THE IMPACTS OF PARTNER ABUNDANCE ON BENEFITS FROM

FACULTATIVE POLLINATION MUTUALISM

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The undersigned, appointed by the dean of the Graduate School, have examined the

dissertation entitled

THE IMPACTS OF PARTNER ABUNDANCE ON BENEFITS FROM

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presented by Jennifer C. Geib,

a candidate for the degree of doctor of philosophy,

and hereby certify that, in their opinion, it is worthy of acceptance.

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Professor Candace Galen

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To all those without whose support I could not have made it this far: Mom, Dad, Johanna, Michael,

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Partner abundance affects costs and benefits in obligate mutualisms, but its role in facultative partnerships is less clear. I investigated this topic in a small alpine pollination web in the Colorado Rocky Mountains consisting of two clovers, *Trifolium dasyphyllum and T. parryi*, that vary in specialization on a shared bumblebee pollinator, *Bombus balteatus*. I examined a) foraging choices of queen *B. balteatus* among the clovers and explanatory mechanisms behind observed foraging biases, b) how intraspecific and interspecific variation in pollination niche breadth impacts individual- to population-level plant responses to manipulated pollinator density, c) habitat-scale relationships between natural bumblebee colony abundance and clover reproductive rates, and d) the broader impacts of this research in the setting of an outreach program using pollinator gardens at a local high school.

Results showed that architectural trait differences between the clovers leading to differences in foraging likely mediate preference of *B. balteatus* for *T. parryi*. Because of bees' preferences and the lack of co-pollinators for much of its blooming season, *T. parryi* benefits more than *T. dasyphyllum* from increases in *B. balteatus* density at the individual plant level, at life stages linking individual success with population growth, at population levels, and across habitats; however benefits even for the specialist, *T. parryi*, are not unlimited. In addressing the broader impacts of science research in a K-12 setting, components of the participant teachers' beliefs about teaching science mediate the implementation and outcome of outreach activities.

CHAPTER 1

INTRODUCTION TO THE STUDY SYSTEM

Jennifer C. Geib

Mutualisms are interspecies interactions, ubiquitous to all ecosystems, which, by definition, benefit their interspecific partners. The goods and services exchanged by partners vary widely. For example, in an aquatic mutualism clown fish exchange food scraps for protection from predators by an anemone. On land, mycorrhizal fungi help plants absorb water and nutrients in exchange for a share of carbon. Through it is clear that these interactions help individuals acquire goods or accomplish tasks they cannot do as well or at all on their own, we now know that actual benefits to each partner are context-dependent, varying in strength and even sign depending on biotic and abiotic factors in the environment (Bronstein 1994a).

Partner abundance has been implicated as one key factor affecting the balance of trade in a variety of mutualisms, from those involving pollinating seed parasites to ant-membracid and ant-plant associations (Cushman and Whitham 1989, Herre 1989, Breton and Addicott 1992, Nefdt and Compton 1996, Morales 2000, Ness et al. 2006, Anderson and Midgley 2007). However, with few exceptions these studies have mainly utilized systems in which partners' life cycles are tightly and obligately linked. The objective of my dissertation has been to move beyond this pair-wise perspective (Stanton 2003) and examine the impacts of partner abundance within a facultative, multi-species mutualism, a system that better represents the complexity of the natural world.

Pollination mutualisms provide a model system in which to address the implications of varying partner abundance, both because of their global importance and because of the breadth and depth of current knowledge about pollination as an interaction. Pollination is a keystone interaction in both native and agricultural ecosystems; over one third of global crop production depends on services provided by pollinators (Klein et al. 2007), and most native plants (60-90%) require or benefit from pollination (Kearns et al. 1998). Declines in populations of native and commercial pollinators (National Research Council 1996, Goulson et al. 2008, Johnson 2010), and the suggested link between declines of linked plant and pollinator species (Biesmeijer et al. 2006), have recently brought this mutualism to the forefront of concern to researchers, governments, and the general public. Though restoration of lost native pollinators may be possible, lack of applicable information has precluded inclusion of pollinators in most land management plans (Corbet 2000). Most importantly, there is little empirical evidence regarding the effects of increased abundance of any one pollinator on diverse plant species within communities. Despite this important gaps in understanding plant-pollinator interactions, pollination is historically well-studied compared to other mutualisms (Bronstein 1994b, Bronstein et al. 2006), especially in obligate species-specific systems. Consequently, there is large body of theoretical work regarding the impacts of partner abundance in obligate pollination mutualisms (Bronstein 2001, Holland et al. 2002), which can be used as a starting point to make predictions about the messier domain of facultative interactions.

My dissertation research addresses the impacts of partner abundance in facultative pollination mutualism within a small alpine pollination web in the Colorado Rockies, consisting of two native clovers, *T. dasyphyllum* and *T. parryi*, that differ in specialization on a shared pollinator, the native bumblebee *Bombus balteatus*. Alpine pollination webs are a convenient system in which to empirically test predictions of the impacts of pollinator abundance, as they contain fewer species than webs in more temperate habitats (Bingham 1998), yet provide a step beyond the pair-wise studies done to date. The field investigations for my dissertation took place in the Front Range of the Colorado Rocky Mountains, primarily on Pennsylvania Mountain (near Fairplay), Park County, CO, during June-August 2005-9.

Though alpine communities contain many interesting genera worthy of study, I selected *Trifolium* for a number of reasons: As nitrogen-fixers, *Trifolium* are ecologically important, particularly in alpine habitats where cold temperatures and slow decomposition make nitrogen a limiting nutrient for plants

(Korner 1999). Resident alpine fauna depend on legumes, including clovers, to meet their nitrogen needs (Clarke and Johnson 2005). *Trifolium* are also important agriculturally world-wide (Kaleem Abbasi and Nasim Khan 2004). The two *Trifolium* species selected for my studies, *T. dasyphyllum* and *T. parryi* (Fabaceae, subfamily Faboideae), are closely related native clovers (co-sectional: Involucarium; Ellison et al. 2006) abundant in the central Rocky Mountains (USA). They are found at the Pennsylvania Mountain study site from treeline upward and flower prolifically from snowmelt in June to late August. Plants of both clovers are perennial and lack clonal spread (Weber and Wittmann 2001). *T. dasyphyllum* is more common in open dry meadow habitats whereas *T. parry*i is more abundant in moist snowbeds. The two species co-occur extensively near treeline.

Both *T. dasyphyllum* and *T. parryi* are obligate outcrossers with gametophytic self-incompatibility systems (Dhar et al. 2006). Prior studies indicate that both species depend mainly on *Bombus* for pollination (Macior 1974, Bauer 1983) but differ in the composition of their bumblebee guild; both clovers are extensively visited by a long-tongued bumblebee, *B. balteatus*, but *T. dasyphyllum* also has a frequent short-tongued co-pollinator, *B. sylvicola*. Both *Bombus* species are abundant from timberline upward at the Pennsylvania Mountain study site (Byron 1980). Queens emerge in mid-June and forage throughout the summer, and workers emerge 2-3 wk after colony establishment in early July. More recent observations of the clover visitor assemblages show that both species also receive non-*Bombus* visitors, including solitary bees and flies, early in the season (June-early July) and increasing in frequency July-August (Appendix 1). However the contribution of non-*Bombus* visitors to pollination is unknown. At the very least, the clovers differ in their specialization on the shared long-tongued bumblebee, *B. balteatus*. This system is therefore characterized by two features of broader pollination networks: Redundancy or substitutability of alternative mutualist partners and potential competition within the pollinator guild.

Given the clovers' differences in specialization on *B. balteatus*, one might ask how benefits to the clovers would be impacted by varying density of this shared pollinator. The chapters of my dissertation address this question from a number of perspectives. Chapter 2 starts with the premise that predicting how pollinator population variations may impact plants requires understanding of pollinator foraging decisions. For bumblebees, and bumblebee-pollinated flowers, a morphological matching of tongue and flower tube length is thought to be one factor mediating species interactions, and resource partitioning among

competitors (Inouye 1978, Pyke 1982). I present experiments testing whether foraging of the long-tongued bumblebee, *B. balteatus*, in mixed clover patches is biased and fits with a long-standing pattern pairing long-tongued bees with long-tubed flowers. I examine two hypotheses that may explain bees' foraging choices based on foraging efficiency: 1) that foraging speed of a long-tongued pollinator on flowers with longer tubes is faster than when foraging on shorter-tubed flowers, thus increasing the nectar gain rate; and 2) that differences in clover traits relevant to supporting a heavy foraging bee impacts the biomechanics of bees' foraging, and consequently the energy expended to collect nectar.

Chapter 3 describes experimental tests of predictions about the impacts of partner abundance from ecological theory: If partners are a primary limiting factor, increasing abundance of one partner will increase benefits to the other, with the potential for a corresponding increase in population size. However, in most cases extrinsic factors will eventually limit this response causing benefits to saturate. Depending on the ratio of gross benefits to costs, benefits may even decrease at high levels of partners, resulting in maximum benefit at an intermediate partner density (Bronstein 2001, Holland 2002). However, these predictions are based on a pair-wise, species-specific perspective, when most mutualisms involve guilds of species competing for partners on the other side (Stanton 2003). To examine the functional response of one partner to varying density of the other in a more generalized system, I experimentally manipulated *B. balteatus* density for mixed patches of *T. dasyphyllum* and *T. parryi*. I traced the pathway of individual-level benefits from increasing partner density, including visitation rates, pollination services, seed set. I also look at the effects of pollinator density at life stages linking individual and population levels, including germination and dormancy. To explore whether impacts of bumblebee density on individual performance carry over to population dynamics, I developed demographic models for the clovers. I discuss the patterns of benefits observed at each life stage, as well as costs, and explore potential explanatory mechanisms.

