abundant flowers, and lower nectar and pollen rewards (Anderson and Busch, 2006; Fishman and Willis, 2006; Kennedy and Elle, 2008; Foxe et al., 2009); making them less attractive to pollinators (Kudo and Harder, 2005; Dickson and Petit, 2006; Ishii et al., 2008; Parachnowitsch and Kessler, 2010). Therefore, this hypothesis would predict that rare taxa are poor competitors for pollinators. The principle of reproductive assurance is based on one of the seven forms of rarity (Rabinowitz et al., 1981), which are defined by a combination of local population size, geographical extent, and habitat specificity (Table 4-1). One important assumption underlying this model, specifically low pollen availability, is applicable when local populations are small or pollination success is low or unpredictable. On the other hand, locally abundant, regionally-rare (LAHS) taxa may not be limited by pollen availability if pollination is sufficient and predictable. This leads to the possibility that factors other than low pollen availability can affect selection on floral traits, such as stressful conditions or competition for pollinator services.

If rare species specialize on the stressful (defined here as hot and xeric) habitat in which they occur, they should be locally-adapted and better able to tolerate those conditions than species that are not restricted to these habitats or that are adapted to more benign habitats (Grime, 1977; Chapin et al., 1993). The widespread species may have lower photosynthetic rates and a greater reduction in floral output and floral attraction

67

traits when stress is high. A reduction in floral attraction traits could diminish their ability to attract pollinators and compete for pollinator services under stressful conditions. Plants with larger floral displays and greater reward output are better competitors for pollinator services (Brown and Mitchell, 2001; Bell et al., 2005) and have greater reproductive success (Bell et al., 2005; Kudo and Harder, 2005). Stressful conditions may also select for higher offspring quality and outcrossing rates, in response to high mortality or inbreeding depression. Therefore, I predict that LAHS species that are endemic to stressful environments should exhibit traits associated with increased outcrossing rates and competitive ability for pollinators. Individuals with fewer, larger flowers are known to have higher outcrossing rates (Karron et al., 2004; Eckert et al., 2009; Karron et al., 2009) mediated by pollinator behavior, and pollinators are attracted to individuals with greater floral display, either via flower number or flower size (Kudo and Harder, 2005; Dickson and Petit, 2006; Ishii et al., 2008; Parachnowitsch and Kessler, 2010). According to this hypothesis, and contrary to traditional theory, LAHS taxa should be better competitors for shared pollinators than widespread, closely-related species when in high stress habitats.

In this study, I test the hypothesis that floral attraction traits affect the relative competitive ability of two locally abundant, regional rare taxa (LAHS) in comparison to their widespread, common congeners (CC) for pollinators in the stressful (i.e., hot and xeric) habitats of the Ozark glades. Specifically, I will test two alternative predictions. First, LAHS taxa have traits associated with increased attractiveness to pollinators and are better competitors for pollinators than their common congeners (CC). Secondly, LAHS species have traits associated with reduced competitive ability (e.g., fewer, smaller

flowers) and are poorer competitors than their CC, as traditional competitive exclusion theory would predict. I tested these hypotheses via a modified De Witt replacement competition experiment that maintained density of inflorescences but manipulated the relative proportion of conspecific and heterospecific individuals. I introduce naïve individuals to pollinators in a common habitat to assess their ability to attract pollinators as measured by visitation rate and reproductive success.

MATERIALS AND METHODS

Study system—I chose two plant species endemic to glades, *Delphinium treleasei* (Ranunculaceae) and *Echinacea paradoxa* (Asteraceae), which are restricted to glades in the Ozark Region in Missouri and Arkansas and have common congeners (CC; *D. carolinianum* and *E. pallida*, respectively) that can grow on glades but are not restricted to them. Glades are hot, dry habitats with a high-proportion of exposed bedrock dominated by herbaceous vegetation, which occur within a matrix of more mesic oak-hickory woodland (Kucera and Martin, 1957; Yatskievych, 1999). Comparing congeneric pairs provides insight into the factors that restrict one species to glades and not the other, and multiple congeneric comparisons also make the results of this study more broadly applicable (Bevill and Louda, 1999). Moreover, comparing restricted species to their widespread congeners mitigates the potential influence of evolutionary history on the biological traits correlated with competitive ability for coevolved pollinators.

Experimental design—I conducted a field competition experiment in order to explicitly test the relative competitive abilities of the LAHS and CC taxa. In order to incorporate spatial variation in pollinator assemblage and abundance, I conducted the experiment at multiple field sites (two for *Delphinium* and three for *Echinacea*; Table 4-

69

2) across a broad geographic range relative to the distribution of the LAHS taxa. Sites were chosen based on two criteria: (1) both species of a congeneric pair occur on the same glade and (2) the density of each species was roughly similar. Inflorescences from elsewhere in the same glade were bagged before flowering in order to exclude visiting insects. Because successful transplantation into glades is difficult due to shallow soils and extreme conditions, all inflorescences were collected, placed in glass containers filled with water and wrapped with a light, neutral-colored material in order to keep the inflorescence fresh and erect. Each individual was randomly assigned to one of three competition treatments: interspecific competition, focal individuals in the intraspecific competition, and non-focal individuals in the intraspecific competition treatment. In other words, each species competed with individuals of the same species (intraspecific, 0% proportion to heterospecifics) and with individuals of its congener (interspecific, 50% proportion to conspecifics) while plant density remained constant (Figure 4-1). The intraspecific competition treatment was subdivided into focal and non-focal individuals in order to maintain equal replication per species per treatment (i.e., two individuals per species per treatment replicate), despite a difference in relative abundance of conspecifics. Only those individuals assigned to the focal group were used for statistical analyses in order to maintain a balanced comparison between treatments. The experiment was repeated two to three times per site with different individuals, depending on availability of inflorescences, for a total of up to 36 individuals per field site [(4 individuals for the intraspecific competition treatment + 2 individuals for the interspecific competition treatment) x 3 replicates x 2 species; Figure 4.1].

Floral attraction traits—I measured the following floral attraction traits: plant height, number of open flowers (*Delphinium*) or florets (*Echinacea*), flower size (*Delphinium* only), and total display size (TDS). Due to morphological differences, TDS was quantified differently for each genus. For the *Delphinium* taxa, TDS is defined as the product of the total number of flowers open times corolla area (i.e., flower width x flower height), and TDS for the *Echinacea* taxa the product of number ray petals times corolla area (i.e., ray petal width times ray petal length). Pollinator attraction traits were measured following observations, in order to avoid damaging flowers or altering reward quality or quantity (e.g., pollen quantity).

Pollination and reproductive success—Plants were exposed to pollinators (i.e., bagging removed) after they were placed in the competition array in order to ensure that pollination occurred only when in the arrays, and they remained in the arrays for six to eight hours. I observed the number, type, and behavior (e.g., visit duration, anther and stigma contact) of all floral visitors during peak periods of activity for 30 minutes. The arrays were placed in a part of the glade where both species occur in equal abundance in order to reduce any potential density-dependence effect on pollinator behavior. Pollination was quantified as visitation rate, visit duration, and the number of flowers probed per visit (for *Delphinium* species only). Because seed set from cut inflorescences is variable and often unsuccessful, I quantified reproductive success as the number of pollen grains germinated and the number of pollen tubes reaching the ovary. Once the inflorescences were removed from the field, they remained undisturbed for 24 hours to allow for pollen tube growth, after which each ovary was dissected from the inflorescence. At one site (SNR; Table 4-2), the abundance of individuals blooming

concurrently was low and insufficient to conduct the experiment. Therefore, individuals that were collected at a different site (HHTSP) were randomly re-assigned to a new treatment and the experiment was repeated at SNR. Since *Echinacea* species have florets that are only receptive for one day, I was able to collect the achenes in between experiments and ensure that pollen deposition and pollen tube growth were not the result of previous exposure to pollinators. I also allowed two days between observation periods, during which the inflorescences were excluded from pollinators and kept in cool (roughly 23ºC) conditions to reduce wilting. I carefully removed those florets that were exposed to pollinators after each treatment, without damaging the capitula.

Once removed, the ovaries were fixed in 3:1, 95% ethanol:glacial acetic acid for 2 hours, rinsed and stored in 70% ethanol. They were then immersed in 10% (w/v) solution of sodium sulfite and autoclaved for 30 or 40 minutes at 60 or 70°Celsius for *Delphinium* and *Echinacea* taxa, respectively. After cooling, each pistil was mounted on a slide, covered with 3-5 drops of decolorized aniline blue, refrigerated for 24 hours, and illuminated using a 100 W fluorescent source (Lipow et al. , 2002) on a Zeiss Universal microscope. In order to determine reproductive success, I counted the number of pollen grains on the stigma, the number of pollen tubes growing down the style, and the number of pollen tubes reaching the ovary (see Lipow et al., 2002). Competitive ability was quantified as differences in visitation rate, number of pollen on the stigma, number of pollen tubes germinating down the style, and number of pollen tubes to reach the ovary in response to the competition treatments.

Statistical analyses—I tested for differences in morphological traits between species of each genus via a two-way, nested ANOVA with Treatment and Distribution as

factors in order to ensure that the randomization procedure was not biased (i.e., traits did

not vary by treatment). Distribution was nested within Site to control for potential spatial

variation in species' traits. All three treatments (i.e., intraspecific, interspecific focal

plants, interspecific non-focal plants) were included to determine the potential for a bias

in the randomization procedure. Upon finding a significant interaction term, I used a

Tukey's HSD *posthoc* test to determine paired differences between the significant factors.

The effects of competition treatment on pollination and reproductive success were

tested as a two-way, nested ANOVA with treatment and distribution nested within site.

Treatment was nested within site to control for among-site variation in pollinator

composition and abundance. Since some morphological traits varied spatially,

distribution was also nested within site, in order to differentiate between the effects of

site-level variation in morphological traits and pollinator assemblages. Due to low

visitation rates and pollen deposition, significant differences in the competitive ability of

congeners were also tested using a two-way nested, permutation ANOVA (*aovp* in R

Statistical Package (R Development Core Team, 2008)) with Treatment and Distribution

nested within Site as above. I used a Monte Carlo resampling approach (i.e., without

replacement) to construct a null expectation based on a random sampling of the available

data and compared each of 5000 runs to the null. Statistical significance is quantified as

the number of runs for which the F-statistic is greater than or equal to the observed value.

Response variables were tested for normality and transformed as necessary. Variables

that could not be transformed to meet the assumption of normality for parametric

statistics were also tested for differences using the permutation approach described

above. Results did not differ between the parametric and nonparametric analyses; therefore, I report the results of the parametric tests.

RESULTS

Morphological traits—For the *Delphinium* taxa, morphological traits differed between congeners and across sites, but there was no treatment effect, which verified the randomization procedure. Height and display size of both *Delphinium* taxa differed significantly between sites ($DF = 1$, *F* stat = 6.33, *P* value = 0.0197; $DF = 1$, *F* stat = 26.0, *P* value < 0.0001, respectively; Figure 4-2 A and B), which means that the morphological traits differed between the replicates and could reflect different abiotic conditions between sites. There was no difference between congeners ($DF = 2$, *F* stat = 1.18, *P* value > 0.05; $DF = 2$, *F* stat = 1.98, *P* value > 0.05; respectively). There was a significant interaction between site and distribution for the number of flowers open (NFO) concurrently ($DF = 2$, *F* stat = 5.54, *P* value = 0.0105; Figure 4-3A). The LAHS *Delphinium treleasei* had a higher NFO at one site (RBCA) than the other, but there was no difference between sites for the CC or between congeners within a site. Corolla height (CH), which includes the landing pad of the flower, also differed among species and sites for the *Delphinium* taxa ($DF = 2$, *F* stat = 7.04, *P* value = 0.0043; Figure 4-3B). The LAHS species had greater CH than its CC at DCA but not at RBCA, which suggests that CH is as variable by geography as it with between congeners. Distance to nectar was marginally different between *Delphinium* species at different sites ($DF = 2$, *F* stat = 2.87, *P* value = 0.0780), which was driven by a marginal difference between taxa at one site (DCA).

The *Echinacea* taxa are not different in their morphological traits among the interspecific, focal-individuals of the intraspecific treatment, and the non-focal individuals of the intraspecific treatment, again verifying the randomization procedure. They also differed in their morphological traits among sites and species; in other words, species traits were different at some sites and not at others. The number of flowers open and display size differed significantly among sites for both species, indicated the geographic variation is greater than any differences between congeners. There were more flowers open at PLH than either HHT or SNR ($DF = 2$, F stat = 19.15, P value < 0.0001), and total display size was successively larger at PLH, HHT, and SNR ($DF = 2$, F stat = 42.92, P value <0.0001). Height was significantly different between congeners among sites ($DF = 2$, F stat = 4.55, P value = 0.0091; Figure 4-4A), which was driven by differences between congeners at one site (HHTSP). *Echinacea paradoxa* (LAHS) was significantly shorter than its CC at HHTSP, which is consistent with the prediction of greater competitive ability.

Competitive ability—Neither pollination nor reproductive success differed between the intra- and interspecific competition treatments for either genus. However, both genera had significant differences among sites and distributions. Both *Delphinium* species had marginally lower visitation rates at DCA than RBCA ($DF = 1$, F stat = 3.48, P value = 0.0833; Figure 4-2C), but there was no difference between species or treatments ($DF = 2$, F stat = 0.54, P value > 0.05; $DF = 2$, F stat = 0.36, P value > 0.05; respectively). There were no differences in visit duration or the number of flowers probed per visit for site, distribution or treatment. The *Delphinium* species differed across sites in the number of pollen grains germinating on the stigma ($DF = 2$, F stat = 11.33, P value =

75

0.0017); the LAHS species at RBCA had a greater number in comparison to the LAHS species at DCA and to its CC at either site. The number of tubes developing down the style was significantly different between species within sites ($DF = 3$, F stat $= 4.61$, P value $= 0.0327$; Figure 4-3C); the LAHS species at RBCA had more tubes in comparison to its CC at either site and to itself at DCA. However, there were no differences in the number of pollen tubes reaching the ovaries ($DF = 3$, F stat $= 0.56$, P value > 0.05), which may reflect low pollen quality or maternal effects, since the inflorescences were removed from the rest of the individual which could impede pollen tube growth.