In 2006, a study was published that demonstrated landscape-level correlations between declines of linked plant and pollinator species in Britain and the Netherlands (Biesmeijer et al. 2006). Chapter 3 tests the causal mechanisms operating within populations that may be driving these patterns. However, it is still unclear at what other scales these impacts can be seen. Chapter 4, therefore, changes the resolution of observation, using molecular methods to assess the number of natural bumblebee colonies servicing different habitats across a landscape. I then examine colony abundance relationships with reproductive

rates of bumblebee-dependent plants. In addition, I discuss whether bumblebee foraging ranges or natural landscape features may contribute to patterns observed.

The research activities in Chapters 2-4 aim to contribute intellectually to the scientific understanding of mutualisms, yet there have also been recent calls for scientists to better consider the broader implications of their research for society (Frodeman and Holbrook 2007). Chapter 5 explores the broader impacts of my dissertation research within the realm of K-12 science teacher education. I present a case study describing a pollinator gardens outreach program, conducted with secondary students, designed to model effective incorporation of investigative research into K-12 classrooms. Using naturalistic qualitative research traditions (Denzin and Lincoln 2005), I explore how the collaborating teacher's knowledge of the guiding frameworks and purpose for teaching science (teacher "orientations"; Abell 2007) may have played a role in the implementation and outcome of the pollinator outreach program.

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CHAPTER 2

WHY SIZE MATTERS: A NEW HYPOTHESIS TO EXPLAIN SPECIES ASSOCIATIONS IN POLLINATION MUTUALISMS

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ABSTRACT

Though mechanisms underlying pollinator foraging choices have received prolific theoretical and empirical consideration, a general explanation for long-standing species association patterns, such as that between long-tongued pollinators and long-tubed flowers, is still lacking. Selection for flower handling efficiency is thought to give rise to this association, despite the fact that proboscis and corolla tube length often covary with other determinants of foraging success. In this paper I revisit the tongue-tube length association using a long-tongued bumblebee, *Bombus balteatus*, and two co-occurring clovers *Trifolium dasyphyllum* and *T. parryi,* which differ in corolla tube length. I tested whether differences in handling time or handling effort between species give rise to bees' choice of the longer-tubed clover and addressed whether correlated traits influence foraging energetics by altering support for perched bees under windy alpine conditions.

Flower handling time did not contribute to foraging efficiency; however, differences in clover scape and floret pedicel strength impacted the mechanics of foraging giving rise to differences in handling effort. Results suggest that in exposed habitats biomechanical aspects of fit may have important consequences for foraging energetics, driving floral preferences of long-tongued pollinators.

Key words: pollinator, optimal foraging, foraging biomechanics, handling energy, foraging efficiency, *Bombus balteatus, Trifolium dasyphyllum, Trifolium parryi*

INTRODUCTION

For outcrossing plants, sexual production requires gene exchange with other conspecific members of the population. Because plants are root-anchored to one location over the time scale that pollinators are present, it is the pollinators whose choices determine which plants mate and produce offspring. Pollinator foraging decisions have received much empirical and theoretical treatment, and non-random choices among available plant species are well documented. Bumblebees, for example, though generalist pollinators as a whole, individually "major and minor" on particular species, often changing their preferences over a season (floral visitation frequency, Heinrich 1979). Floral species fidelity during inflorescence-to-inflorescence transitions within foraging bouts is also a ubiquitously observed phenomenon among pollinator taxa (Free 1963, Heinrich et al. 1977, Lewis 1989, Goulson and Cory 1993). The individual roles that floral traits such as color, shape, display, odor, and reward play in triggering such foraging biases have been explored (Chittka et al. 1999, Hill et al. 2001, Raguso 2004, Ishii 2006), and studies have also shown that increasing numbers of trait differences among available flower choices lead to increased non-random foraging (Gegear and Laverty 2001).

Pollinators with a long "feeding apparatus" (proboscis or bill) tend to forage mainly on long-tubed flowers. Though not without exception, this pattern has been repeatedly documented over the years in taxa as diverse as bumblebees, moths, flies, and hummingbirds (Brian 1957, Inouye 1978, Ranta and Lundberg 1980, Pyke 1982, Snow and Teixeira 1982, Fussell and Corbet 1992, Anderson et al. 2009). The relationship between feeding apparatus and corolla length seems non-intuitive, given that long-tongued (or long-billed) pollinators can just as easily reach nectar from short-tubed flowers. What potential mechanisms could explain this correlation? In general, non-random foraging among floral species is thought to be an adaptive strategy promoting foraging efficiency (Bateman 1951).

For pollinators, the ultimate currency of foraging efficiency is the energy needed to sustain daily activity and contribute to reproduction of self or the colony (Heinrich 1975). When pollinators forage on

flowers that are compiled into inflorescences, relative energetic efficiency among floral species depends on

the potential net energy gain at the inflorescence level ($E_{net.}$):

$$E_{net.} = [(E_{sugar} - E_{handling})\,(\text{flowers/inflorescence})] - E_{transition} \qquad (1)$$

E_{sugar} is energy available from the per-flower sugar content, or standing crop, $E_{handling}$ is the per-flower

energy expended during handling flowers and extracting nectar, and $E_{transition}$ is the energy expended

during forward flight while searching for and transitioning to each inflorescence. Each of these terms is

also a function of others: $E_{transition}$ depends on the inflorescence abundance in a given area, the distance

between inflorescences, and their apparancy to the pollinator. $E_{handling}$ is a function of both the floral

handling time (time spent manipulating the flower and extracting nectar, as well as interfloral travel time

within an inflorescence; Inouye 1980), and the *metabolic cost* of handling (Corbet 2000, Rodriguez-

Girones and Santamaria 2006).

Numerous empirical trials have shown that matching between the length of the proboscis or bill

and flower tube increases flower handling speed (Inouye 1980, Temeles 1996). This should increase these

pollinators' bias for long-corolla species because, all else being equal, it increases the net energy gain from

nectar (Equation 1). However, genetic analyses of floral display traits suggest that corolla length is

correlated with many other traits that could influence foraging efficacy (Conner and Sterling 1995, Galen

and Cuba 2001). Here I consider how differences in plant physical traits correlated with corolla length

could affect pollinators' metabolic cost while foraging from short vs. long tubed flowers, prompting them

to make choices that also give rise to widely observed differences in foraging speed.

Metabolic costs of foraging are a function of the biomechanics involved during foraging activities,

the forces acting upon and enacted by the pollinator as well as its motion (Giancoli 2000). For example,

hovering, common during foraging among hummingbirds, bats, and insects (Chai and Millard 1997,

Willmott and Ellington 1997, Voigt and Winter 1999), is the costliest locomotion an organism engages in

because the power required to maintain lift and overcome the force of gravity acting on its body mass is

generated entirely by flapping or oscillating the wings (Weis-Fogh 1977, Norberg 1990). However, on

inflorescences composed of closely spaced flowers, pollinators, rather than hovering, often "perch" during

nectar extraction and walk between flowers to avoid the high energy cost of engaging their flight muscles (Heinrich 1975, Corbet 2000). To date, no study has examined in detail the mechanics of perched nectar foraging from the pollinator's perspective, which has implications for metabolic expenditure and the energetic efficiency of the interaction (Equation 1).

Perching is an interaction between the pollinator and an architectural structure, here, a plant. Thus, the biomechanics of perching are likely to depend on particular characteristics of inflorescences and flowers. There are a number of floral "interaction" traits that could generate differences in the biomechanics of perch-foraging. For example, at least two studies have demonstrated that some bee-pollinated plant species require a minimum force for flower entry (Brantjes 1981, Reith et al. 2006), limiting access to only relatively powerful species. For relatively heavy foragers, plant species may differ in the capacity of flower and inflorescence architecture to support the pollinator's body weight, generating differences in the forces acting on and enacted by the pollinator. From the pollinator's perspective such forces could be considered components of handling "effort". Here I examine the potential for biomechanics of foraging rather than variation in attractants or rewards to drive pollinator choices among floral species.

I chose to investigate the potential role of biomechanics in shaping long-tongued pollinator foraging choices in a system where floral trait differences between plant species are very few: a small alpine pollination web consisting of two clovers, *Trifolium dasyphyllum* and *T. parryi* (Fabaceae, subfamily Faboideae) and a shared pollinator, the long-tongued bumblebee *Bombus balteatus*. Inflorescences of these clovers share the same structure, complexity, and available reward, but differ in corolla length (Table 1). I examine foraging of queen *B. balteatus* in mixed species arrays of the two clovers to test whether flower choices are biased toward long corollas and to elucidate possible mechanisms explaining such bias. In *T. dasyphyllum* and *T. parryi* inflorescences, individual flowers are closely aggregated, being borne on ovoid heads at the top of a scape. Densely clustered inflorescences are common among insect-pollinated plants of many families (e.g. Fabaceae, Asteraceae), and often instigate perch foraging. Thus clovers make a good general case in which to examine the biomechanics of perched foragers on a common inflorescence type.