The *Echinacea* taxa did not differ in visitation rate among sites ($DF = 2$, F stat $= 2.36$, P value > 0.05) or by distribution within sites ($DF = 3$, F stat $= 1.80$, P value > 0.05). The duration of the visits received was also not different among sites ($DF = 2$, F stat $= 1.21$, P value > 0.05) or by distribution within sites ($DF = 3$, F stat $= 2.04$, P value > 0.05). The number of pollen grains deposited on the stigma was significantly different between species across sites ($DF = 3$, F stat $= 4.75$, P value $= 0.0232$; Figure 4-4B), but there were no differences between congeners within a given site. There was also no difference in the number of pollen tubes reaching the ovaries at any site ($DF = 3$, F stat $= 1.10$, P value > 0.05).

DISCUSSION

For decades, the relative importance of biotic and abiotic factors in determining species distributions has been debated in ecology. Competition for shared pollinators may influence distributions via reproductive success. Here, I tested the alternative hypotheses that two rare species are (1) poorer competitors for pollinators in comparison to their common congeners (CC), based on the predictions of traditional reproductive theory

which state that rare taxa have higher selfing rates and are therefore less attractive to pollinators or (2) better competitors for pollinators in the plant specialized for increased attractiveness in stressful environments. There was no significant effect of the competition treatment on pollination or reproductive success of any species tested. However, there was significant variation among sites in the floral traits and reproductive success of endemics and their CC, which indicates the important role of scale on interspecific interactions and, potentially, the mechanisms restricting a species' range.

Contrary to my predictions, neither the CC nor the LAHS species were consistently better competitors for pollinators. There were no significant differences in visitation rate, pollen deposition, or pollen tube growth between intra- and interspecific competition treatments. The lack of a treatment effect suggests that competition for pollinator services is either not occurring between these congeneric pairs on glades or they have equal competitive abilities. In a previous study (Chapter 2), I demonstrate that pollen limitation did not differ between *Delphinium* congeners and was moderately low (*D. treleasei*: 3.18 seeds per fruit (12.3%) and *D. carolinianum*: 3.69 seeds per fruit (20.84%)), which indicates that competition for pollinator services may not be occurring since pollen is not strongly limiting. The LAHS *E. paradoxa*, on the other hand, was marginally more pollen limited than *E. pallida* (Chapter 2), and these species could be competing for limited pollen resources. Overall visitation and fertilization rates were quite low (0.87 visits/30 min and 1.02 pollen tubes that reached the ovary) and may have been too low to detect competition in either species pair. In habitats with more abundant pollinators or less-stressful conditions, competition for pollinator services may be greater. In order to eliminate the potential effects of pollinator identity and experience, I

conducted this experiment on glades at which both species occur. These species are often found on glades without the other; therefore, these sites may represent a subset of ecological space in which both species can occur. In order to fully understand the potential role of competition for pollinators in restricting the distribution of glade endemic plants, studies that test for differences in relative competitive ability for pollinators in non-glade habitats and in glades with varying relative population density of each congener are required. While the LAHS species' coevolved pollinators may not occur off of glades, a comparison of competitive ability for pollinators on and off glades will determine whether or not the distributions of LAHS species are influenced by their plant-pollinator interactions, either via a lack of effective pollinators or poor competitive ability for shared pollinators in comparison to their CC.

While there was no treatment effect on pollination or reproductive success, all species showed significant spatial variation in floral traits and pollination success. The traits of both *Delphinium* species indicate a tradeoff between allocation to reproduction and vegetative output that varies across space. Plants of both species were shorter but had greater floral displays at RBCA than at DCA. The insects respond to the increase in floral display seen at RBCA with higher visitation rates to plants at RBCA in comparison to DCA. Differences in allocation to reproduction could be a function of variation in soil nutrient content or habitat suitability between sites. For instance, if DCA is hotter and drier than RBCA, the difference seen in floral and vegetative traits would support the prediction that allocation to reproduction increases with stress. Higher visitation rates at RBCA than at DCA also indicate a role of larger spatial-scale variation in floral density on insect behavior. While I chose an area within each glade where both species were in

equal abundance, density on the glade as a whole can vary considerably across glades. Anecdotally, relative abundance and floral density of the *Delphinium* species was much lower at DCA in comparison to RBCA. Insects are known to travel large distances (van Nieuwstadt and Iraheta, 1996; Pasquet et al., 2008; Elliott, 2009; Rader et al., 2011), sometimes moving distances greater than the size of many glade habitat (e.g., more than a kilometer; Osborne et al., 2008; Pasquet et al., 2008; Wikelski et al., 2010), which can range in size from less than one hectare to greater than 200 hectares (Nelson and Ladd, 1982). Therefore, the pollinators may be responding to inflorescence density on the glade and not directly to individual variation in floral traits. Future studies will document floral density and abiotic conditions across glades in order to disentangle the relative importance of these two mechanisms.

The *Delphinium* congeners also differed significantly in reproductive success (i.e., number germinating pollen grains and pollen tube growth) at RBCA. While there was no difference in visitation rate or the number of total pollen grains on the stigma, the LAHS plant had more pollen tubes that were germinating and developing down the style. This pattern suggests that the visits to the LAHS *Delphinium* species may have been more effective and resulted in the transfer of higher quality or more conspecific pollen in comparison to its common congener. The Delphinium congeners are known to hybridize (Warnock, 1987), and germination rates may reflect differences in the ability of conspecific pollen to cross-pollinate. However, pollen quality of each species, *per se*, was not quantified, and further research is needed to determine the mechanism behind the greater proportion of pollen grains that successfully fertilized ovaries in *D. treleasei*. In a previous study conducted at a larger spatial scale (Chapter 1), I showed that the endemic

Delphinium species had a more specialized pollination system (i.e., fewer species of pollinators accounted for the majority of pollen flow) than its congener, which could account for the difference seen in pollen germination rate and pollen tube growth. Moreover, spatial variation in pollen tube growth is likely a result of low visitation rates and not variation in pollinator effectiveness. While differences were only marginally statistical significant due to low pollinator abundance and replication, visitation rates were nearly four times (3.8) higher at RBCA than DCA, and visit duration was more than two and a half times greater.

The *Echinacea* species also differed in their floral traits and reproductive success among sites. However, there was no difference within a given site, with the exception of height, which suggests that the differences were dominated by site-level effects. The LAHS species was shorter than its CC at HHTSP. They were also shorter at SNR (which were individuals collected from HHTSP due to low abundance of blooming individuals at SNR), but the pollinators did not respond differently to the congeners at SNR. I predicted that the pollinators would be more attracted to the taller individuals, as has been shown in other taxa (Dickson and Petit, 2006), which would be the CC at both HHTSP and SNR. Contrary to my predictions, pollen deposition rate was higher to the LAHS species at HHTSP, and there was no difference at SNR. Pollen deposition rate may be related to the variation in pollinator fidelity or effectiveness of the pollinators among sites, as seen in the *Delphinium* species. However, the LAHS *E. paradoxa* does not have a more specialized pollination system than *E. pallida*. Moreover, the disparity in pollen deposition did not lead to differences in pollen tube growth or fertilization rates, which suggests there may be a trade-off between pollen abundance vs. pollen viability. In other

words, *E. paradoxa* pollen may be more abundant but of lower quality, resulting in a negligible difference in reproductive success. On the other hand, the pollen deposited on the stigmas of *E. paradoxa* could have had a greater proportion of heterospecific pollen, resulting in reduced fertilization rates. Unfortunately, the pollen of the *Echinacea* species could not be distinguished from each other, so I was unable to test for this mechanism. In future studies, pollen of each individual could be tagged with fluorescent dye in order to distinguish between the transfer of heterospecific and conspecific pollen deposition.

Conclusion

This study illuminates the potential influence of site-level characteristics on local reproductive success and pollination specialization for greater pollen transfer. In contrast to our hypotheses, there was no difference in competitive ability between two endemic glade plants and their congeners. Competition for pollinator services has been documented in some habitats (Brown and Mitchell, 2001; Bell et al., 2005; Internicola et al., 2007) but there is no evidence of competition for pollinator services between these congeners in glade habitats. While these congeners did not compete for pollinators, there were differences in the reproductive success of the *Delphinium* congeners when pollination visitation was relatively high. The *Delphinium* endemic glade plant has a more specialized pollination system and may have more effective pollinators. Pollination specialization is predicted to increase conspecific pollen transfer (Darwin, 1877; Rymer et al., 2005; Galloni, 2008) and increases reproductive success (Rymer et al. 2005; Galloni 2008) as documented in this study. Moreover, differences in pollination success and reproductive success appear to be influenced by habitat-level factors, such as inflorescence abundance or pollinator assemblages, and less by individual morphological

traits. The structure of habitat within a landscape is known to affect the distribution and behavior of pollinating insects in experimental and agricultural settings (Kremen et al., 2002; Ricketts et al., 2008). This study demonstrates the potential significance of landscape-scale factors on plant-pollinator interactions and plant reproductive success of LAHS plants in natural habitats. Understanding the relative importance of landscape-scale factors on local interactions is increasingly important given high rates of habitat alteration and climatic change around the world.

Table 4-1. Description of the Rabinowitz's Seven Forms of Rarity based on geographic extent, population size and habitat specificity (modified from Rabinowitz et al., 1981). Bold font represents the locally abundant, regionally rare (LAHS) taxa in this study.

	Geographic Extent (GE)			
	Large		Small	
Population Size (PS)				
Somewhere large	<u>Common</u>	Large PS Large GE Narrow HS	Large PS Small GE Broad HS	**Large PS (LA)** **Small GE** **Narrow HS (HS)**
Somewhere small	Small PS Large GE Broad HS	Small PS Large GE Narrow HS	Small PS Small GE Broad HS	Small PS Small GE Narrow HS
	Broad	Narrow	Broad	Narrow
	Habitat Specificity (HS)			

Figure 4.1. A schematic of a representative glade site (A) and the competition treatments (B; including intraspecific and interspecific competition treatments. In order to maintain equal replication between intra- and interspecific competition treatments only two individuals per intraspecific treatment (the *focal* individuals, solid and outlined circles) were used to test for differences between LAHS and CC species in response to the competition treatments. The non-focal species (striped circles) were excluded from the analyses in order to maintain equal replication between treatments), and (C) a map of sites at which I conducted a pollination competition experiment with two congeneric species pairs, *Delphinium treleasei* and *D. carolinianum* (□) and *Echincaea paradoxa*

and *E. pallida* (○ ; LAHS and CC, respectively). Glade density data was from Nelson

and Ladd (1982) coded in to a heat-density map. The original data represented a range in

glade density per 7.5 degree minute quadrats (approximately 144 km^2 in Missouri);

therefore the heat map reflects a rough estimate of glade density per 144 km^2 as of 1982.

Figure 4-2. Morphology and visitation rates of two *Delphinium* species at two field sites in south western, Missouri Drury Conservation Area (DCA) and Rocky Barrens Conservation Area (RBCA), including (A) Height (cm), (B) Display size (floral output x corolla area), and (C) visitation rate per 30 minute observation period. * denotes statistically significant differences.

Figure 4-3. Floral traits and reproductive success of a locally abundant, regionally rare (LAHS) *Delphinium treleasei*, and its common congener (*D. carolinianum*, CC) at two field sites in south western Missouri (i.e., Drury Conservation Area (DCA) and Rocky Barrens Conservation Area (RBCA)), including (A) floral display (the number of open flowers), (B) corolla height, including the landing pad, and (C) the number of pollen tubes developing down the style of the experimental plants. Different letters denote statistically significant differences.
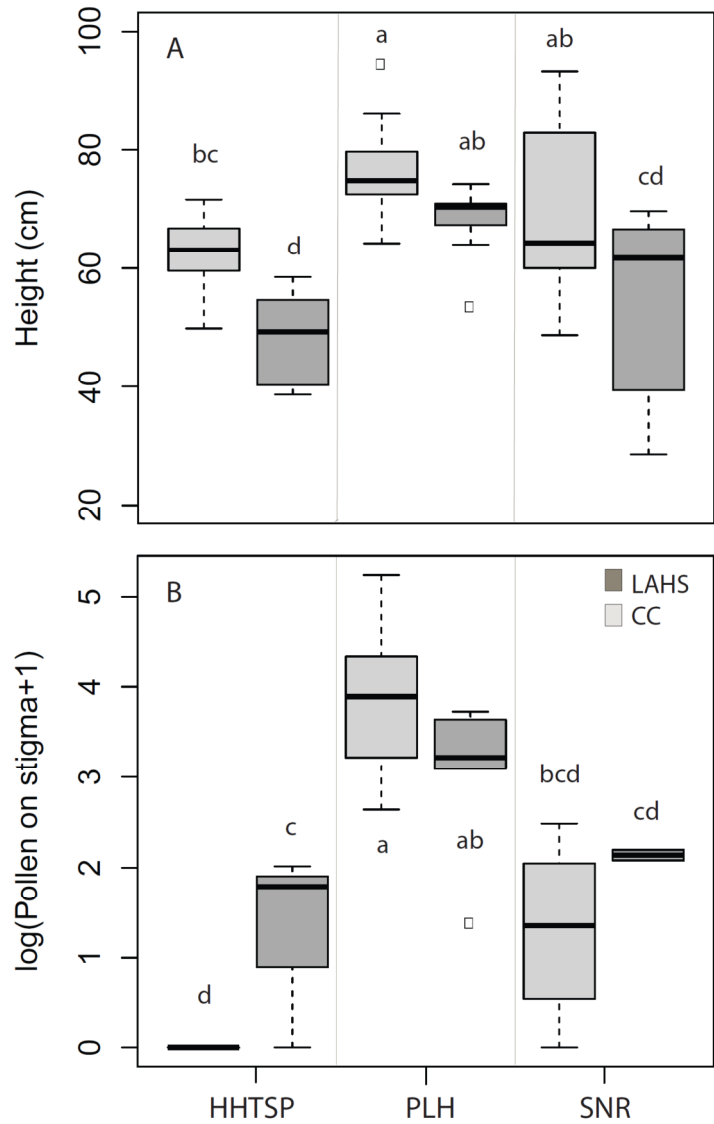
Figure 4-4. Height (A) and reproductive success (B; number of pollen grains on the stigma) of a locally abundant, regionally rare (LAHS) species, *Echinacea paradoxa*, and its common congener (*E. pallida*, CC) at three field sites in Missouri (HaHaTonka State Park (HHTSP), Private glade (PLH), and Shaw Nature Reserve (SNR)). Different letters represent statistical differences between