I examined two floral traits mediating support of perching pollinators, floret pedicel strength and the width-to-length ratio of the inflorescence scape (a proxy for scape strength). If scape and pedicel

strength differ between floral species, there are a number of predictions that can be made related to foraging mechanics: For example, perching on an architecturally stronger inflorescence likely increases time elapsed when weight (the force of gravity acting on mass) is fully supported by the normal force of the inflorescence (the force equal but opposite to the pollinator's weight; Fig 1A). Conversely, weaker inflorescences increase time spent hanging. A hanging pollinator has to exert greater effort; it experiences a tension force equal to its own weight divided over the number of legs it's using to grip the flower, and must lift its own body mass against the force of gravity to move between flowers. Unless it has hooked feet, it must also grip tightly enough that the friction between its feet and the petals is greater than its weight to keep from falling off (Fig. 1B). Perching on stronger inflorescences also likely reduces lateral motion. Weaker inflorescences or flowers comprising them are more likely to bend or "flop over" under the pollinator's weight. Floppiness is important because a pollinator riding on a moving object has velocity and linear momentum in the direction of movement. If that object is pivoting around a point, the pollinator's velocity and linear momentum are tangent to the rotation (Giancoli 2000). A pollinator experiences further tension in its legs, and exerts even greater effort to hold on and move between flowers as the scape and/or flower flops around and changes direction (Fig. 1C, 1D). Lastly, and specifically to bees, if a perching pollinator both lacks support and experiences greater motion while on a weaker inflorescence, it might be forced to buzz its wings to maintain its position, i.e. transition to hovering. Hovering requires the most effort and is the costliest of foraging activities.

Here I experimentally evaluate whether differences in inflorescence scape and flower pedicel strength impact pollinator handling effort by changing the forces and motion of pollinator foraging giving rise to nonrandom foraging in *B. balteatus*. The specific questions addressed in this study were:

1. While foraging on mixed clover patches, do long-tongued *B. balteatus* prefer flowers with longer corolla tubes?

2. Which hypothesized mechanism behind *B. balteatus* foraging choices provides a better fit to the behavioral data:

    a. Flower handling speed?

    b. Flower handling effort, relating to differences in foraging mechanics arising from flower and inflorescence strength and floppiness?

METHODS

Study Site

Studies were conducted in Park County, Colorado, on Pennsylvania Mountain except as noted. The plant species studied, *T. dasyphyllum* and *T. parryi,* are closely related (congeneric, co-sectional; Ellison et al. 2006), long-lived alpine perennials that flower prolifically from treeline upward. *T. dasyphyllum* and *T. parryi* have both sympatric and allopatric populations. *T. parryi* primarily occurs in moist meadow and snow bed habitats while *T. dasyphyllum* primarily occupies dry open areas; sympatric zones occur at these habit boundaries. In these sympatric zones, *T. dasyphyllum* inflorescence density is higher compared to *T. parryi* (86% vs. 14%, respectively, Table 1), due to differences in growth form; *T. dasyphyllum* plants are cushion-like and produce, on average, 8.1 heads each, whereas *T. parryi* plants produces 1-2 heads (Table 1). Inflorescences of both species have similar structure and complexity, average number of flowers, and nectar standing crop (Table 1). In addition, floral advertisements to pollinators are very similar; neither has a UV reflection to guide bees in to the "landing pad" (P. Kevan, personal communication). However corolla tubes of *T. parryi* florets average 1.4 times longer than those of *T. dasyphyllum* (Table 1). Preliminary investigations also suggest that floral scent composition differs between the clovers (R. Raguso, unpublished data).

From mid-June to mid-July queen bumblebees, primarily *Bombus balteatus* and *B. sylvicola,* are the dominant pollinators of both clovers, with increasing visitation by worker bees and smaller co-pollinators from mid-July onward (Appendix 1). *B. balteatus* queens have the longest tongues of all these visitors (Macior 1974, Pleasants 1980, Bauer 1983), and are by far the largest, averaging 1.4 times longer and two times heavier than the next largest clover visitor, *B. sylvicola* queens (Body length = 20.2 mm, 15.98 mm; Mass = 0.525 g, 0.339 g for *B. balteatus* and *B. sylvicola,* respectively; Appendix 2).

B. balteatus foraging choices

To test for foraging biases of the long-tongued pollinator *B. balteatus* among *T. dasyphyllum* and *T. parryi*, in June-August, 2007, I documented bee foraging in two situations: Queens caught and allowed to forage on mixed cover patches in mesh enclosures, and queens foraging naturally on mixed clover

patches in the field. All observations were conducted on a south-facing ridge near treeline (elevation 3600 m) on Pennsylvania Mountain.

Enclosures: I caught queen *B. balteatus* (N=47 bees) within 1 km of the aforementioned ridge and allowed them to forage individually on naturally occurring mixed patches of the two clover species within a 2 m x 2 m mesh tent (Cerf Brothers Bag Co., USA). Experimental patches contained five plants each of *T. dasyphyllum* and *T. parryi*. Natural floral display of each experimental plant was maintained, but florets were removed from any extra plants (always *T. dasyphyllum*). Bees were brought to patches in vials on ice and allowed to forage individually within the mesh tent. Each bee was placed on a "starter" consisting of one virgin inflorescence of each clover species held in a waterpik. The starter was removed as soon as the bee moved to other inflorescences in the patch. Each bee foraged for one bout during which species choices were recorded with a digital voice recorder. The bout ended when a bee left the patch by flying to the tent wall. No bout lasted longer than 30 minutes. Observations were conducted from 9 am-5 pm during at least partly sunny weather.   Bees were marked before release with a non-toxic paint pen (Testors Corporation) to prevent reuse and released at the site of capture the same day.

Field: To test whether bees' foraged similarly in tents vs. natural populations queen *B. balteatus* (N=9) were observed when visiting mixed clover patches in the field. To track each bee's path I dropped numbered poker chips next to each visited inflorescence and concurrently recorded the bee's choices on a digital voice recorder. Each bout ended when the bee left the patch on a long-distance flight.

I analyzed all foraging bouts from enclosures and the field for two measures of foraging bias: 1) Preference: defined as a significant deviation between observed floral visitation frequency to each clover species and that predicted from their relative abundance (Slaa and Biesmeijer 2005), and 2) fidelity: recognized as a deviation in frequency of species-constant vs. species-switching transitions between inflorescences from predictions based on relative inflorescence abundance of *T. dasyphyllum* and *T. parryi* (Bateman 1951, Slaa and Biesmeijer 2005).

Clover architectural traits

To assess interspecific variation in architectural traits related to pollinator foraging effort, I surveyed populations at two sites during June-July, 2008: The aforementioned sympatric zone on the south-

14

facing ridge on Pennsylvania Mountain and a second site at Kite Lake near Alma (Park Co.), Colorado,

3666 m asl. At Kite Lake clover species are allopatric, but less than 500 m apart. At both sites I ran a 50 m

transect through each clover population and randomly selected 20 plants having at least one head with ≥ 10

florets. For plants displaying more than one flowering head, I haphazardly selected among those with ≥ 10

florets to standardize trait comparisons.

On each inflorescence I measured scape length between the ground and the base of the head and

scape width at the midpoint of the scape length. I also measured the force required to pull the most upright

floret perpendicular to the scape (floret "pedicel strength"). To measure pedicel strength I looped a small

wire of known mass over the tip of the floret and added small washers and beads until the floret was

perpendicular to the scape. To standardize the pedicel tests all scapes were held erect at 90^0 from level.

Washers and beads were collected and weighed using an Acculab V-200 electronic balance (Acculab,

Newtown, PA). Pedicel strength was calculated in Newtons (N): total mass (kg) x 9.8 m/s^2, the

acceleration due to gravity.

Flower handling and foraging mechanics

In June-August 2008 I conducted an experiment to test whether 1) differences in scape and pedicel

strength between *T. dasyphyllum* and *T. parryi* impact the biomechanics and handling time of bees foraging

for nectar and 2) strengthening the scape and/or pedicel of the weaker clover eliminates differences in bee

foraging effort between species. It was not possible to weaken the stronger clover's scape and pedicels

without damaging the flowers or inflorescences. Captured queens of *B. balteatus* (N=10) were allowed to

forage on 1 m^2 mixed patches of clover. The patches contained at least four *T. dasyphyllum* plants with four

inflorescences each and at least four *T. parryi* inflorescences. All but four inflorescences were removed

from each *T. dasyphyllum*, and inflorescences were also removed from any extra *T. dasyphyllum* plants.

The four inflorescences on each *T. dasyphyllum* plant were randomly assigned among unmanipulated (TD);

stem support (TDS), consisting of a cut straw slit lengthwise and placed around the scape to prevent

bending, pedicel support (TDP), achieved by wrapping a narrow strip of tape around the pedicels of the

outer ring of florets, and stem and pedicel support (TDSP), subject to both treatments. *T. parryi*

inflorescences (TP) were left unmanipulated as a second control.

I placed a 2 m x 2 m mesh tent over the patch then released a bee in the center where it was allowed to forage on a starter as previously described. The starter was removed when the bee moved to inflorescences in the patch. As before, each bee was allowed to forage for one "bout". I videotaped all foraging bouts for later analysis. For each bee I calculated the following measures by treatment: 1) mean handling time/flower, 2) mean proportion of foraging time spent fully supported by the inflorescence, 3) maximum stem deviation from the starting point (Fig. 1C), 4) maximum pedicel deviation from the starting point (Fig. 1D), and 5) hover-foraging incidents. "Fully supported" was defined as having the points of leg attachment to the bee's body and the bees' center of mass within the boundary of a clover inflorescence (Fig. 1A). "Hover-foraging" was defined as engaging flight muscles during foraging.

STATISTICAL ANALYSES

B. balteatus foraging choices

Expectations for the null hypothesis of random foraging were based on relative inflorescence abundances for the clover species, averaging 86% *T. dasyphyllum*: 14% *T. parryi* (Table 1). For transitions between successive inflorescences within a foraging bout (species fidelity/switching), relative expected proportions were as follows: 0.74 for *T. dasyphyllum* to *T. dasyphyllum* transitions (equal to the probability of choosing two *T. dasyphyllum* inflorescences in a row; $.86^2$), 0.12 for *T. dasyphyllum* to *T. parryi* (.86 x.14), 0.02 for *T. parryi* to *T. parryi* ($.14^2$), 0.12 for *T. parryi* to *T. dasyphyllum* (.14 x .86). In each instance, I fit parametric distributions (PROC UNIVARIATE, SAS 9.2; Curtis 1999), and used Student's t test to determine whether the observed distribution mean differed from the expected value. All proportions were arcsine-square-root transformed to improve normality before analysis.