CHAPTER FIVE

Pages 90 to 112

PHENOLOGICAL SHIFTS IN CONGENERIC PAIRS OF RARE AND WIDESPREAD

SPECIES: THE ROLE OF INTRINSIC AND EXTRINSIC FACTORS

INTRODUCTION

Phenological shifts in response to climate change have been well documented (IPCC, 2007, Badeck et al., 2004; Dose and Menzel, 2004; Ibanez et al., 2010; Kudo and Hirao, 2005; Lambert et al., 2010; Sherry et al., 2007). While species responses are consistent with the direction of climate change, there is considerable variation in the degree to which species are responding (Kudo and Hirao, 2005; Cleland et al., 2006; Miller-Rushing et al., 2007; Miller-Rushing and Primack, 2008; Miller-Rushing and Inouye, 2009; Forrest et al., 2010). For instance, many plant species in the same genus respond differently to changes in climate (Miller-Rushing and Primack, 2008; Miller-Rushing and Inouye, 2009), and differential responses may affect interspecific interactions (Forrest et al, 2010, Rafferty and Ives, 2011). Both intrinsic and extrinsic factors, for example pre-adaptation or micro-climate effects, respectively, may contribute to the variation in species responses to climate change. The mechanisms behind interspecies variation are rarely assessed (but see Kudo and Hirao, 2005; Miller-Rushing and Inouye, 2009) and could inform our understanding of the relative importance of the biological processes that underlie them.

Variation in species responses to climate change may be partially explained by historical factors, such as habitat specialization, genetic composition, or pre-adaptation to a particular climatic change. Species that specialize on stressful habitats may also have lower phenotypic plasticity (Ellison and Parker, 2002; Pohlman et al., 2005), either in their morphological traits or in their phenological patterns. Species that are adapted to a narrow suite of abiotic conditions may exhibit limited phenotypic plasticity that would allow them to respond to climate change. Strong selection for local adaptation can lead to

reduced phenotypic plasticity (Ellison and Parker, 2002; Pohlman et al., 2005), and many rare and endemic plants are thought to be habitat specialists (Rabinowitz et al., 1981; Izco, 1998, Espeland and Emam, 2011) that are restricted in their ranges due to the lack of suitable habitat. Therefore, stress-adapted specialist plant species could be less responsive to climate change in comparison to species that occur in multiple habitat types. Alternatively, local adaptation to a stressful environment may include the ability to track short periods of favorable abiotic conditions, such as cooler temperatures or rain events (Aronson, 1992; Angert et al., 2010). Stress-adapted species would then be more responsive to climate change in order to avoid higher-stress conditions later in the season.

In addition to intrinsic factors, extrinsic mechanisms, such as local abiotic conditions, may affect the impact of regional climate change on individuals (Kudo and Hirao, 2006) and the evolution of populations in unpredicted ways. Many local factors, such as plant cover, soil composition, and exposed bedrock, may also influence microclimate conditions, such as the retention and distribution of heat and water throughout a habitat. Evaporative cooling of plants via transpiration can buffer temperatures in the surrounding habitat (Georgescu et al., 2011), vegetation structure can reduce the wind exposure experienced by other plants near them (Lortiek et al. 2002), and the latent heat of exposed bedrock may increase soil and air temperatures close to the ground. These local factors can indirectly influence the degree to which an individual is impacted by regional climate change by either mitigating or intensifying climate conditions. For instance, plants that occur in soils with low water-holding capacity may be more responsive to increased temperature, which should reduce plant-available water.

In this study, I address three hypotheses that potentially influence differential responses of endemic species and their closely-related congeners to regional warming based on two principles, pre-adaptation and local habitat effects. The first predicts that plants that are specialize on high-stress habitats are less responsive to regional climate change than closely-related, widespread species. Alternatively, the second hypothesis predicts that stress-adapted species are more responsive to changes in abiotic conditions via greater ability to track suitable habitat in time. The third predicts that all plants in exposed, xeric habitats have greater response to regional warming, regardless of geographic range size. I test these predictions using a novel, continuous descriptor of developmental stage that was calculated for each herbarium specimen and a categorical variable describing the habitat in which the individual was collected. Using data collected from 21 weather stations over the 110 years throughout the region to which these species are endemic (Missouri and Arkansas), I determined which factors (range, habitat, weather or change in climate through time) are the primary drivers of phenology in all species.

METHODS

Study System—I selected two species restricted to glades in the Ozark Region of Missouri and Arkansas, *Delphinium treleasei* (Ranunculaceae) and *Scutellaria bushii* (Lamiaceae); each of which has at least one widespread congener (*D. carolinianum ssp. carolinianum*, *S. ovata*, and *S. parvula*) that grows on glades but is not restricted to them. Glades are hot, dry habitats with exposed bedrock, thin soils, and limited herbaceous plant cover that occur within a matrix of more mesic oak-hickory woodlands. They are xeric habitats, which are hotter than the surrounding woodland and prairie habitats during the spring and summer months (data not shown) when these plants are photosynthetically

93

active and blooming. Comparing these congeneric pairs provides insight into the factors

that restrict one species to glades and not the other. Moreover, comparing restricted

species to their widespread congeners mitigates the potential influence of evolutionary

history on the biological traits correlated with endemism. Multiple congeneric

comparisons also make the results of this study more broadly applicable (Bevill and

Louda, 1999).

Climatic data—In order to document potential changes in climate, I acquired

climate data from fourteen weather stations throughout Missouri (Figure 5-1), seven

weather stations in Arkansas, and one in Kansas, for a total of 22 weather stations.

Stations were chosen based on the duration of available data and location, in order to

accurately describe the climate variables throughout the range of our target species.

Climate data were available for at least five, spatially distributed weather stations

between 1897 and 1905; between 1905 and 1931, roughly half of the weather stations had

available data; and after 1931 all weather stations contributed data for most years. The

data from all weather stations were checked for missing values. If any weather station did

not have data for every month in a given year, the data from that weather station for that

year were excluded from the analyses. The climatic data were reduced to annual averages

across all sites in order to control for spatial variation in climate. I conducted a principle

components analysis (PCA) to reduce the multiple abiotic variables (i.e., average

temperature, average high temperature, average minimum temperature, and yearly

precipitation) into 2 principle components describing the climate in multivariate space.

Since average minimum and maximum temperatures are inherently correlated with

average annual temperature, I first regressed minimum and maximum temperatures

against average annual temperature, and the residuals from this analysis were used in the PCA. In this way, I was able to more accurately describe climate space by isolating the effects of maximum and minimum temperatures on phenology, independent of annual average temperature. This is particularly important if the range of temperatures has an influence on phenology. The summary of these data represented by the first two principle components allowed me to explore the response of each species to a more meaningful variable that incorporated multiple interacting factors contributing to climate.

Plant phenology—All available herbarium specimens for our target species were acquired from six herbaria that were considered to have the largest holdings of these species, the Missouri Botanical Garden, University of Arkansas Herbarium, Illinois Natural History Museum, United States National Herbarium, New York Botanical Gardens, and Field Museum. The developmental stage of each specimen was calculated using a continuous variable that standardized for floral output. All floral buds, open flowers, and fruits were counted, and I ranked each unit (i.e., bud as zero, flower as 1, and fruit as 2), summed them, and divided by the total floral output. Developmental stage was calculated as,

$$DS = \frac{b*0 + f*1 + r*2}{2(b+f+r)} \qquad\qquad \text{Equation 1.0}$$

where b is the number of buds, f is the number of flowers and r is the number of fruits. Therefore, phenological stage ranges between zero and one, where zero denotes a plant that is in full bud, 0.5 denotes a plant in full bloom, and one reflects an individual in full fruit. Developmental stage of all *Scutellaria* taxa was biased by developmental stage; many more individuals were collected at the developmental limits (i.e., zero and one). Individuals at these limits were excluded from the analyses, in order to remove this bias

and acquire a normal distribution. All collection dates were converted into a continuous Julian date.

In order to control for latitudinal variation in climatic conditions, I used latitude as a covariate for all analyses in order to account for this variation. Many modern specimens were georeferenced and for other specimens with sufficient descriptive locality information on the herbarium labels, I estimated latitude via satellite imagery in Google Earth (Google, 2011). Specimens that could not be georeferenced were excluded from the analyses. Using the descriptors on the herbarium labels, habitat was categorized into four main types, Glade, Prairie, Woodland and Disturbed, representing the major habitat types in which these species were found. The designation "Glade" included all specimens with habitat described as upland prairies, rocky prairies, bald knobs, rocky outcrops, barrens, cedar glades, limestone glades, etc. "Woodland" refers to specimens collected from habitats characterized as rocky woodlands, wet woodland, upland woodland, forests, etc. "Prairie" included specimens collected from meadows, prairies and wet prairies, and "Disturbed" denotes habitats along roadsides, railroads, and power-lines, unless otherwise specified on the herbarium specimen. There were only 3 specimens in woodland habitats for *S. parvula*; therefore I pooled all non-glade habitats in order to increase replication.

Statistical Analyses—Regression analyses were conducted to determine whether and which abiotic factors and their composite climate variables (PC 1 and 2) were changing through time. In order to test for nonlinearity in the relationship between abiotic factors and year, I compared the Akiaike Information Criterion (AICc; a measure of relative goodness of fit that penalizes model complexity and controls for replication) of

96

the linear model to those constructed using nonparametric smoothing models. Those variables with a nonlinear relationship with time were fitted using nonparametric smoothing methods via generalized additive models (gam; R Development Core Team, 2008) and I then compared the AICc between models and rejected the linear model if the difference between the AICc's (ΔAICc) was greater than six. If there was no difference (i.e., less than 6 ΔAICc), I report the results from the more parsimonious, linear model

I then explored the potential for shifts in the relative importance of each factor (i.e., average temperature, maximum temperature (residuals), minimum temperature (residuals) and precipitation) in determining each principle competent (PC) through time. I separated the time variable into decades and conducted independent principle component analyses for each decade (i.e., 1897-1909, 1910-1919, 1920-1929, 1930-1939, 1940-1949, 1950-1959, 1960-1969, 1970-1979, 1980-1989, 1990-1999, and 2000-2009). I extracted the weightings for each variable in a given decade and tested for changes in the absolute value of the weightings (which refer to the correlation between the factor and PC of interest) through time via regression analysis. A large negative and positive weighting denote high correlation between the variable and the PC; therefore, I use the absolute value in order to explore the strength of the relationship – whether the relationship is negative or positive. In this way, I was able to determine if the importance of a variable is changing through time.

Multiple regression was used to test which variable and their interactions explained the most variation in phenological stage for each species. Specifically, I tested for the effects of climate in a given year (defined as PC 1), change in climate through time (represented by time in years), habitat (the common species only), and the

97

interactions of these factors on the relationship between developmental stage and

collection date. I focus on PC 1 since it has changed linearly through time and explained

the majority (57.54%) of climatic variation in the Ozark region. The interaction between

climate (PC 1) and collection date tested the prediction that the phenology of these

species is controlled by climate and not (for instance if the species is photoperiodic).

Change in phenology due to climate change was documented as the interaction between

collection date and year. Latitude was included as a covariate in order to account for

spatial variation in phenology. Model reduction was used (*step* in the R Statistical

Package (R Development Core Team 2008) in order to refine the statistical result. The

step function systematically removes higher-order terms from the model structure,

compares the Akaike information criterion (AIC; a measure of relative goodness of fit

that penalizes model complexity) between models, and reduces the model to that with the

best fit (i.e., lowest AIC with a threshold of 6).

All variables were transformed for normality, and if normality could not be

achieved, the statistical test was repeated with summarized data or via nonparametric

tests. Time in years could be not normalized for either *Delphinium* taxa or for *S. ovata*,

but the results were consistent when I conducted the analyses when time in years was

categorized into pre- and post-1960 time periods. I report the results of the analysis with

time as a categorical variable for the *Delphinium* taxa for ease of interpretation. For the

Scutellaria species, I report the results of the analysis with time as a continuous variable,

because the developmental stage of *S. bushii* was biased by time (all pre-1960 individuals

were in full fruit). Analyses could only be conducted for the latter part of the century.

Latitude was non-normal for *D. treleasei*, and analyses were conducted with and without

the covariate. There was no difference in the analyses for *D. treleasei*, so I report the

analyses conducted on the categorical time variable here.

RESULTS

Regional climate change—The climate in Missouri and Arkansas has become

warmer and wetter over the past 112 years. When tested independently, average annual

temperature has fluctuated nonlinearly through time ($DF = 110$, $F = 13.88$, $P < 0.0001$,

AICc = 256.80, ΔAICc = 30.44; Figure 5-2A). Maximum temperatures are decreasing

nonlinearly ($DF = 110$, $F = 8.77$, $P < 0.0001$, AICc = 40.65, ΔAICc = 18.28; Figure 5-

2B), and minimum temperatures are increasing nonlinearly ($DF = 110$, $F = 21.72$, $P =$

0.0006, ΔAICc = 11.90; Figure 5-2C). Precipitation is increasing linearly through time

($DF = 109$, $R^2 = 0.0938$, $F = 11.29$, $P = 0.0011$; Figure 5-2D).

The first principle component of climate (PC 1) in Missouri and Arkansas was

primarily influenced by maximum temperature, minimum temperature, and total

precipitation (Table 1), and it explained 57.54% variation in climate. The second PC was

described primarily by average temperature (Table 1) and explained an additional 25.62%

for a total of 83.16% of variation explained by the first two principle components. The

third PC explained an additional 12.11% of variation for a total of 95.27% variation

explained. The first principle component of climate has changed linearly over the last 112

years ($DF = 109$, $R^2 = 0.179$, $F = 23.73$, $P < 0.0001$, AICc = 390.78; Figure 5-3A), and

the second PC has fluctuated through time in a nonlinear fashion ($DF = 110$, $F = 10.90$, P

< 0.0001, AICc = 297.52, ΔAICc = 23.52). I focused on the effect of changes in PC 1 on

phenology as the climate change factor, since PC 2 did not show a a clear directional shift

and explained less than half of the variation in climate in comparison to PC 1.

The strength of correlations (i.e., the absolute value of the weighting) between PC1 and average and maximum temperatures did not change through time (Average temperature: $DF = 9$, $R^2 = 0.0278$, $F = 0.26$, $P < 0.0001$; Maximum temperature: $DF = 9$, $R^2 = 0.112$, $F = 1.14$, $P > 0.05$). There was a marginal change in the variation explained by minimum temperatures ($DF = 9$, $R^2 = 0.324$, $F = 4.29$, $P = 0.0681$), but this was driven by the outlier decade, average of the years 1897-2009. There was no significant difference when the outlier was removed ($DF = 9$, $R^2 = 0.125$, $F = 1.14$, $P > 0.05$). The correlation between PC1 and precipitation did change marginally through time ($DF = 9$, $R^2 = 0.325$, $F = 4.34$, $P = 0.067$; Figure 5-3B) and was highly significant when the outlier, average of the years 1910-1919, was removed ($DF = 9$, $R^2 = 0.840$, $F = 42.07$, $P = 0.0002$). There were no changes in the relationships between PC2 and any climatic factor through time (Average temperature: $DF = 9$, $R^2 < 0.0001$, $F = 0.0008$, $P > 0.05$; Maximum temperature: $DF = 9$, $R^2 = 0.142$, $F = 1.49$, $P > 0.05$; Minimum temperature: $DF = 9$, $R^2 = 0.187$, $F = 2.07$, $P > 0.05$; Precipitation: $DF = 9$, $R^2 = 0.237$, $F = 2.79$, $P > 0.05$). The strength of the relationship between PC3 and maximum temperature decreased through time ($DF = 9$, $R^2 = 0.439$, $F = 7.04$, $P = 0.0264$), but there was no change for any other factor (Average temperature: $DF = 9$, $R^2 = 0.100$, $F = 1.01$, $P > 0.05$; Minimum temperature: $DF = 9$, $R^2 = 0.0017$, $F = 0.015$, $P > 0.05$; Precipitation: $DF = 9$, $R^2 = 0.090$, $F = 0.895$, $P > 0.05$).

Phenological responses to climate change—The developmental stage of *D. treleasei*, the rare species, was significantly affected by collection date (N = 20, $F = 23.11$, $P = 0.0002$, AICc = -44.37) and climate (N = 20, $F = 8.13$, $P = 0.0116$, AICc = -44.37), and had a marginal response to climate change (N = 20, $F = 3.59$, $P = 0.0764$,

AICc = -44.37; Figure 5-4A), in congruence with my hypotheses. Latitude also explained a marginally significant amount of variation in the developmental stage of *D. treleasei* (N = 20, *F* = 4.34, *P* = 0.0535, AICc = -44.37). Not surprisingly, collection date explained a significant amount of the variation in the developmental stage of *D. carolinianum* (N = 79; *F* = 11.26; *P* = 0.0013, AICc = -113.39). The variation in developmental stage of *D. carolinianum* was not significantly described by latitude or any interaction term. For instance, there was no effect of habitat on response to climate change (N = 79, *F* = 1.99; *P* > 0.05, AICc = -113.39). Neither latitude nor climate was a significant factor (N = 79, *F* = 0.03, *P* > 0.05, AICc = -113.39; N = 79, *F* = 0.76, *P* > 0.05, AICc = -113. 39; respectively) in explaining the developmental stage of *D. carolinianum*.

Due to bias in the age of individuals of *Scutellaria bushii* throughout the century (all individuals from the early-20th century were in fruit), only analyses based on modern collections (1960 to present) could be analyzed, and the replication was reduced to 11. During this period, variation in the phenophase of *S. bushii* was explained by collection date (N = 11, *F* = 20.17, *P* = 0.0020, AICc = -22.39). Under the reduced model, phenology was marginally affected by time (N = 11, *F* = 3.69, *P* = 0.0912, AICc= -22.39; Figure 5-5A), my measure of climate change, but there was no significant effect of time in the full model (N=11, *F* = 2.17, *P* = 0.1909, AICc = -18.73). Since there was no difference between the reduced and full model (ΔAICc = 3.66), both models should be considered as potentially accurate. There were no other qualitative differences between the models. Latitude (N = 11, *F* = 0.189, *P* > 0.05, AICc = -18.73; removed from the reduced model via *step*) and climate (N = 11, *F* = 2.45, *P* > 0.05, AICc = -18.73) did not

explain a significant amount of variation in the phenophase of *S. bushii*. Low replication and the lack of early-century data make these analyses inconclusive.

Variation in the developmental stage of *S. ovata* was explained by latitude (N = 55, $F = 9.76$, $P = 0.0031$, AICc = -25.13) and marginally by collection date (N = 55, $F = 3.64$, $P = 0.0611$, AICc = -25.13). Neither climate (N = 55, $F = 1.96$, $P > 0.05$, AICc = -25.13) nor time (N = 55, $F = 1.93$, $P > 0.05$, AICc = -25.13; Figure 5-5B) explained a significant amount of the variation in phenology for *S. ovata*. There was no significant interaction between habitat and collection date (N = 55, $F = 1.81$, $P > 0.05$, AICc = -25.13) or between habitat and response to climate change (i.e., Habitat by Time by Julian date; N = 55, $F = 0.41$, $P > 0.05$, AICc = -25.13). Therefore, spatial variation in day length or temperature appears to account for the phenology of *S. ovata*.

Scutellaria parvula's developmental stage was correlated with collection date (N = 22, $F = 6.12$, $P = 0.0223$, AICc = -33.30), and varied marginally across habitats (N = 22, $F = 3.08$, $P = 0.0950$, AICc = -33.30). Specifically, individuals in the glade habitat bloomed marginally earlier than in nonglade habitas. There was no interaction between climate and collection date (N = 22, $F = 0.70$, $P > 0.05$, AICc = -21.86; removed from final model (ΔAICc = 11.44)) or between collection date and change through time (N = 22, $F = 0.88$, $P > 0.05$, AICc = -21.86; Figure 5-5C; removed from final model (ΔAICc = 11.44)), suggesting that other factors are stabilizing the phenological response of *S. parvula* to climate change, such as day length, genetic constraints, or interspecific interactions. There was no effect of an interaction between habitat and time (N = 22, $F = 0.013$, $P > 0.05$, AICc = -21.86; removed from final model (ΔAICc = 11.44)) on the phenology of *S. parvula*. Latitude also did not explain a significant amount of variation

seen in the developmental stage of *S. parvula* (N = 22, *F* = 0.12, *P* > 0.05, AICc = -21.86; removed from final model (ΔAICc = 11.44)).

DISCUSSION

In this study, I find that related pairs of widespread and rare endemic species respond differently to regional climate change, and intrinsic factors appear to drive their phenological patterns. The climate of the Ozark region has seen an increase in minimum temperatures and precipitation over the past century and a decrease in maximum temperatures. On the whole, the Ozark region is becoming wetter and warmer, with a reduction in the severity of temperature extremes (maximum and minimum temperatures). Moreover, precipitation is less correlated with PC1 through time, as indicated by a decline in the weighting through time. In other words, the inter-annual variation in climate seen today is less influenced by variation in precipitation than it was 100 years ago.

One of the rare plants, *Delphinium treleasei*, is responding to changes in climate with phenological change. I predicted that species that are endemic to high-stress environments would be less responsive to climatic change, assuming habitat specialization to xeric glade habitats, in comparison to CC species with broader habitat use. However, the results indicate the opposite. The endemic *D. treleasei* is blooming earlier but the phenology of the widespread species, *D. carolinianum*, has not changed within any habitat, including glades. The lack of a habitat effect in *D. carolinianum* suggests that local abiotic conditions do not influence its phenology. Therefore, the phenological change documented in *D. treleasei* is not driven by local abiotic conditions in the glade habitat, contrary to my prediction. Greater plasticity in phenology allows

individuals to track temporal variation in climate and either avoid more stressful conditions or capitalize on favorable conditions. For instance, if a species specializes on hot, dry environments, it may be more responsive to a reduction in stress, such as an increase in precipitation (Aaronson, 1992; Angert et al., 2010). Alternatively, genetic homogeneity across habitats could reduce the ability of *D. carolinianum* to respond to local climatic conditions (Lerner, 1954). In order to test for these mechanisms, future studies should explore the genetic basis for these phenological trends.

The differential responses of sympatric congeners may induce changes in their interspecific interactions, regardless of the mechanism driving the pattern. On average, *D. carolinianum* blooms roughly 2.8 days earlier than *D. treleasei*. Therefore within native habitats, the phenologies of the *Delphinium* species are converging. These species of *Delphinium* can hybridize (Warnock, 1987), and they share some pollinators (Miller-Struttmann, data not published). Therefore, hybridization rates and competition for shared pollinators could increase in the future if the phenology of *D. treleasei* continues to advance.

Neither of the common *Scutellaria* species responded to changes in climate with shifts in phenology. Variation in the phenology of one common congener, *S. ovata*, was influenced by spatial variation and marginally by collection date, but neither climate nor climate change affected phenology. *Scutellaria ovata* may be responding to day length or to climatic factors that are not changing in this region, such as high temperatures. The developmental stage of another widespread species, *S. parvula*, was correlated with collection date and habitat, and there was no effect of spatial variation or climate. While the individuals in the glade habitats had an earlier phenology than individuals in the

nonglade habitats, there was no change in phenology for individuals in either habitat type. The climatic factors to which *S. parvula* is responsive may not be changing in this region, or the degree of change in key climatic factors may not be sufficient to affect the phenology of this species. Moreover, additional factors such as interspecific interactions could be stabilizing the response of *S. parvula* to climate change if they are exerting selective pressure on phenology in opposing directions (Elzinga et al., 2007; Strauss and Irwin, 2004). The phenology of the endemic *S. bushii* was marginally described by climate change, as indicated by difference in phenology since 1970. However, this model did not fit the data any better than the full model, under which there was no effect of time on phenology of *S. bushii*. Interestingly, the direction of the relationship between climate change and phenology is counter-intuitive. *Scutellaria bushii* is blooming marginally later through time, not earlier as I predicted and as seen in *D. treleasei*. Climate change in Missouri and Arkansas is driven by two main factors, warming temperatures and increased precipitation. Unlike many species, the phenology of *S. bushii* may not be responding to temperature, since temperatures are increasing and should result in the advancement of flowering. Precipitation has increased by roughly 18.2 cm (17.8%) over the last century (comparison of average precipitation between 1897-1909 and 2000-2009), and may be driving the marginal change in the phenology of *S. bushii*. An increase in precipitation could lead to later onset of flowering or to a longer flowering period. However, the low replication of scorable specimens reduced my ability to rigorously test the prediction that intrinsic factors drive the phenological response of *S. bushii* to climate change, and this pattern should be interpreted with caution. Detailed field experiments

should be conducted to establish if this pattern is real and to explicitly test the mechanisms potentially leading to the phenological shifts seen in *S bushii*.

This is the first study of which I am aware that documents the phenological shifts of plant species using a continuous phenophase measure. A continuous measure allowed me to disentangle the effect of collection date from phenological stage, which is a confounding factor in most phenological studies using herbarium specimens. Most studies rely on more general categories, such as in fruit or in flower, and could be misleading if collection date and phenophase are correlated through time. Herbarium records are an abundant and important resource for understanding the effects of climate change on phenology. However, data collected from these records should be analyzed carefully, and the results interpreted with caution. Incorporating vital biological information, such as the developmental stage of the individual, will improve our understanding and prediction of species responses to climate change. Understanding the determinants of plant phenology allows scientists to summarize and categorize the types of species that will respond to climate change and in which direction they will respond. This broader scale understanding will improve our ability to predict which species should be of future conservation concern and those that may have lower risk of decline.