Clover architectural traits

Comparisons of clover scape width-to-length ratio and floret pedicel strength were conducted using ANOVA (Proc GLM: SAS 9.2) with site, species, and site by species interaction as fixed effects. Stem width-to-length ratio was log transformed to improve normality and equalize variances.

Flower handling and foraging mechanics

Analyses of differences in handling time per flower, proportion of time supported during foraging, stem deviation, and pedicel deviation among treatment were conducted using ANOVA with treatment as a fixed effect and bee as a random effect. Planned pair-wise comparisons among treatments were conducted using Least Squares Means. An exact multinomial test for goodness of fit (McDonald 2009) was used to assess whether or not hover-foraging incidents were randomly distributed among treatments (Proc FREQ: SAS).

RESULTS

B. balteatus foraging choices

Foraging of queen *B. balteatus* in mixed clover patches was biased toward *T. parryi*. Bees foraging in mesh enclosures and in the open field visited *T. parryi* inflorescences more often than expected based on their relative abundance (t_{46}=2.31, P=.026 and t_8=17.58, P<.0001 for tent and field trials, respectively; Fig. 2A and B). In addition, bees were more constant than expected on *T. parryi*; *T. parryi* to *T. parryi* movements made up a greater proportion of transitions than expected both in the tents and in the open field (t_{46}=4.14, P=.0001 and t_8=12.63, P<.0001, respectively; Fig. 2C and D).

Clover architectural traits

T. parryi has significantly greater structural strength than *T. dasyphyllum* (Fig 3; Table 2). Scape width-to-length ratio was significantly greater for *T. parryi* than *T. dasyphyllum* (SPECIES $F_{1,76}$= 5.15, P=.026). In addition, flower pedicels were significantly stronger in plants of *T. parryi* than those of *T. dasyphyllum* (SPECIES $F_{1,76}$= 522.4, P<.0001), requiring four times more force to pull perpendicular to the scape. Site effects on species differences in scape and pedicel strength were non significant (SITE*SPECIES for scape strength and pedicel strength, respectively: $F_{1,76}$=0,68, P=.413 and $F_{1,76}$=1.97, P=.165).

Flower handling and foraging mechanics

Variation in *B. balteatus* handling time per flower visit was lacking among any experimental groups (TREATMENT $F_{4, 242}$=.32, P>.80; Fig. 4, Table 3). Conversely, foraging biomechanics varied among groups in ways that should impact handing energy expenditure (proportion time supported: TREATMENT $F_{4,251}$=12.73, P<.0001; scape deviation: TREATMENT $F_{4,260}$=57.22, P<.0001; pedicel deviation: TREATMENT $F_{4,244}$=31.02, P<.0001; Table 3). Specifically, bees spent a significantly greater proportion of time in the energetically efficient state of full support while foraging on *T. parryi* inflorescences compared to *T. dasyphyllum* (planned comparison P<.0001; Figure 5A). Strengthening *T. dasyphyllum* pedicels or both stems and pedicels increased the proportion of time that bees were fully supported to levels observed for bees foraging on *T. parryi* (planned comparisons TP vs. TDP and TDSP P>.10).

Moreover, inflorescences of *T. parryi* were significantly less "floppy" than those of *T. dasyphyllum* (planned comparison TD vs. TP for both stem and pedicel deviation P<.0001; Fig. 5B and C), imparting little momentum to bees during foraging. Strengthening *T. dasyphyllum* scapes reduced scape deviation and strengthening pedicels eliminated the interspecific difference in pedicel deviation (a priori comparisons: Scape deviation TP vs. TDS, P=.12; pedicel deviation TP vs. TDP, P=.89; Fig. 5B and C), reducing bees' linear momentum and tension forces in the legs in ways that should impact energy spent foraging on the flowers.

Energetically costly hover-foraging incidents were not randomly distributed among treatments (Exact test P=.008); nine out of ten occurred for bees foraging on unmanipulated *T. dasyphyllum* inflorescences (Fig. 5D).

DISCUSSION

Foraging biases of the long-tongued alpine bumblebee, *Bombus balteatus*, on mixed patches of alpine clovers follow the widely observed pattern pairing a long proboscis with long corolla tube length. This pattern held under multiple foraging scenarios. However, the traditional mechanism predicted to underlie the association, handling time efficiency, did not contribute to bees' bias toward long-tubed flowers. Instead, two traits correlated with tube length (clover scape and pedicel strength) impacted

support for perching bees and in turn the mechanics of bee foraging. Thus in this scenario pollinator weight and floral strength matching have greater implications for foraging efficiency and likely play a greater role in driving the species association than proboscis and corolla tube length.

Predicted and observed foraging patterns of B. balteatus

A pollinator foraging at random should visit available floral species in ratios predicted by their abundance (Levin and Anderson 1970). Optimal foraging theory however, suggests that pollinators should be "choosy" and more often pick resources that provide the greatest net reward (Macarthur and Pianka 1966). If pollinators vary in which floral resources are optimal, this allows for resource partitioning based on the mediating trait or traits (Rodriguez-Girones and Santamaria 2006). In many cases plant-pollinator associations within habitats are nonrandom, with length of the feeding apparatus matched to that of the corolla tube. At first glance, results here also conform to this scenario. Floral visitation frequencies and inflorescence transition frequencies demonstrated that queens of the long-tongued bumblebee *B. balteatus* were strongly biased toward the less abundant but longer-tubed clover, *T. parryi*.

The role of floral traits in mediating foraging choices

To optimize energetic efficiency pollinators should forage in ways that maximize the ratio of energy gain to energy spent searching for and handling flowers (Equation 1). Because environments are complex, animals can rarely make truly optimal choices (McNamara and Houston 2009). Never-the-less, foraging is costly, and thus pollinators still need to maintain an overall positive energy balance (Heinrich 1975). Any floral species on which foraging consistently decreases the cost-benefit ratio should be less favored. Matching between flower tubes and the feeding apparatus of the pollinator increases floral handling speed in a variety of systems (Holm 1966, Benedek 1973, Inouye 1980, Temeles 1996, Plowright and Plowright 1997), and consequently should increase the rate of nectar gain (Equation 1). That pollinator preference often aligns with flowers minimizing handling time and that these flowers have matched corolla-to-proboscis length suggests that such aspects of morphological fit contribute to pollinator choice. However, results here suggest that when long-tongued pollinators are relatively heavy and when plants differ in floral traits that impact perching effort, traits underlying mechanical support may be as or more important to energetic efficiency. Foraging choices should depend on which suite of traits creates the

19

greatest handling energy differential between the floral species, and whether time or effort is most closely associated with energy gain for the pollinator. Of course, these mechanisms are not mutually exclusive and likely act synergistically to promote nonrandom foraging in nectivores.

Others have suggested that the tongue-corolla tube matching may be an overemphasized "niche dimension" (Goulson 2003) in understanding plant-pollinator associations. Factors influencing pollinator biases could be confounded if, as in the current study, other traits are correlated with pollinator proboscis length (e.g., Harder 1985, Inoue and Yokoyama 2006). By testing the handling time hypothesis for tongue-tube matching, as well as the impact of flower and inflorescence resistance to pollinators' weight on foraging biomechanics (the forces and motion of the interaction), I distinguished between these potential confounding influences on pollinators' foraging biases.

Structural resistance of flowers, particularly, is known to be important in determining other plant-pollinator associations, especially as a mechanism for screening plant visitors, by requiring them to be either strong enough to push open a resistant flower (Brantjes 1981, Reith et al. 2006) or heavy enough to deform a strong keel (Lovell 1919). Though presented from the plant's point of view, such results imply that pollinators are faced with choices requiring differential exertion to handle flowers and access nectar.

Updated models of optimal foraging (Rodriguez-Girones and Santamaria 2006) predict that, where handling time is equivalent between two floral species, differential metabolic costs to the pollinator should drive species association patterns. Results here support this prediction, and suggest that biomechanics is the missing link in this and many past discussions of how plant-pollinator traits impact the energetics of foraging. As with all phenomena in the physical universe, the laws of physics govern biological interactions. Here I show how forces and motion during perching should affect a pollinator's foraging effort, and thus its metabolic costs. Respiration rates while hovering vs. resting have been estimated for many pollinators (Bartholomew and Casey 1978, Heinrich 1979, Withers 1981, Silvola 1984, Bartholomew and Lighton 1986, Voigt and Winter 1999). For hovering bumblebees, oxygen consumption rates vary from five to twenty-five times that at rest depending on ambient temperature and species (Heinrich 1979, Silvola 1984). The energy differential between hovering and resting is likely even greater for bees foraging at high elevation where air density is lower and requires greater power generation to produce enough uplift to offset body weight (Dudley 2000, Dillon et al. 2006). Pollinator metabolism during more sedentary

activities (e.g. walking) is fairly similar to that at rest especially when compared to the cost of engaging the flight muscles (Heinrich 1975), suggesting that foraging with weight fully supported is probably not highly taxing for a pollinator. As bees in this study spent significantly less time fully supported while foraging on *T. dasyphyllum*, and nine out of ten bees had at least one hovering incident while foraging on *T. dasyphyllum*, metabolic costs while foraging are likely significantly greater on *T. dasyphyllum*. Less certain is whether observed energy differentials such as these are perceivable to pollinators.