Table 5-1. The weightings of each variable (Average temperature, residuals of maximum temperature, residuals of minimum temperature, and total precipitation) toward the first four principle components from a principle components analyses across all years and by decade. The residuals from a multiple regression of maximum and minimum temperature against average temperature were used in order to remove any potential correlation between maximum and minimum temperatures with average temperatures; therefore, I was able to isolate the influence of maximum and minimum temperatures from average temperature on the multivariate climate axes.

|	PC	All years	1897-1909	1910-19	1920-29	1930-39	1940-49	1950-59	1960-69	1970-79	1980-89	1990-99	2000-09
Weightings													
Average	PC1	0.0414	0.3881	0.4213	0.1409	0.1329	0.3314	0.4029	0.3305	-0.4388	-0.0753	-0.3378	-0.2305
temperature	PC2	-0.9739	-0.5966	-0.6142	0.9355	0.9900	-0.9428	0.9149	0.8666	0.7312	-0.9235	-0.6603	0.6760
(ºC)	PC3	-0.2221	0.5028	0.6456	-0.2581	-0.0264	0.0025	-0.0263	0.3731	-0.5113	0.3760	-0.6697	-0.6991
	PC4	0.0209	-0.4906	-0.1688	0.1957	0.0391	0.0364	-0.0005	0.0242	-0.1066	0.0032	0.0378	-0.0350
Maximum	PC1	0.6110	-0.6217	-0.6034	-0.5213	0.5781	-0.5738	0.5341	0.5654	0.5470	0.6122	0.6282	-0.6554
temperature	PC2	-0.0497	-0.3304	0.1746	-0.1932	-0.0935	-0.2280	-0.2268	-0.3157	0.0389	0.0894	-0.0175	-0.2565
(residuals)	PC3	0.2614	-0.4491	0.6654	-0.7910	0.4249	0.3481	0.2766	0.2782	-0.5458	0.3481	-0.3391	-0.0673
	PC4	-0.7456	-0.5502	0.4034	0.2556	0.6903	-0.7054	0.7660	-0.7094	0.6335	-0.7043	-0.7001	0.7073
Minimum	PC1	-0.5932	0.0958	0.6024	0.5838	-0.5819	0.5770	-0.5305	-0.5608	-0.5500	-0.6134	-0.6341	0.6422
temperature	PC2	0.0668	0.7285	0.2270	-0.1155	0.0397	0.1746	0.2187	0.3467	0.0168	-0.0887	-0.0191	0.2958
(residuals)	PC3	-0.4653	0.1435	0.0229	-0.5468	-0.3723	-0.3686	-0.5287	-0.2630	0.3368	-0.3347	0.2984	0.0390
	PC4	-0.6536	-0.6629	0.7649	-0.5890	0.7219	-0.7077	0.6255	-0.7044	0.7640	-0.7098	-0.7131	0.7061
Total	PC1	0.5226	0.6736	0.3091	0.6063	-0.5563	0.4775	-0.5206	-0.5065	-0.4536	-0.4933	-0.2987	0.3239
precipitation	PC2	0.2111	-0.0649	0.7354	-0.2722	0.0979	0.1695	0.2524	-0.1707	-0.6808	0.3622	0.7505	-0.6243
(cm)	PC3	-0.8160	-0.7245	0.3742	-0.0936	0.8247	0.8620	0.8021	0.8451	-0.5720	0.7908	-0.5895	-0.7108
	PC4	0.1282	-0.1308	-0.4729	0.7413	-0.0285	-0.0178	0.1481	0.0037	-0.0594	0.0081	-0.0012	0.0062
Variation explained													
Proportion	PC1	0.5754	0.3956	0.5696	0.4776	0.7204	0.6832	0.8427	0.6925	0.8196	0.6295	0.5843	0.5177
	PC2	0.2562	0.3766	0.3282	0.2579	0.2421	0.1968	0.1353	0.2150	0.1655	0.2727	0.2476	0.4155
	PC3	0.1211	0.1281	0.0571	0.1666	0.0364	0.1192	0.0201	0.0925	0.0142	0.0976	0.1681	0.0668
	PC4	0.0473	0.0997	0.0451	0.0980	0.0012	0.0008	0.0019	0.00002	0.0007	0.0002	0.00003	0.00003
Cumulative	PC1	0.5754	0.3956	0.5696	0.4776	0.7204	0.6832	0.8427	0.6925	0.8196	0.6295	0.5843	0.5177
	PC2	0.8316	0.7722	0.8978	0.7354	0.9624	0.8800	0.9780	0.9075	0.9852	0.9022	0.8319	0.9332
	PC3	0.9527	0.9003	0.9549	0.9020	0.9988	0.9992	0.9981	0.99998	0.9993	0.9999	1.0000	0.99997
	PC4	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
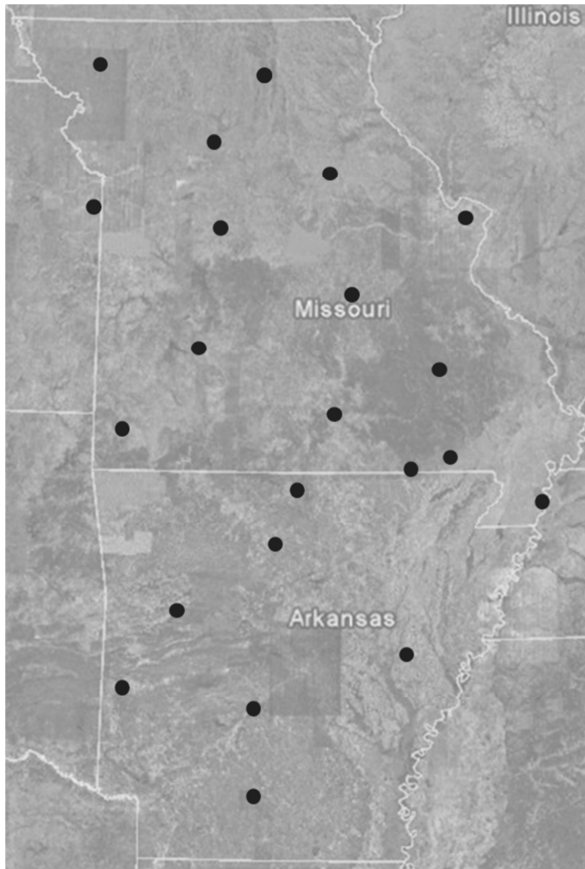
Figure 5-1. A map of the 21 weather stations (●) in Missouri and Arkansas from which data were summarized in order to investigate changes in climate through time (1987-2009). Stations were chosen based on duration of data and location in order to account for spatial variation in climate throughout the region in which the herbarium specimens were collected.
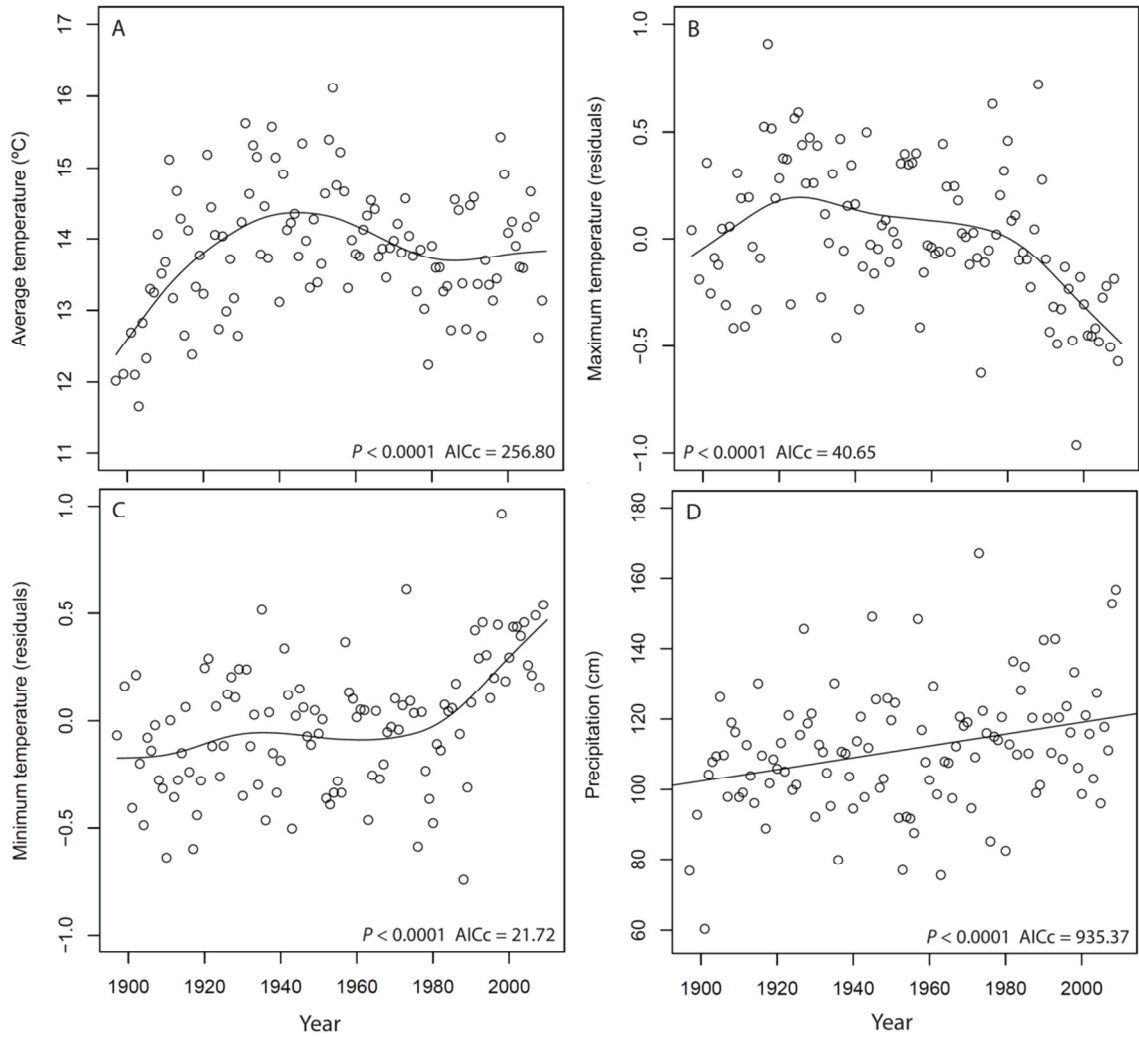
Figure 5-2. Changes in climatic variables between 1897 and 2009 in Missouri and Arkansas as documented by 21 weather stations, including (A) average monthly minimum temperature (°C), (B) maximum temperatures (residuals after regression against average temperatures in order to remove the confounding factor of average temperature), (C) minimum temperatures (residuals from regression with average temperature as above), and (D) total annual precipitation (cm).
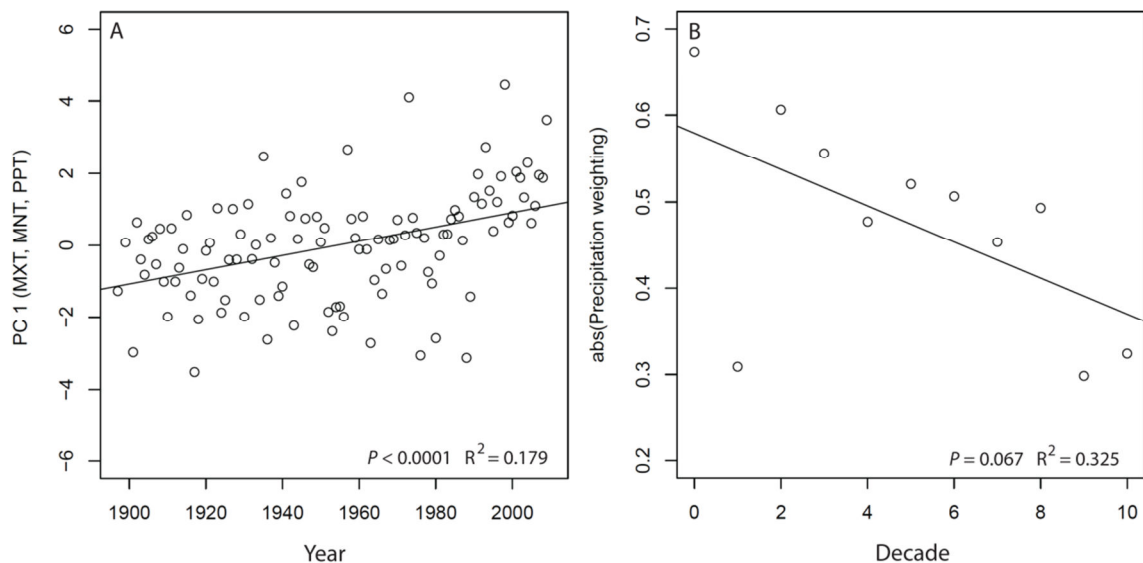
Figure 5-3. Changes in the composite climate variable and in the strength of correlation between one factor and PC 1 as documented by 21 weather stations in Missouri and Arkansas, including (A) the composite variable Climate, defined as the first principle component (PC 1) from a principle components analysis of four abiotic variables (i.e., mean temperature, maximum temperature (residuals), minimum temperature (residuals), and precipitation), and (B) the change in weightings of precipitation for PC1 by decade between 1897 and 2009.
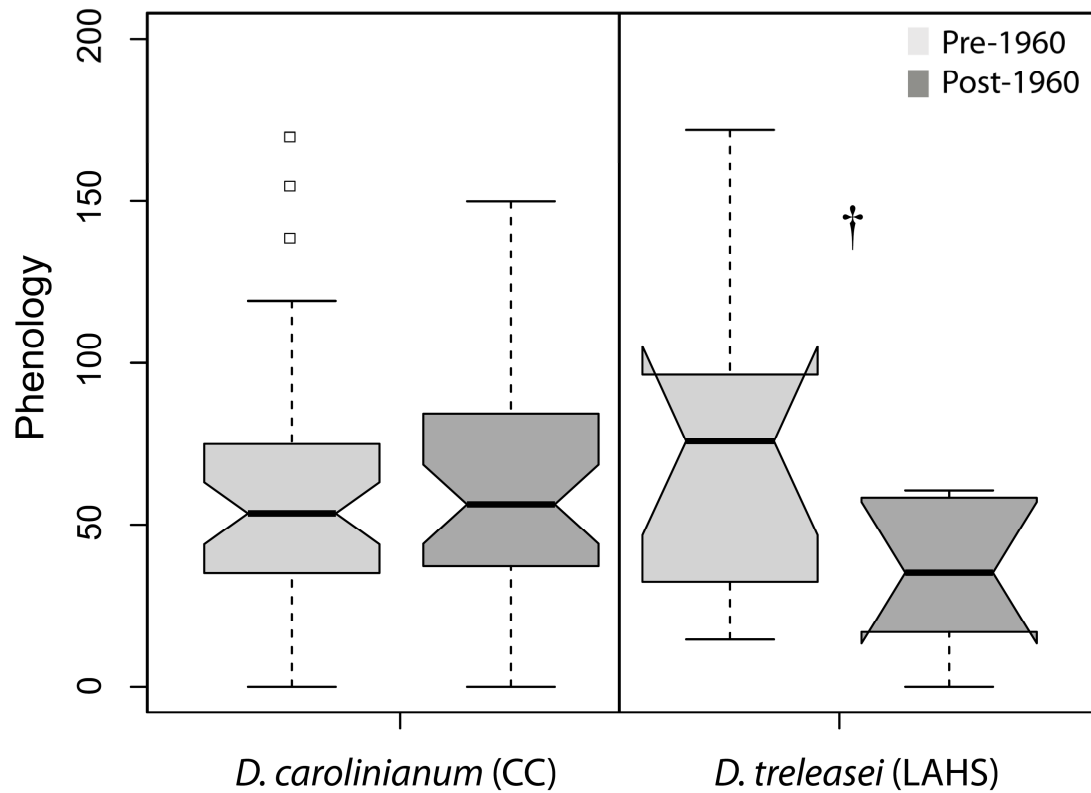
Figure 5-4. Phenology of an endemic plant, *Delphinium treleasei*, and its widespread

congener, *D. carolinianum*, during two period of time, before and after 1960 (pre- and

post-1960, respectively). Phenology is defined as the product of collection date and

phenophase, which is a continuous variable that describes the developmental stage of an

individual. Statistical significance is denoted by a dagger (†) for results with $P < 0.075$.