By conducting foraging trials in tents my results may have actually under-estimated the effect of scape and pedicel strength on foraging mechanics and energetics. Though air density is lower, alpine habitats are never-the-less characterized by strong gusts of wind, and scape architecture is known to mediate wind effects on inflorescences (Etnier and Vogel 2000). The open windy conditions of the natural clover environments should exacerbate scape motion, especially for plants with "weaker" stems, further increasing the likelihood of pollinator foraging bias towards a more motion resistant species.

This study demonstrates one way that forces and motion in plant-pollinator interactions may influence foraging choices, but there are likely others. Even in systems where tongue-tube length matching does increase floral handling speed, biomechanics may be the mediating factor. For example, Kugler (1940, in Inouye 1980 and Goulson 2003) observed that long tongue bees foraging on a drop of sugar water placed on a flat surface (akin to a short open corolla) had great "difficulty" potentially because the long tongue is "unwieldy" in those situations. If we think of the tongue as a lever arm (Giancoli 2000), a small movement of the bee's head should move a long tongue a greater distance than a short tongue, making it more difficult to accurately position the point of the glossa on the sugary drop. Inserting a long tongue into a long tubed flower may stabilize the bees head, reducing motion, and making it easier to place its tongue directly into the nectar. Borrell and Krenn (2006) have also considered how varying proboscid architecture among pollinator taxa may affect the mechanics and fluid dynamics of nectar foraging.

Examining species traits and their implications for ecological interactions from a biomechanical perspective should provide fruitful areas of new research. Here I have considered the physics involved in a biological interaction, an area of research that has recently produced new insights in a number of ecological areas (Herrel et al. 2006). In this study, biomechanics links plant architecture to energetics, behavioral ecology, and species associations in a community. More research is needed to understand the broader

implications of biomechanics for specific interacting partners and to identify environments like the high

alpine tundra, where mechanics may figure strongly in foraging choices.

ACKNOWLEDGMENTS

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Table 1. Comparison of floral traits in sympatric alpine clovers, *T. dasyphyllum and T. parryi.* Species means are given with standard errors in parentheses.

Trait	*T. dasyphyllum*	*T. parryi*	
Relative inflorescence abundance per patch	0.86 (.02)	0.14 (.02)	***
Inflorescences per plant	8.01 (1.20)	1.25 (.099)	***
Florets per inflorescence	17.2 (.405)	16.9 (.371)	
Nectar standing crop (ug sugar/ml)	79.7 (13.2)	77.0 (17.6)	
Corolla length (mm)	15.5 (.161)	21.6 (.280)	***

*** indicates significant differences at p<.0001

Source: Appendix 1

Table 2. ANOVAs testing the effect of clover species and site on clover architectural traits: (A) stem width:length ratio and (B) pedicel strength.

Source	DF	SS	F	Prob > F
(A) Stem width:length ratio				
species	1	0.417	5.15	0.0261
site	1	0.187	2.3	0.1332
species*site	1	0.055	0.678	0.4130
error	76	6.818		
(B) Pedicel strength				
species	1	32.1	522.4	<.0001
site	1	1.01	16.4	0.0001
species*site	1	0.121	1.97	0.1646
error	76	37.860		

Table 3. ANOVA showing the effect of inflorescence treatment on *B. balteatus* floret handling time, biomechanics, and clover scape movement.

Source	DF	F	Prob > F
Handling time per floret			
inflorescence treatment	4	0.32	0.8654
error	242		
Proportion of time fully supported			
inflorescence treatment	4	12.73	<.0001
error	251		
Maximum stem deviation			
inflorescence treatment	4	57.22	<.0001
error	260		
Maximum pedicel deviation			
inflorescence treatment	4	31.02	<.0001
error	244		

FIGURE LEGENDS

Figure 1. Mechanics of perching for bumblebees on clover inflorescences. A) Bee fully supported by the normal force of the inflorescence. B) Unsupported, hanging bee. C) Scape deviation. D) Pedicel deviation. Arrows show direction of vector quantities. Circle near middle of bee body represents the center of mass. w = weight, F_N = normal force, T = tension, mv = momentum, θ = angle of maximum deviation from starting point.

Figure 2. *B. balteatus* foraging choices among mixed patches of alpine clover. Mean inflorescence visitation frequencies to *T. dasyphyllum* and *T. parryi* for queen bees (A) foraging within mesh enclosures and (B) foraging naturally in the field. Mean frequencies of transitions between inflorescence types (C) within mesh enclosures and (D) in the field. Error bars represent 95% confidence intervals. Dashed lines show expectations with random foraging. * p<.05; *** p<.0005.

Figure 3. Traits of *T. parryi and T. dasyphyllum* related to support for perching bees. Scape width-to-length ratio plotted against floret pedicel strength (in Newtons). Open diamond is *T. dasyphyllum*, filled diamond is *T. parryi*. * p<.05, *** p<.0005.

Figure 4. *B. balteatus* handling time per clover floret. TDS, TDP, and TDSP (textured bars) are experimentally manipulated *T. dasyphyllum* inflorescences with stem support, pedicel support, and stem + pedicel support, respectively. TD (white bar) and TP (black bar) are unmanipulated *T. dasyphyllum* and *T. parryi* control inflorescences, respectively. Line above bars indicates treatments that did not differ significantly (p≥.05).

Figure 5. Biomechanics of queen *B. balteatus* foraging on alpine clover. A) Proportion of foraging time spent fully supported by clover inflorescences. B) Maximum deviation, on average, of scapes during bee foraging. C) Maximum deviation, on average, of floret pedicels during bee foraging. D) Hover-foraging incident frequencies across treatments. See Fig 4 legend for treatment designations. A through C: Lower case letters indicate treatments that differ significantly at p<.05. D: Dashed line represents expectations under the null hypothesis of equal frequency of hover-foraging incidents across treatment groups.

Figure 1.
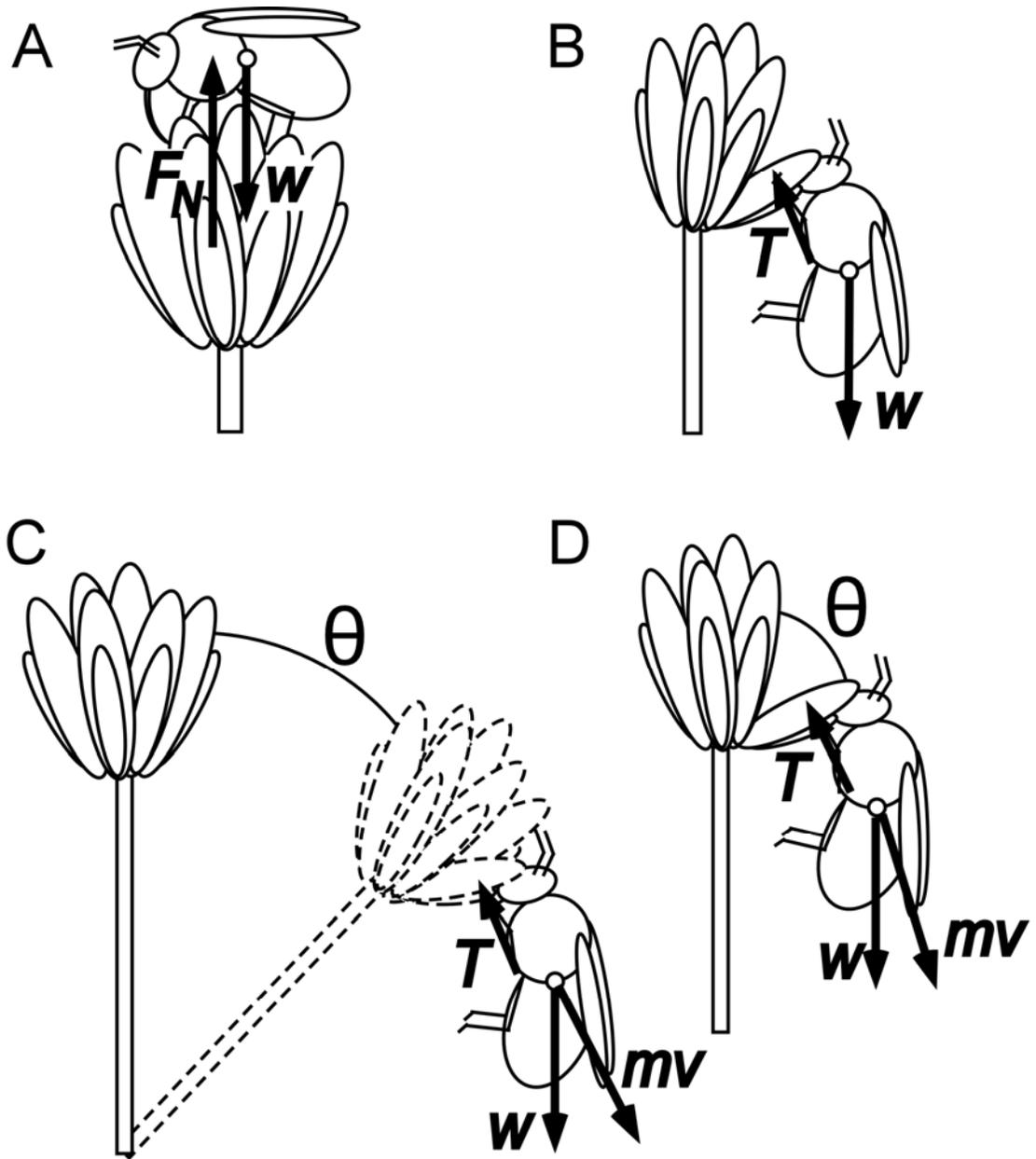
Figure 2. *B. balteatus* foraging choices among mixed patches of alpine clover.
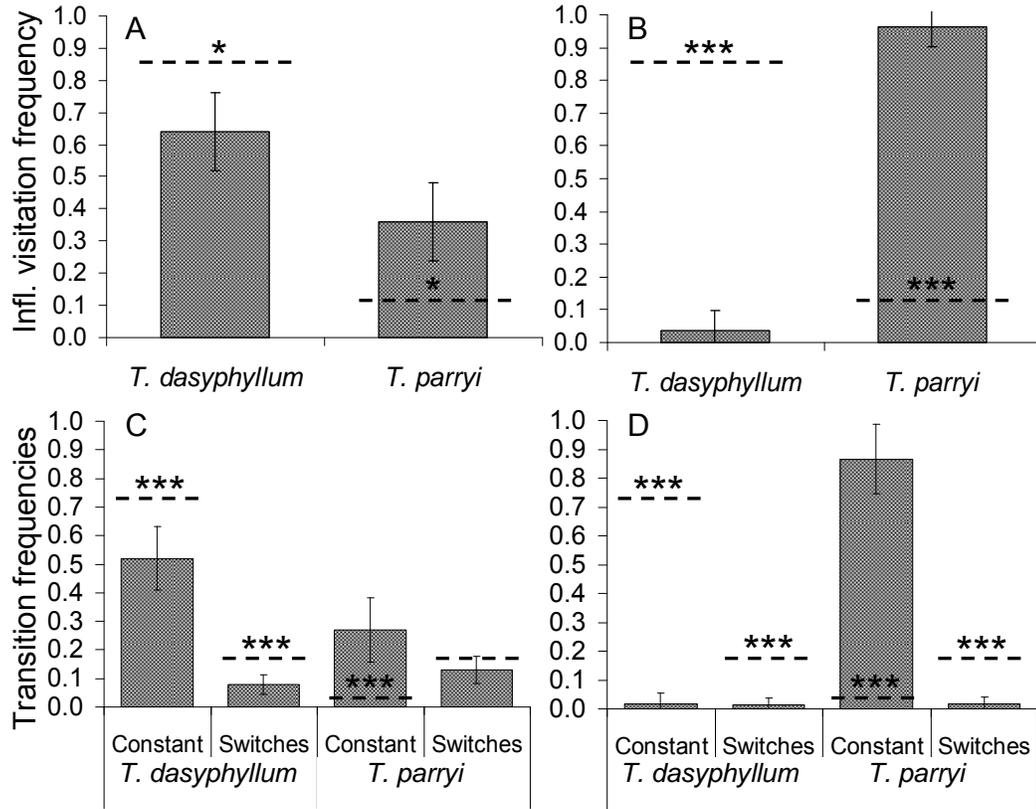
Figure 3. Traits of *T. parryi* and *T. dasyphyllum* related to supporting foraging bees.
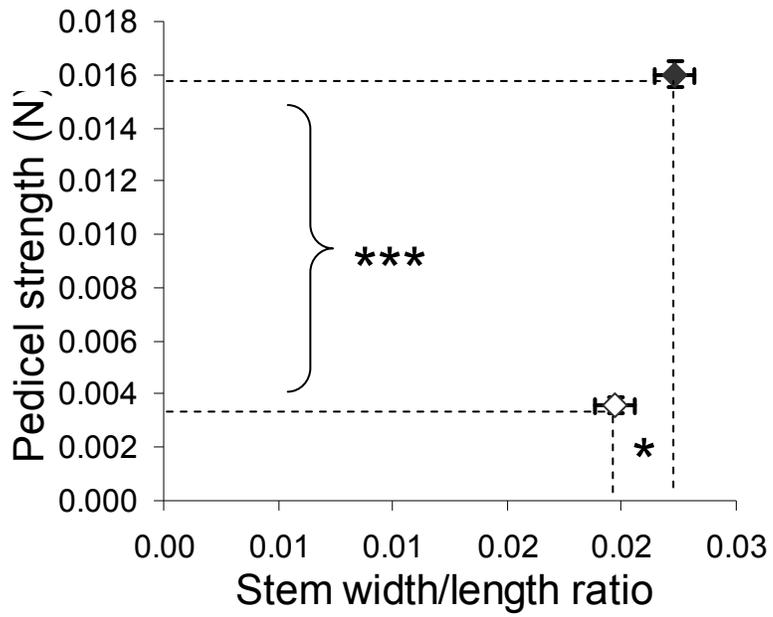
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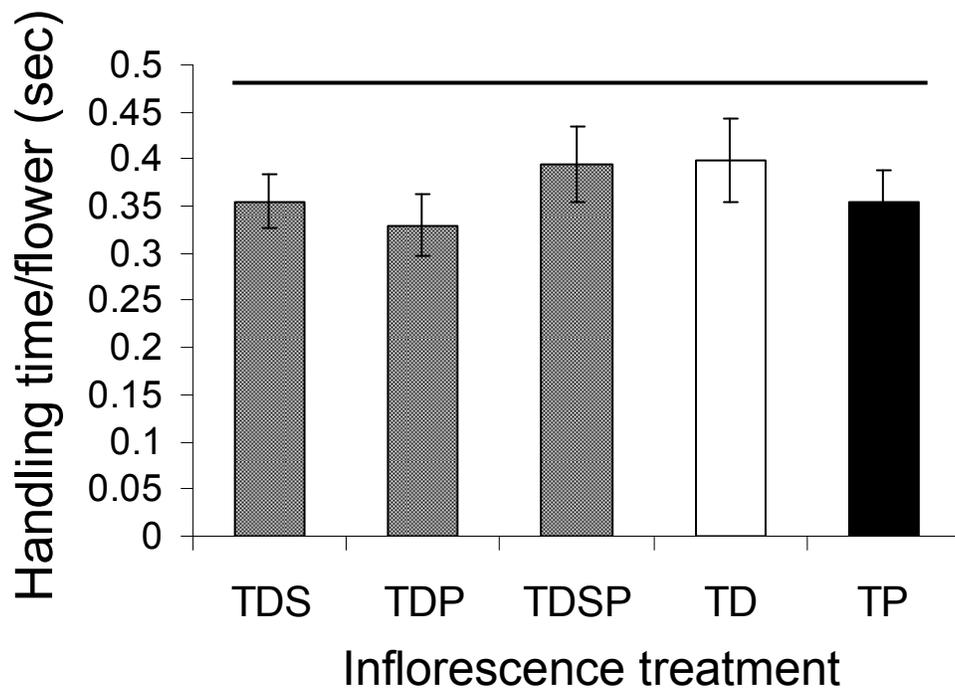
Figure 5.
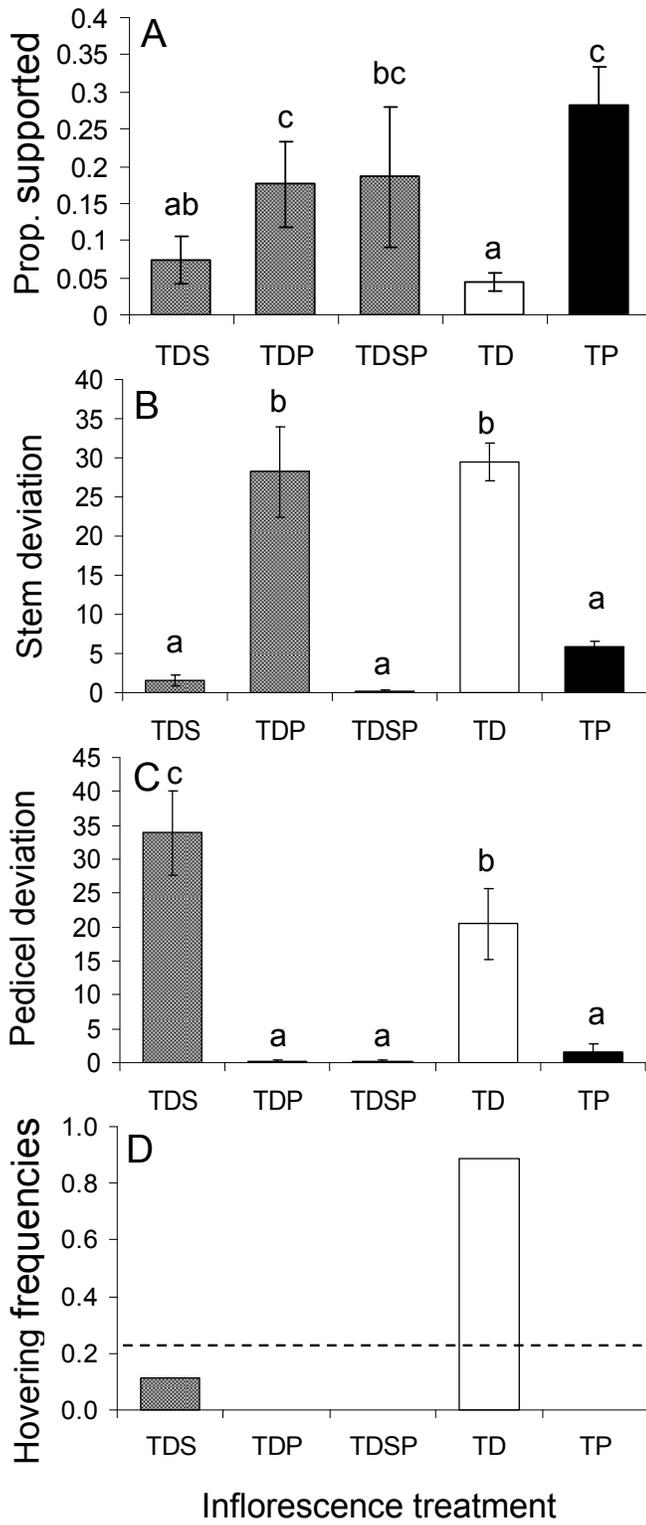
CHAPTER 3