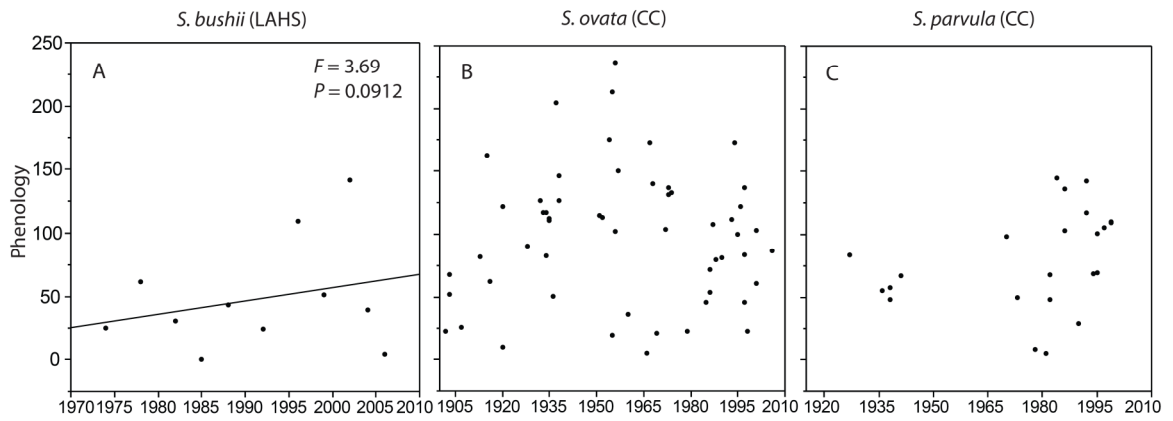Figure 5-5. Phenology of an endemic plant, *Scutellaria bushii* (A), and two widespread congeners, *S. ovata* (B) and *S. parvula* (C) during the past 40 to 105 years in Missouri and Arkansas. Phenology is defined as the product of phenophase and collection date, and only *S. bushii* had a marginally significant response to climate change through time.

CHAPTER SIX

Pages 113 to 119

CONCLUSION

The goal of this dissertation was to determine the relationship between reproduction and rarity in three glade-endemic plants based on predictions developed from two prominent ecological principles, reproductive assurance and stress-adaptation. I confirm the prediction that locally abundant, regionally-rare species (LAHS) are more resistant to stressful abiotic conditions (Chapter 3) and have more specialized reproductive systems than the CC species (Chapter 2). Moreover, the LAHS and CC species responded differently to regional climate change, which could lead to a shift in their interspecific interactions. By coalescing abiotic and biotic mechanisms into a suite of predictions that could be tested simultaneously, this research is more biologically realistic than previous studies of rarity and reproduction and corroborates the necessity for a more nuanced theory relating the two.

Contrary to the traditional prediction that rare species have more generalized reproductive systems, two of the three LAHS species studied in this dissertation had larger flowers and more specialized pollination systems in comparison to their CC species. This pattern confirms my predictions that optimal resource allocation differed between congeners and that the LAHS taxa had traits that are associated with higher outcrossing rates. Therefore, factors other than reproductive assurance via low pollen availability may be influencing the pollination biology and reproductive success of these species, such as offspring quality and local-adaptation. The LAHS species did not differ consistently from the CC species in vegetative traits that are associated with stress-adaptation and do not appear to be more stress tolerant than the CC species. While some traits indicate that the LAHS species are not locally adapted to the glade environment, I

could not measure certain key traits (i.e., biomass and root to shoot ratios) that may affect their ability to tolerate stressful conditions.

Experimental manipulations of abiotic conditions (i.e., water availability and temperature) confirm that the glade endemic plants are more resistant to high stress conditions. The CC species had reductions in biomass and growth parameters with an increase in stress, and the LAHS species did not. Greater phenotypic plasticity in the CC species suggests a potential role for genetic assimilation, or the canalization of xeric-adaptive traits, in habitat specialization. Greater plasticity may allow the CC species to persist in multiple habitats, but the LAHS species, with their more refined traits, are better adapted to the abiotic conditions on the glades. Moreover, allocation to reproductive output differed between two of the three pairs of congeners. The LAHS species had fewer, higher-quality offspring than their CC species, and stress level did not affect offspring quality or quantity in the LAHS species. Offspring quality may increase survival rates and population viability in more stressful habitats. When under low stress conditions, the CC species produced many more seeds than the LAHS species, but seed set was equivalent to their LAHS congeners when stress was high. Differences in reproductive allocation between congeners corroborate my prediction that production of high quality offspring is adaptive in stressful environments. Greater offspring abundance in the CC species may allow them to invade novel habitats with a greater range of abiotic conditions, and therefore may contribute to their larger bigeographic ranges. The CC species also had traits associated with greater competitive ability for pollinators, such as being taller and producing more flowers. However, total display size did not differ, since the LAHS species had fewer but marginally larger flowers.

Field competition experiments determined that the LAHS and CC species did not differ in their ability to compete for pollinators and that pollinator behavior (visit duration) also did not differ either between congeners or treatments. Landscape-scale spatial variation in floral traits was mirrored by pollinator behavior; plants at sites with showier plants were visited more frequently than those at sites with less attractive individuals. Interestingly, one LAHS species, *D. treleasei*, had greater reproductive success (more pollen tubes germinating down the style) than its CC species at the site with higher visitation rates, despite the fact that visitation rate and visit duration were not different between congeners. More germinating pollen tubes without a difference in pollinator effort suggests that the LAHS species has greater proportional pollination success (e.g., higher fertilization rates per unit of pollination effort). This pattern supports my prediction that specialized pollination systems are more efficient and result in the transfer of high quality pollen. Similarly, the *Echinacea* species differed in pollen deposition rates at one site. *Echinacea paradoxa* (LAHS) had more pollen on their stigmas, and neither visitation rate nor visit duration differed between species. However, this difference did not result in greater reproductive success of the LAHS species. Pollen tube growth was low for both species, which could reflect low pollen quality or a reduction of pollen tube growth in cut inflorescences. These results indicate an important role for large-scale mechanisms and pollination specialization in determining local reproductive success of LAHS species and their CC species.

Finally, this work documents the differential responses of LAHS and CC species in their responses to climate change. The CC species, which I predicted would be more responsive to regional climate change, have not changed in their phenological patterns.

This pattern is congruent with genetic homeostasis, which occurs through adaptation to varied or multiple environments. Therefore while the xeric-adapted traits of the CC species have a plastic response to stress (Chapter3), their phenological patterns are less variable. The LAHS species, on the other hand, are responding to climate change, but their phenological shifts are in opposing directions. One species, *D. treleasei*, is advancing in flowering date and the other, *S. bushii*, is delaying in one of the two models. The variation in responses between the LAHS species suggests that they are responding to different climatic variables. *Delphinium treleasei* is blooming earlier, which is consistent with higher minimum temperatures. *Scutellaria bushii*, which is blooming later, may be responding to an increase in precipitation that has occurred in this region. Few studies have looked at the role of precipitation on floral phenology, and this study indicates that it warrants more exploration. The differences between congeners in response to climate change imply that interspecific interactions may also shift with the new abiotic conditions. The *Delphinium* species are converging in their phenologies, which could result in competition for shared pollinators or greater hybridization rates.

The research in this dissertation advances our understanding of how locally abundant, regionally-rare species differ in their intrinsic traits from and in their interactions with their common congeners. I demonstrate that both biotic and abiotic factors, specifically pollination biology and stressful abiotic conditions, may be contributing synergistically to the range restriction of three glade endemic plant species. Determining how biotic and abiotic factors interact to affect species traits, and potentially their distributions, is particularly important for rare and endemic species, many of which are at increasing risk of extinction. Differences in the responses of LAHS and CC species

117

to regional climate change further support the need for a more nuanced understanding of the seven types of rarity. In light of the current rate of climate change, a more informative framework with which to predict which rare species are most vulnerable to changes in climate is imperative.

Future work

In this dissertation, I document the unique attributes of three species that fall into one of the seven types of rarity, locally abundant and regionally rare taxa. Hitherto, the focus of most research into rarity has focused on those species that are locally-rare, and a robust body of theory and empirical studies explores the ecological and evolutionary causes and consequences of this type of rarity. However, the theory is less developed for the other six types of rarity, which warrant closer examination. In future work I will expand this theory in order to better understand the ecological and evolutionary mechanisms that drive variation in species distributions. Specifically, future work should explore the mechanisms behind the disparity in offspring quality and quantity between congeners by documenting the relationship between mating system and offspring survival in endemic and widespread species.

My research shows that LAHS and CC species differ in resource allocation to reproduction, which indicates that offspring quality may be adaptive in stressful habitats. In order to more fully understand what excludes the LAHS species from non-glade habitats, I advise that future research explore the role of propagule pressure and offspring quality on the invasion success of the LAHS and CC species into novel habitats and on survival in high stress environments. While greenhouse studies can inform how species respond to stress under controlled conditions, the abiotic conditions on the glades cannot

be reproduced. Field studies should be conducted in order to confirm that these patterns are consistent in more realistic conditions and in the context of natural ecological communities.

Research into additional pairs of related species, which vary in their distributions and in the habits on which they specialize, will determine how generalizable the results from this research are. If rare species that are habitat specialists are more dependent on their coevolved pollinators for persistence, they may be more susceptible to pollinator declines. The concomitant decline of oligolectic (specialist) pollinating insects and pollinator-dependent plants in Europe (Biesmeijer et al., 2006) illustrates the need for a more holistic understanding of the mechanisms determining species biogeographical ranges, particularly for rare and endemic species, many of which are at increasing risk of extinction. Coalescing abiotic and biotic mechanisms into a suite of predictions that can be tested simultaneously will not only make the results more biologically realistic, but it will also make them more relevant. With a greater understanding of the factors that determine variation in species distributions, we will be better equipped to protect, conserve and restore Earth's declining biodiversity in a rapidly changing environment.

LITERATURE CITED

Abrams, P.A., Rueffler, C. & Kim, G. (2008) Determinants of the strength of disruptive and/or divergent selection arising from resource competition. *Evolution*, **62**, 1571-1586.

Anderson, B. & Johnson, S.D. (2008) The geographical mosaic of coevolution in a plant-pollinator mutualism. *Evolution*, **62**, 220-225.

Anderson, I.A. & Busch, J.W. (2006) Pollinator-mediated selection weakens floral integration in self-compatible taxa of *Leavenworthia* (Brassicaceae). *American Journal of Botany*, **93**, 860-867.

Angert, A.L. (2009) The niche, limits to species distributions, and spatiotemporal variation in demography across the elevation ranges of two monkeyflowers. *Proceedings of the National Academy of Sciences of the United States of America*, **106 Suppl**, 19693-19698.

Angert, A.L., Horst, J., Huxman, T.E., & Venable, D.L. (2010) Phenotypic plasticity and precipitation response in Sonoran desert winter annuals. *American Journal of Botany*, **97**, 405-411.

Aragón, C.F., Escudero, A. & Valladares, F. (2008) Stress-induced dynamic adjustments of reproduction differentially affect fitness components of a semi-arid plant. *Journal of Ecology*, **96**, 222-229.

Aronson, J., Kigel, A., Shmida, A., & Klein, J. (1992) Adaptive phenology of desert and Mediterranean populations of annual plants grown with and without water stress. *Oecologia*, **89**, 17-26.

Armbruster, P. & Reed, D.H. (2005) Inbreeding depression in benign and stressful environments. *Heredity*, **95**, 235-242.

Badeck, F.W., Bondeau, A., Bottcher, K., Doktor, D., Lucht, W., Schaber, J., & Sitch, S. (2004) Responses of spring phenology to climate change. *New Phytologist*, **162**, 295-309.

Baker, H.G. (1955) Self-compatibility and establishment after "long-distance" dispersal. *Evolution,* **9**, 347-349

Baskin, J.M. & Baskin, C.C. (1974) Some aspects of the autecology of prairie larkspur (*Delphinium virescens*) in Tennessee cedar glades. *American Midland Naturalist*, **92**, 58-71.

Baskin, J.M. & Baskin, C.C. (1982) Ecological life cycle and temperature relations of seed germination and bud growth of *Scutellaria parvula*. *Bulletin of the Torrey Botanical Club*, **109**, 1-6.

Baskin, J.M. & Baskin, C.C. (1985) Photosynthetic pathway in 14 southeastern cedar glade endemics, as revealed by leaf anatomy. *American Midland Naturalist*, **114**, 205-208.