TRACING THE IMPACTS OF PARTNER ABUNDANCE IN FACULTATIVE POLLINATION MUTUALISMS: FROM INDIVIDUALS TO POPULATIONS

Jennifer C. Geib

ABSTRACT

Partner abundance affects costs and benefits in obligate mutualisms, but its role in facultative partnerships is less clear. I address this gap by tracing impacts of partner abundance in a pollination web consisting of two clovers, *Trifolium dasyphyllum and T. parryi*, that vary in specialization on a shared bumblebee pollinator, *Bombus balteatus*. We examine how intraspecific and interspecific variation in pollination niche breadth impacts plant responses to pollinator abundance, comparing responses to bumblebee density between early- (specialized) and late- (generalized) flowering cohorts of *T. parryi* and early *T. parryi* to *T. dasyphyllu*m, a pollination generalist. From individual-level benefits of increases in bumblebee density population outcomes are modeled. Co-pollinators disrupt the link between *B. balteatus* visitation and pollination rate for both clovers. However, early-flowering plants of *T. parryi* receive more visits, pollination, and seed set with increases in density of *B. balteatus*. In addition, bumblebee density alters timing and extent of seed germination in *T. parryi*. Seeds from plants receiving additional *B. balteatus*

germinate sooner and more often than open-pollinated counterparts. Benefits accrue nonlinearly, satiating at intermediate bumblebee densities. Models suggest that population persistence depends partly on *B. balteatus* density for *T. parryi,* but not *T. dasyphyllum.* Overall, findings support the prediction that functional redundancy among guild members mediates species' responses to partner density in facultative mutualisms. Nonetheless, as in more specialized interactions, the hypothesis of diminishing returns in pollination benefits is supported. Effects of pollinator density on life history strategies represent a novel result with implications for further models of pollination mutualism.

Key words: mutualism, facultative, density dependence, pollination, pollination niche, specialization, *Trifolium dasyphyllum*, *Trifolium parryi*, alpine clover, *Bombus balteatus*

INTRODUCTION

Mutualisms benefit interspecific partners, yet realized benefits are context-dependent, depending on biotic and abiotic environmental factors (Bronstein 1994). Partner abundance is an intrinsic factor contributing to this variation (Holland et al. 2002). If partners are rare, increasing abundance of one should increase benefits to the other, with the potential for a corresponding increase in population size (Stanton 2003). Ultimately, however, extrinsic limits to this response should cause returns from the interaction to satiate and even decline (e.g. Bronstein 1994; Bronstein 2001). Studies demonstrating the importance of partner abundance for costs and benefits of mutualism have been of limited generality for two reasons. Most have utilized relatively simple presence/absence manipulations which fail to characterize the full, potentially non-linear, range of responses possible in natural systems (e.g. Breton and Addicott 1992; Morales 2000). Additionally, these studies have mainly focused on systems in which partners' lifecycles are tightly and obligately linked (e.g. Anderson and Midgley 2007; Pellmyr and Huth 1994). This approach simplifies predictions and experimental manipulations but ignores the facultative nature of most mutualisms. As of yet, few studies have tested density dependence over a range of partner density within facultative, multispecies mutualisms.

Facultative mutualisms are the more general case in nature (Holland and Fleming 2002; Stanton 2003) and range from highly specialized interactions, dependent on one or a few closely related partners, to broadly generalized relationships, involving multiple highly substitutable unrelated partner species

(Bascompte et al. 2003; Thompson 2006). Generalized interactions are more complex than specialized

ones, because competition and functional redundancy among possible partners may impact the outcome of

mutualism. Pollination mutualisms, which often comprise multispecies guilds on either side of the

interaction (Stanton 2003) exemplify this complexity. Holland and Fleming (2002), for example, show that

the availability of co-pollinating bees can influence the dynamics of interactions between senita cacti and

their primary moth pollinators. Although studies of ecological networks suggest that potential partners in

pollination assemblages are highly substitutable and generalized, much less is known about how

generalization affects the population dynamics of the interacting species. A major goal of this study was to

extend their approach by comparing responses to pollinator abundance between closely related host plant

species that differ in exploitation of co-pollinator taxa.

One challenge in comparisons of specialist and generalist life histories is that niche breadth is not

static over time. For example, requirements for survival change from seedling to adult life stages (Grubb

1977). The pollination niche can vary over an even shorter time scale due to annual changes in climate and

seasonal schedules of pollinator emergence. Olesen et al. (2008) show that temporal dynamics of pollinator

assemblages vary annually as well as seasonally and that network linkages increase, indicating expanding

pollination niche breadth, with flowering span. I take advantage of seasonal trends in co-pollinator

availability to compare effects of pollinator density on early- (specialist) vs. late- (generalist) flowering

cohorts.

Increased pollinator abundance should confer greater benefits to a specialist host plant (Bronstein

1994). However, during successive pair-wise steps in the full pathway from partner density to recruitment

each step may be affected by external abiotic and biotic factors. Consequently, indirect effects from

increased pollinator density may not propagate through to recruitment, even for a specialist; instead, effects

may attenuate rather than cascade (Gruner 2004; McCann et al. 1998; Polis and Strong 1996). While

abundance of pollinators is widely assumed to affect plant population growth, the validity of this thesis

depends on the strength of causal linkages between pollinator density, pollination services, and host plant

demography (Fig. 1). Studies using hand-pollination as a proxy for increased pollinator visits show that

pollination deficiencies exist in diverse plant communities (Burd 1994; Kearns et al. 1998; Knight et al.

2005; Mayfield 1998; Thomson 2001), suggesting that increased pollinator abundance may enhance

population growth in this manner. Others have shown correlations between pollinator density and seed quantity (Plowright and Hartling 1981), or between population declines of linked plant and pollinator species (Biesmeijer et al. 2006). However, little direct evidence exists supporting the set of pair-wise links connecting pollinator density and plant population growth.

There is a particular dearth of evidence for the effects of pollinator density on life stages beyond fecundity. Yet, if pollinators alter their foraging behavior as density increases by adding floral resources of lower rank, then changes in mating patterns within populations and potential costs of heterospecific pollen transfer may ensue. Effects on outcrossing rate, seed viability, germination timing, and offspring quality are plausible (e.g. Bell et al. 2005; Brock 2004; Sobrevila 1988; Waser and Price 1989). Because germination and seedling establishment function as the gateway to entry of new offspring into a population, there are potential demographic implications. Germination timing may also be impacted by the pollen parent (e.g. Galloway 2001; García-Gusano et al. 2005), altering generation time and population growth rate. The impacts of pollinator density on germination, seedling establishment, and dormancy are untested, as well as the consequences that may follow at the population level. In my study I address these gaps, examining impacts of increased pollinator density on individual performance, and creating models that link these effects to likelihood of population persistence.

This study addresses the impact of partner density in facultative mutualisms within the context of a small alpine pollination web, consisting of two native clovers, *Trifolium dasyphyllum* and *T. parryi* and a shared pollinator, the native bumblebee, *Bombus balteatus*. I experimentally vary the abundance of the pollinator in order to trace the impacts on sequential steps in the pathway from pollinator density to plant population growth (Fig. 1). Past descriptions of this pollination network describe a co-pollinator, *B. sylvicola,* for *T. dasyphyllum* (Byron 1980; Macior 1974). More recent studies (Appendix 1) also revealed late season increases in visits by solitary bees, flies, and worker bumblebees for both clovers. Thus, this plant/pollinator system is characterized by features of broader pollination networks: redundancy or substitutability of alternative mutualist partners and potential competition within the pollinator guild (Bascompte et al. 2003; Pleasants 1980). It provides an opportunity to move beyond pair-wise studies of pollination mutualism in a relatively simple experimental system.

The primary objectives of my study were to determine 1) steps in the transition from pollinator visitation to recruitment that depend on pollinator density, 2) the impact of interspecific and intraspecific (seasonal) variation in pollinator niche breadth on plant functional response to changes in density of a primary pollinator, and 3) the impact of pollinator abundance on population growth rate (λ) and persistence.

METHODS

Study system

T. parryi and *T. dasyphyllum* (Fabaceae, subfamily Faboideae) are closely related native clovers (Ellison et al. 2006) abundant in the central Rocky Mountains (USA). They are found on my Pennsylvania Mountain study site (Park Co., CO) from treeline upward and flower prolifically from snowmelt in June to late August. Plants of both clovers are perennial and lack clonal spread (Weber and Wittmann 2001). *T. dasyphyllum* has a cushion-like growth habit averaging about eight inflorescences (heads) per plant, whereas plants of *T. parryi* produce 1-2 heads. *T. dasyphyllum* is more common in open dry meadow habitats whereas *T. parryi* is more abundant in moist snowbeds. Though the two species co-occur extensively near treeline, fertile interspecific hybrids of any *Trifolium* species are rare (Taylor et al. 1980), and no evidence of hybridization has been found in this system. Both species are obligate outcrossers with gametophytic self-incompatibility (Dhar et al. 2006).

T. dasyphyllum and *T. parryi* depend mainly on *Bombus* for pollination (Macior 1974, Bauer 1983). *T. dasyphyllum* has a shorter flower tube and is extensively visited by *B. balteatus* (a long-tongued species) and *B. sylvicola* (short-tongued). In contrast, *T. parryi* (longer-tubed) is visited almost exclusively by *B. balteatus*. Queen bumblebees emerge in mid-June and forage throughout the summer. Workers emerge 2-3 wk after colony establishment in early July. Both clovers are also visited by solitary bees and flies with increasing frequency over the summer (Appendix 1).