Baskin, J.M. & Baskin, C.C. (1988) Endemism in rock outcrop plant communities of unglaciated eastern United States: An evaluation of the roles of the edaphic, genetic and light factors. *Journal of Biogeography*, **15**, 829-840.

Baskin, C.C., Baskin, J.M., & Quarterman, E. (1972) Observations on the Ecology of *Astragalus tennesseensis*. *American Midland Naturalist*, **88**, 167-182.

Bell, J.M., Karron, J.D. & Mitchell, R.J. (2005) Interspecific competition for pollination lowers seed production and outcrossing in *Mimulus ringens*. *Ecology*, **86**, 762-771.

Bevill, R.L. & Louda, S.M. (1999) Comparisons of related rare and common species in the study of plant rarity. *Conservation Biology*, **13**, 493-498.

Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemuller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J., & Kunin, W.E. (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, **313**, 351-354

Brisson, J.A, Strasburg, J.L. & Templeton, A.R. (2003) Impact of fire management on the ecology of collared lizard (*Crotaphytus collaris*) populations living on the Ozark Plateau. *Animal Conservation*, **6**, 247-254.

Brown, B.J., Mitchell, R.J. & Graham, S.A. (2002) Competition for pollination between an invasive species (purple loosestrife) and a native congener. *Ecology*, **83**, 2328-2336.

Brown, B. & Mitchell, R. (2001) Competition for pollination: Effects of pollen of an invasive plant on seed set of a native congener. *Oecologia*, **129**, 43-49.

Bryant, J.P., Chapin, F.S., & Klein, D.R. (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, **40**, 357-368.

Chapin, F. S. (1980) The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics*, **11**, 233-260.

Chapin, F. S., I., Autumn, K., & Pugnaire, F. (1993) Evolution of suites of traits in response to environmental stress. *The American Naturalist*, **142**, S78-S92.

Chase, J. & Leibold, M. (2003) *Ecological niches: linking classical and contemporary approaches (interspecific interactions)*. University of Chicago Press, Chicago, IL.

Chase, J.M., Abrams, P.A., Grover, J.P., Diehl, S., Chesson, P., Holt, R.D., Richards, S.A., Nisbet, R.M., & Case, T.J. (2002) The interaction between predation and competition: A review and synthesis. *Ecology Letters*, **5**, 302-315.

Cheptou, P.O., Berger, A., Blanchard, A., Collin, C., & Escarre, J. (2000) The effect of drought stress on inbreeding depression in four populations of the Mediterranean outcrossing plant *Crepis sancta* (Asteraceae). *Heredity*, **85 Pt 3**, 294-302.

Cleland, E.E., Chiariello, N.R., Loarie, S.R., Mooney, H.A., & Field, C.B. (2006) Diverse responses of phenology to global changes in a grassland ecosystem. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 13740-13744.

Clinebell, R.R.I. & Bernhardt, P. (1998) The pollination ecology of five species of *Penstemon* (Scrophulariaceae) in the tallgrass prairie. *Annals of the Missouri Botanical Garden*, **85**, 126-136.

Dafni, A. (1993) *Pollination ecology: A practical approach.* Oxford University Press, Oxford, U. K.

Darwin, C. (1877) *On the various contrivances by which British and foreign orchids are fertilized* (ed. D. Appleton). New York.

Del Castillo, R.F. & Trujillo, S. (2008) Effect of inbreeding depression on outcrossing rates among populations of a tropical pine. *The New Phytologist*, **177**, 517-24.

Devaux, C. & Lande, R. (2009) Displacement of flowering phenologies among plant species by competition for generalist pollinators. *Journal of evolutionary biology*, **22**, 1460-1470.

Dickson, C.E. & Petit, S. (2006) Effect of individual height and labellum colour on the pollination of *Caladenia* (syn. Arachnorchis) *behrii* (Orchidaceae) in the northern Adelaide region, South Austrailia. *Plant Systematics and Evolution*, **262**, 65-74.

Domínguez Lozano, F. & Schwartz, M.W. (2005) Patterns of rarity and taxonomic group size in plants. *Biological Conservation*, **126**, 146-154.

Dose, V. & Menzel, A. (2004) Bayesian analysis of climate change impacts in phenology. *Global Change Biology*, **10**, 259-272.

Eckert, C.G., Ozimec, B., Herlihy, C.R., Griffin, C.A., & Routley, M.B. (2009) Floral morphology mediates temporal variation in the mating system of a self-compatible plant. *Ecology*, **90**, 1540-1548.

Elliott, S.E. (2009) Subalpine bumble bee foraging distances and densities in relation to flower availability. *Environmental Entomology*, **38**, 748-756.

Ellison, A.M. & Parker, J.N. (2002) Seed dispersal and seedling establishment of *Sarracenia purpurea* (Sarraceniaceae). *American Journal of Botany*, **89**, 1024-1026.

Elzinga, J.A., Atlan, A., Biere, A., Gigord, L., Weis, A.E., & Bernasconi, G. (2007) Time after time: Flowering phenology and biotic interactions. *Trends in Ecology & Evolution*, **22**, 432-439.

Erhardt, A. & Rusterholz, H.P. (1998) Do Peacock butterflies (*Inachis io* L.) detect and prefer nectar amino acids and other nitrogenous compounds? *Oecologia*, **117**, 536-542.

Espeland, E.K. & Emam, T.M. (2011) The value of structuring rarity: The seven types and links to reproductive ecology. *Biodiversity and Conservation*, 963-985.

Fargione, J., Brown, C.S., & Tilman, D. (2003) Community assembly and invasion: An experimental test of neutral versus niche processes. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 8916-8920.

Fausto , Jr ., J.A., Eckhart, V.M., & Geber, M.A. (2001) Reproductive assurance and the evolutionary ecology of self-pollination in *Clarkia xantiana*. *America Journal of Botany*, **88**, 1794-1800.

Fenster, C.B., Armbruster, W.S., Wilson, P., Dudash, M.R., & Thomson, J.D. (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics*, **35**, 375-403.

Firman, R.C. & Simmons, L.W. (2008) Polyandry facilitates postcopulatory inbreeding avoidance in house mice. *Evolution*, **62**, 603-611.

Fishman, L. & Willis, J.H. (2008) Pollen limitation and natural selection on floral characters in the yellow monkeyflower, *Mimulus guttatus*. *New Phytologist*, **3**, 820-810.

Flanagan, R.J., Mitchell, R.J., & Karron, J.D. (2010) Increased relative abundance of an invasive competitor for pollination, *Lythrum salicaria*, reduces seed number in *Mimulus ringens*. *Oecologia*, **164**, 445-454.

Forrest, J., Inouye, D.W., & Thomson, J.D. (2010) Flowering phenology in subalpine meadows: Does climate variation influence community co-flowering patterns? *Ecology*, **91**, 431-440.

Fox, C.W. & Reed, D.H. (2011) Inbreeding depression increases with environmental stress: An experimental study and meta-analysis. *Evolution*, **65**, 246-258.

Foxe, J.P., Slotte, T., Stahl, E.A., Neuffer, B., Hurka, H., & Wright, S.I. (2009) Recent speciation associated with the evolution of selfing in *Capsella*. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 5241-5245.

Galloni, M. (2008) Visitor diversity and pollinator specialization in Mediterranean legumes. *Flora*, **203**, 94-102.

Georgescu, M., Lobell, D.B., & Field, C.B. (2011) Direct climate effects of perennial bioenergy crops in the United States. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 4307-4312.

Gregory, R.D. & Gaston, K.J. (2000) Explanations of commonness and rarity in British breeding birds: Separating resource use and resource availability. *Oikos*, **88**, 515-526.

Grime, J.P. (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist*, **111**, 1169–1194.

Halpern, S.L., Adler, L.S., & Wink, M. (2010) Leaf herbivory and drought stress affect floral attractive and defensive traits in *Nicotiana quadrivalvis*. *Oecologia*, **163**, 961-971.

Harder, L.D., Richards, S.A., & Routley, M.B. (2008) Effects of reproductive compensation, gamete discounting and reproductive assurance on mating-system diversity in hermaphrodites. *Evolution*, **62**, 157-172.

Hardin, G. (1960) The competitive exclusion principle. *Science*, **131**, 1292-1297.

Hauser, T.P. & Loeschcke, V. (1996) Drought stress and inbreeding depression in *Lychnis flos-cuculi* (Caryophyllaceae). *Evolution*, **50**, 1119-1126.

Heschel, M.S., Hausmann, N., & Schmitt, J. (2005) Testing for stress-dependent inbreeding depression in *Impatiens capensis* (Balsaminaceae). *American Journal of Botany*, **92**, 1322-1329.

Ibanez, I., Primack, R.B., Miller-Rushing, A.J., Ellwood, E., Higuchi, H., Lee, S.D., Kobori, H., & Silander, J.A. (2010) Forecasting phenology under global warming. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365**, 3247-3260.

Internicola, A.I., Page, P.A., Bernasconi, G., & Gigord, L.D.B. (2007) Competition for pollinator visitation between deceptive and rewarding artificial inflorescences: An experimental test of the effects of floral colour similarity and spatial mingling. *Functional Ecology*, **21**, 864-872.

Ishii, H.S., Hirabayashi, Y. & Kudo, G., (2008) Combined effects of inflorescence architecture, display size, plant density and empty flowers on bumble bee behaviour: Experimental study with artificial inflorescences. *Oecologia*, **156**, 341-350.

Izco, J. (1998) Types of rarity of plant communities. *Journal of Vegetation Science*, **9**, 641-646.

Jacquemyn, H. (2005) Does nectar reward affect rarity and extinction probabilities of orchid species? An assessment using historical records from Belgium and the Netherlands. *Biological Conservation*, **121**, 257-263.

Jacquemyn, H. & Brys, R. (2008) Density-dependent mating and reproductive assurance in the temperate forest herb *Paris quadriolia* (Trilliaceae). *American Journal of Botany*, **95**, 294-298.

Jenkins, S.E. & Jenkins, M.A. (2006) Effects of prescribed fire on the vegetation of a savanna-glade complex in northern Arkansas. *Southeastern Naturalist*, **5**, 113-126.

Hegland, S.J. & Totland, Ø. (2008) Is the magnitude of pollen limitation in a plant community affected by pollinator visitation and plant species specialisation levels? *Oikos*, **117**, 883-891.

Karron, J.D., Mitchell, R.J., Holmquist, K.G., Bell, J.M., & Funk, B. (2004) The influence of floral display size on selfing rates in *Mimulus ringens*. *Heredity*, **92**, 242-248.

Karron, J.D., Holmquist, K.G., Flanagan, R.J. & Mitchell, R.J. (2009) Pollinator visitation patterns strongly influence among-flower variation in selfing rate. *Annals of Botany*, **103**, 1379-1383.

Kelly, C.K., Woodward, F.I., & Crawley, M.J. (1996) Ecological correlates of plant range size: Taxonomies and phylogenies in the study of plant commonness and rarity in Great Britain. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **351**, 1261-1269.

Kennedy, B.F. & Elle, E. (2008) The inbreeding depression cost of selfing: Importance of flower size and population size in *Collinsia parviflora* (Veronicaceae). *American Journal of Botany*, **95**, 1596-1605.

Knight, T.M., Steets, J.A., Vamosi, J.C., Mazer, S.J., Burd, M., Campbell, D.R., Dudash, M.R., Johnston, M.O., Mitchell, R.J., & Ashman, T.L. (2005) Pollen limitation of plant reproduction: Pattern and process. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 467-497.

Kremen, C., Williams, N.M., & Thorp, R.W. (2002) Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 16812-16816.

Kruckeberg, A.R. & Rabinowitz, D. (1985) Biological aspects of endemism in higher plants. *Annual Review of Ecology and Systematics*, **16**, 447-479.

Kucera, C.L. & Martin, S.C. (1957) Vegetation and soil relationships in the glade region of the southwestern Missouri Ozarks. *Ecology*, **38**, 285-291.

Kudo, G. & Harder, L.D. (2005) Floral and inflorescence effects on variation in pollen removal and seed production among six legume species. *Functional Ecology*, **19**, 245-254.

Kudo, G. & Hirao, A.S. (2005) Habitat-specific responses in the flowering phenology and seed set of alpine plants to climate variation: Implications for global-change impacts. *Population Ecology*, **48**, 49-58.

Kudo, G., Ida, T.Y., & Tani, T. (2008) Linkages between phenology, pollination, photosynthesis, and reproduction in deciduous forest understory plants. *Ecology*, **89**, 321-331.

Kunin, W.E. & Gaston, K.J. (1997) Rare-common differences: An overview. *The biology of rarity: causes and consequences of rare-common differences*. (eds. K.J. Kunin, & W.E. Gaston), pp. 12-29. Chapman & Hall, London, UK.

Lambert, A.M., Miller-Rushing, A.J., & Inouye, D.W. (2010) Changes in snowmelt date and summer precipitation affect the flowering phenology of *Erythronium grandiflorum* (glacier lily; Liliaceae). *American Journal of Botany*, **97**, 1431-1437.

Lau, J.A., McCall, A.C., Davies, K.F., McKay, J.K., & Wright, J.W. (2008) Herbivores and edaphic factors constrain the realized niche of a native plant. *Ecology*, **89**, 754-762.

Lavergne, S., Thompson, J.D., Garnier, E., & Debussche, M. (2004) The biology and ecology of narrow endemic and widespread plants: A comparative study of trait variation in 20 congeneric pairs. *Oikos*, **107**, 505-518.

Learn, G.H.J., & Schaal, B.A. (1987) Population subdivision for ribosomal DNA repeat variants in *Clematis fremontii*. *Evolution*, **41**, 433-438.

Lerner, I.M. (1954) *Genetic Homeostasis*. John Wiley & Son, New York

Lipow, S., Bernhardt, P., & Vance, N. (2002) Comparative rates of pollination and fruit set in widely separated populations of a rare orchid (*Cypripedium fasciculatum*). *International Journal of Plant Sciences*, **163**, 775-782.

Lloyd, K.M., Lee, W.G., & Wilson, J.B. (2002) Competitive abilities of rare and common plants: Comparisons using *Acaena* (Rosaceae) and *Chionochloa* (Poaceae) from New Zealand. *Conservation Biology*, **16**, 975-985.

Lortiek, C.J., Michalet, R., Paolini, L., Pugnaireq, F.I., Newingham, B., Aschehoug, E.T., Armasq, C., Kikodze, D., & Cook, B.J. (2002) Positive interactions among alpine plants increase with stress. *Zoologica*, **417**, 844-848.

MacDougall, A.S., Boucher, J., Turkington, R., & Bradfield, G.E. (2006) Patterns of plant invasion along an environmental stress gradient. *Journal of Vegetation Science*, **17**, 47-56.

Maestre, F.T., Callaway, R.M., Valladares, F., & Lortie, C.J. (2009) Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology*, **97**, 199-205.

Martén-Rodríguez, S., & Fenster, C.B. (2010) Pollen limitation and reproductive assurance in Antillean Gesnerieae: A specialists vs. generalist comparison. *Ecology*, **91**, 155-165.

McLernon, S.M., Murphy, S.D., & Aarssen, L.W. (1996) Heterospecific pollen transfer between sympatric species in a midsuccssional old-field community. *American Journal of Botany*, **83**, 1168-1174.

Mevi-Schutz, J. & Erhardt, A. (2005) Amino acids in nectar enhance butterfly fecundity: a long-awaited link. *The American Naturalist*, **165**, 411-419.

Miller-Rushing, A.J. & Inouye, D.W. (2009) Variation in the impact of climate change on flowering phenology and abundance: An examination of two pairs of closely related wildflower species. *American Journal of Botany*, **96**, 1821-1829.

Miller-Rushing, A.J., Katsuki, T., Primack, R.B., Ishii, Y., Lee, S.D., & Higuchi, H. (2007) Impact of global warming on a group of related species and their hybrids: Cherry tree (Rosaceae) flowering at Mt. Takao, Japan. *American Journal of Botany*, **94**, 1470-1478.

Miller-Rushing, A.J. & Primack, R.B. (2008) Global warming and flowering times in Thoreau's Concord: A community perspective. *Ecology*, **89**, 332-341.

Mitchell, R.J., Karron, J.D., Holmquist, K.G. & Bell, J.M. (2004) The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. *Functional Ecology*, **18**, 116-124.

Mitchell, R.J., Flanagan, R.J., Brown, B.J., Waser, N.M., & Karron, J.D. (2009) New frontiers in competition for pollination. *Annals of Botany*, **103**, 1403-1413.

Mitchell, R.J., Irwin, R.E., Flanagan, R.J., & Karron, J.D. (2009) Ecology and evolution of plant-pollinator interactions. *Annals of Botany*, **103**, 1355-1363.

Moeller, D.A. & Geber, M.A. (2005) Ecological context of the evolution of self-pollination in *Clarkia xantiana*: Population size, plant communities, and reproductive assurance. *Evolution*, **59**, 786-799.

Muchhala, N., Brown, Z., Armbruster, W.S., & Potts, M.D. (2010) Competition drives specialization in pollination systems through costs to male fitness. *The American Naturalist*, **176**, 732-743.

Muneepeerakul, R., Bertuzzo, E., Lynch, H.J., Fagan, W.F., Rinaldo, A., & Rodriguez-Iturbe, I. (2008) Neutral metacommunity models predict fish diversity patterns in Mississippi-Missouri basin. *Nature*, **453**, 220-222.

Murray, B.R., Thrall, P.H., Gill, A.M., & Nicotra, A.B. (2002) How plant life-history and ecological traits relate to species rarity and commonness at varying spatial scales. *Austral Ecology*, **27**, 291-310.

Naug, D. & Arathi, H.S. (2007) Receiver bias for exaggerated signals in honeybees and its implications for the evolution of floral displays. *Biology Letters*, **3**, 635-637.

Neiland, M.R.M. & Wilcock, C.C. (1998) Fruit set, nectar reward, and rarity in the Orchidaceae. *American Journal of Botany*, **85**, 1657-1671.

Nelson, P. & Ladd, D. (1982) Preliminatry report on the identification, distribution and classification of Missouri glades. *Proceedings of the Seventh North American*

Prairie Conference. (ed. C. Kucera), pp. 59-76. Southwestern Missouri State University, Springfield, MO.

Ogden, E.C., Raynor, G.S., Hayes, J., DM, L., & Haines, J. (1974) *Manual for sampling air-borne pollen*. Hafner Press, New York.

Osborne, J.L., Martin, A.P., Carreck, N.L., Swain, J.L., Knight, M.E., Goulson, D., Hale, R.J., & Sanderson, R.A. (2008) Bumblebee flight distances in relation to the forage landscape. *The Journal of animal ecology*, **77**, 406-415.

Parachnowitsch, A.L. & Kessler, A. (2010) Pollinators exert natural selection on flower size and floral display in *Penstemon digitalis*. *New Phytologist*, **188**, 393-402.

Pasquet, R.S., Peltier, A., Hufford, M.B., Oudin, E., Saulnier, J., Paul, L., Knudsen, J.T., Herren, H.R., & Gepts, P. (2008) Long-distance pollen flow assessment through evaluation of pollinator foraging range suggests transgene escape distances. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 13456-13461.

Pigliucci, M., Murren, C.J., & Schlichting, C.D. (2006) Phenotypic plasticity and evolution by genetic assimilation. *Journal of Experimental Biology*, **209**, 2362-2367.

Pohlman, C.L., Nicotra, A.B., & Murray, B.R. (2005) Geographic range size, seedling ecophysiology and phenotypic plasticity in Australian *Acacia* species. *Journal of Biogeography*, **32**, 341-351.

Quarterman, E. (2007) Ecology of cedar glades. I. Distribution of glade flora in Tennessee. *Bulletin of the Torrey Botanical Club*, **77**, 1-9.

R Development Core Team. (2008) R: A language and environment for statistical computing.

Rabinowitz, D., Rapp, J.K., Sork, V.L., Rathcke, B.J., Reese, G.A., & Weaver, C, J. (1981) Phenological properties of wind- and insect-pollinated prairie plants. *Ecology*, **62**, 49-56.

Rader, R., Edwards, W., Westcott, D.A., Cunningham, S.A., & Howlett, B.G. (2011) Pollen transport differs among bees and flies in a human-modified landscape. *Diversity and Distributions*, **17**, 519-529.

Rafferty, N.E., & Ives, A.R. (2011) Effects of experimental shifts in flowering phenology on plant-pollinator interactions. *Ecology Letters*, **14**, 69-74.

Ricketts, T.H., Regetz, J., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Bogdanski, A., Gemmill-Herren, B., Greenleaf, S.S., Klein, A.M., Mayfield, M.M., Morandin, L.A., Ochieng, A., Potts, S.G., & Viana, B.F. (2008) Landscape effects on crop pollination services: Are there general patterns? *Ecology Letters*, **11**, 499-515.

Riginos, C., Heschel, M.S., & Schmitt, J. (2007) Maternal effects of drought stress and inbreeding in *Impatiens capensis* (Balsaminaceae). *American Journal of Botany*, **94**, 1984-1991.

Rodríguez-Gironés, M.A., & Santamaría, L. (2007) Resource competition, character displacement, and the evolution of deep corolla tubes. *The American Naturalist*, **170**, 455-464.

Rosenzweig, C., Casassa, G., Karoly, D.J., Imeson, A., Liu, C. Menzel, A., Rawlins, S., Root, T.L., Seguin, B., & Tryjanowski, P. (2007) Assessment of observed changes and responses in natural and managed systems. *Climate Change 2007: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Fourth*

Assessment Report of the Intergovernmental Panel on Climate Change (eds. M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J. van der Linden, & C.E. Hanson), pp. 79-131. Cambridge University Press, Cambridge, U.K.

Ryberg, W.A. & Chase, J.M. (2007) Predator-dependent species-area relationships. *The American Naturalist*, **170**, 636-642.

Rymer, P., Whelan, R., Ayre, D., Weston, P., & Russell, K. (2005) Reproductive success and pollinator effectiveness differ in common and rare species (Proteaceae). *Biological Conservation*, **123**, 521-532.

SAS Institute, Inc. (2009) JMP Statistical Discover Software 8.

Sherry, R.A., Zhou, X., Gu, S., Arnone, J.A., Schimel, D.S., Verburg, P.S., Wallace, L.L., & Luo, Y. (2007) Divergence of reproductive phenology under climate warming. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 198-202.

SigmaScan Pro 5.0. (2002) Systat Software Inc.

Silvertown, J.; Franco M., Pisanty I., and Mendoza, A.M. (1993) Comparative plant demography – relative importance of life-cycle components to the finite rate of increase in woody and herbaceous perennials. *Journal Ecology*, **81**, 465-476.

Soberón, J. (2007) Grinnellian and Eltonian niches and geographic distributions of species. *Ecology Letters*, **10**, 1115-1123.

Sprigler,R. & Chang, S. (2009) Pollen limitation and reproduction varies with population size in experimental populations of *Sabatia angularis* (Gentianacea). *Botany*, **87**, 330-338

Strauss, S.Y. & Irwin, R.E. (2004) Ecological and evolutionary consequences of
multispecies plant-animal interactions. *Annual Review of Ecology, Evolution, and
Systematics*, **35**, 435-466.

Šidàk, Z. (1967). Rectangular confidence region for themeans of multivariate normal
distributions. *Journal of the American Statistical Association*, **62**, 626–633.

Templeton, A.R., Robertson, R.J., Brisson, J., & Strasburg, J. (2001) Disrupting
evolutionary processes: The effect of habitat fragmentation on collared lizards in the
Missouri Ozarks. *Proceedings of the National Academy of Sciences of the United
States of America*, **98**, 5426-5432.

Tilman, D. (1976) Ecological competition between algae: Experimental confirmation of
resource-based competition theory. *Science*, **192**, 463-465.

Tilman, D. (2004) Niche tradeoffs, neutrality, and community structure: a stochastic
theory of resource competition, invasion, and community assembly. *Proceedings of
the National Academy of Sciences of the United States of America*, **101**, 10854-
10861.

Totland, Ø. & Eide, W. (1999) Environmentally-dependent pollen limitation on seed
production in alpine *Ranunculus acris*. *Ecoscience*, **6**, 173-179.

van Nieuwstadt, M.G.L. & Ruano Iraheta, C.E. (1996) Relation between size and
foraging range in stingless bees (Apidae, Meliponinae). *Apidologie*, **27**, 219-228.

Van Zandt, P.A. (2007) Plant defense, growth, and habitat: A comparative assessment of
constitutive and induced resistance. *Ecology*, **88**, 1984-1993.

Van Zandt, P.A., Collins, E., Losos, J.B., & Chase, J.M. (2005) Implications of food web interactions for restoration of Missouri Ozark glade habitats. *Restoration Ecology*, **13**, 312-317.

Volkov, I., Banavar, J.R., Hubbell, S.P., & Maritan, A. (2003) Neutral theory and relative species abundance in ecology. *Nature*, **424**, 1035-1037.

Waddington, C.H. (1953a) Genetic assimilation of an acquired character. *Evolution*, **7**, 118-126.

Waddington, C.H. (1953b) 'Baldwin Effect', 'Genetic Assimilation' and 'Homeostasis'. *Evolution*, **7**, 386-387.

Wagenius, S. & Lyon, S. (2010) Reproduction of *Echinacea angustifolia* in fragmented prairie is pollen-limited but not pollinator-limited. *Ecology*, **91**, 733-742.

Walck, J.L., Baskin, J.M., & Baskin, C.C. (1999) Ecology of the endangered species *Solidago shortii*. VII. Survivorship and flowering, and comparison with common, geographically-widespread *Solidago* species. *Journal of the Torrey Botanical Society*, **126**, 124-132.

Walck, J.L., Baskin, J.M., & Baskin, C.C. (2002) Why is *Solidago shortii* narrowly endemic and *S. altissima* geographically widespread? A comprehensive comparative study of biological traits. *Journal of Biogeography*, **28**, 1221-1237.

Waller, D.M., Dole, J., & Bersch, A.J. (2008) Effects of stress and phenotypic variation on inbreeding depression in *Brassica rapa*. *Evolution*, **62**, 917-931.

Warnock, M.J. (1987) Vicariant distribution of two *Delphinium* species in southeastern United States. *Botanical Gazette*, **148**, 90-95.

Whittall, J.B. & Hodges, S.A. (2007) Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature*, **447**, 706-709.

Wikelski, M., Moxley, J., Eaton-Mordas, A., López-Uribe, M.M., Holland, R., Moskowitz, D., Roubik, D.W., & Kays, R. (2010) Large-range movements of neotropical orchid bees observed via radio telemetry. *PLoS ONE*, **5**, e10738.

Williams, S.E., Williams, Y.M., VanDerWal, J., Isaac, J.L., Shoo, L.P., & Johnson, C.N. (2009) Ecological specialization and population size in a biodiversity hotspot: how rare species avoid extinction. *Proceedings of the National Academy of Sciences of the United States of America*, **106 Suppl**, 19737-19741.

Wilson, P., Castellanos, M.C., Wolfe, A.D., & Thomson, J.D. (2006) Shifts between bee and bird pollination in *Penstemons*. *Plant–Pollinator Interactions: From Specialization to Generalization*. (eds N.M. Waser & J. Ollerton), p. 47–68. The University of Chicago Press, Chicago, IL.

Wright, S. (1922) Coefficients of inbreeding and relationship. *The American Naturalist*, **56**, 330-338.

Yatskievych, G. (1999) *Steyermark's Flora of Missouri, 2nd ed., Vol. 1*. Missouri Department of Conservation, Jefferson City, Missouri.