Bumblebee addition experiment

I experimentally manipulated the number of queen *B. balteatus* foraging on mixed patches of *T. dasyphyllum* and *T. parryi*. Manipulations were conducted in June - July 2007 along a south-facing ridge

near treeline (3600 m elevation) on Pennsylvania Mountain. Experimental patches (n = 16: 12 early-flowering and 4 late-flowering) 2 x 2 m in area contained at least five plants of each species separated by a minimum of 20 cm. On each of five experimental plants per species I tagged one flower head in bud for intensive sampling, but maintained the natural floral display (mean of 8.1 heads/plant of *T. dasyphyllum* and 1.2 heads/plant of *T. parryi*) by leaving other heads intact. I marked the calyx of a haphazardly chosen floret on the focal inflorescence with enamel paint to assay outcross pollen receipt (see below). To standardize plant and flower density among patches I removed inflorescences from all other (background) plants in the plot. The sixteen patches were randomly and evenly assigned among four bumblebee addition treatments: no supplemental bumblebees (control), and one, two, or three queen *B. balteatus* added twice during flowering. The two treatment applications were conducted 48 hours apart, and thus bees in each round had different flowers available on each inflorescence. To confine foragers within a patch, bumblebees were released individually within a temporary 2 m x 2 m screen enclosure (Cerf Brothers Bag Company, Inc., St. Louis, USA) placed over the patch. Enclosures were removed after foraging bouts, leaving all patches open to natural pollination.

For the additions I used *B. balteatus* queens caught >500 m away to ensure that capturing foragers would not alter background visitation. Bumblebees were held in iced vials for transport to patches. Each was allowed to warm up and initiate foraging on a 'starter' bouquet comprised of a *T. parryi* and *T. dasyphyllum* inflorescence collected into a waterpik from the population surrounding experimental patches. Each bee was allowed to visit flowers on experimental plants for one 'foraging bout'. For each bout, I recorded the sequence of inflorescence visits and the number of florets probed on each head. It was not possible to distinguish revisits to florets from initial visits. Visitation rate was calculated as the number of visits received per head divided by the total number of florets. Bouts were terminated when the bee flew to the screen tent and could not be coaxed back onto the starter. All bees were marked with non-toxic paint (Testor Corporation, Inc., Rockford, USA) before release at the original site of capture to prevent re-use.

After flowers senesced, I collected the style from each marked floret for analysis of pollen receipt in the lab. Styles were clipped just above the ovary and preserved in 3:1 (glacial acetic acid: 95% ethanol). Mesh bags were then placed over each head to prevent seed dispersal, and plants were surrounded with plastic caging to exclude elk. In September, all infructescences were collected for seed counts. In the

laboratory, outcross pollen receipt was estimated by counting stylar pollen tubes. Preparation and analysis followed Kearns and Inouye (1993). Styles were cleared in 1 M NaOH for 24-26 h at 50 C, then placed in a drop of 50% decolorized aniline blue in K_2PO_4/50% glycerol on a slide, and carefully squashed. A widefield epifluorescence microscope (Olympus IX70 inverted microscope, CYAN GFP filter #31044) was used to visualize pollen tubes. I counted the number of tubes entering the lower third of the style. I assessed fecundity of experimental infructescences by dividing the total number of seeds produced per head by the number of florets.

Impact of resources and co-pollinators on seed set

To quantify variation in resource limitation of seed set among experimental patches and account for such heterogeneity in analysis of pollinator density effects, I supplemented pollen by hand for a haphazardly chosen inflorescence of each species within 1 m of each patch. Plants were left open to natural pollination. Pollen was taken from a donor located >1 m from the recipient. To assess the contribution of co-pollinators to seed set I used hardware cloth cages (.64 x .64 cm mesh) to exclude all *B. balteatus* queens and workers from a nearby inflorescence of each clover species. Only visitors such as flies, solitary bees, and very small *Bombus* workers, including potentially *B. sylvicola,* could move through cage openings. Fecundity was estimated as above.

Recruitment

To assess impacts of pollinator density on seedling establishment and recruitment, I collected seeds from bumblebee addition plots and, in October 2007, planted them into natural habitats where seedlings of each clover species were found (proven safe sites) within 100 m of the source populations. Seeds from early flowering and late flowering plots were pooled before planting. I planted ten seed grids per species in a randomized complete block design (five blocks, two grids/species each), dividing bulked seed from each addition treatment evenly among grids. All seeds were planted after natural dispersal and before persistent winter snowfall using methods of Galen and Stanton (1999). Before planting, above-ground vegetation was trimmed and soil sieved through 1.5 mm mesh to about 2-3 cm depth to remove *Trifolium* seeds in the soil. Grids made of 96-cell plastic light diffuser screens were pushed 0.5 cm into the soil and secured to the ground with u-hooks. Within each grid, seeds from different bee density levels were

41

randomized spatially (one seed per cell) subject to proportions determined by seed set differences among treatments. Seeds were covered with 1-2 mm of sifted soil. Germination and seedling survival were surveyed weekly from July to early August in 2008 and 2009.

STATISTICAL ANALYSES

Bumblebee addition experiment

All statistical analyses were conducted in SAS (Version 9.1). I used analysis of covariance (ANCOVA; Proc MIXED) to test for relationships between (a) the number of bumblebees added (a continuous variable from 0-3) and visitation rate, (b) visitation rate and pollen tubes per pistil, and (c) pollen tubes and seed set. To test whether species-specific dimensions of pollination niche breadth affects plant responses to bumblebee density, I restricted my analysis to data from early flowering patches where species differences in pollinator composition were greatest (n = 12 patches), and included 'plant species' as a covariate. To explore how seasonal expansion of niche breadth within a given species affects plant responses to bumblebee density I used data from *T. parryi* and compared early flowering and late flowering (n=12 and 4 patches, respectively) cohorts by including 'time of season' as a covariate. In all analyses, visitation rate, pollen tubes per pistil, and seeds per flower were square root transformed to improve homoscedasticity.

Non-linearity in the relationship of bee density to visitation suggested a pattern of diminishing returns, so I tested for linear, quadratic, and cubic relationships. When the polynomial terms for bee abundance were significant, stepwise regression (Proc REG) was used to calculate R^2 values for all models. I then conducted F tests to assess whether including the polynomial term/s significantly increased R^2 (McDonald 2009). 'Patch' was the experimental unit in these analyses since bee addition treatments were applied to entire patches of plants. I analyzed impacts of (b) visitation on pollen tubes per pistil and (c) pollen tubes on seed set with patch as a random factor and 'inflorescence' as the experimental unit. In the analysis of fecundity, variation in resource levels among patches was accounted for by using seed production of hand-pollinated plants near to each patch as an additional covariate.

Impact of resources and co-pollinators on seed set

ANOVA (Proc GLM) was used to assess the effects of resources and co-pollinators on seed set. Pollination treatment (open-pollinated, hand-pollinated, and caged), flowering time (early and late), plant species, and their interactions were fixed effects in the model (for each clover species: n = 15 early, 5 late open-pollinated plants; n= 12 early, 4 late hand-pollinated plants; and 12 early, 4 late caged plants).

Recruitment

Effects of bumblebee density on total germination rate after two years and seedling survival were tested with ANOVA (Proc GLM). I then used a repeated-measures analysis of variance (RMANOVA; Proc GLM) to test whether bumblebee augmentation affected timing of germination. For this analysis, bumblebee addition treatment (0-3) and plant species were fixed effects and seeds germinating yearly the dependent variable. To ask whether seedlings from specific treatments emerged "early" vs. "late" I compared the proportion of seeds germinating in 2008 vs. 2009 within each plant species and treatment group using a paired t-test. All germination rates were arcsine-square-root-transformed before analysis. 'Block' (n=5) was the experimental unit in all analyses.

MATRIX MODEL

To explore how impacts of bumblebee density on individual performance carry over to population dynamics, I developed stage-based (Lefkovitch) projection matrices for each species as described in Caswell (2001) and Morris and Doak (2002). The models were based on an annual time step and utilized a pre-breeding census reflective of a birth-pulse population. Vital rates in the matrices were based on surveys I conducted or unpublished data specific to *T. dasyphyllum* and *T. parryi* when possible. Where no data were available, e.g. age at first flowering, I used means from other alpine plant species with similar life histories. Full descriptions of the life histories (Fig. 1) and parameterization of the projection matrices (Table 1) are described in Appendix 3. For simplicity, models ignore genetic structure within populations, seed dispersal into/out of the population, and assume constant vital rates for each life stage except as described below. Models also assume lack of density dependent effects within each population. This is a

reasonable assumption since interspersed clover populations are located along a ridge characterized by high

disturbance, primarily due to burrowing mammals. New sites open up regularly.

I used "retrospective" perturbation analysis (Caswell 2000) to examine how bumblebee addition

impacted plant population growth rates (λ) and the probability of persistence (probability of $\lambda \geq 1.0$).

Fecundity rate estimates used in all models were adjusted to take into account variation in resources among

plots by calculating least squares means (LSM) estimates of seed production rate per patch with seed set of

the hand-pollinated plant at each patch as a covariate. Due to the pooling of seeds from early and late

flowering cohorts before planting, I could not assess effects of temporal differences in co-pollinator

abundances on population responses. All perturbation analyses were conducted using the popbio package

(Stubben and Milligan 2007) in R statistical software. Code is available upon request from the reprint

author. Parametric bootstrap techniques (N=2000) (Caswell 2001) were used to estimate species-specific

confidence intervals for λ under each level of bumblebee addition. To assess whether the relationship

between *B. balteatus* addition and plant population growth rate differed between *T. dasyphyllum* and *T.*

parryi, I performed ANCOVA (SAS Proc GLM) on λ with plant species as a covariate, testing linear,

quadratic, and cubic effects of bee addition on λ. As before, stepwise regression was used to calculate R^2

for all models and F tests conducted to assess whether including polynomial terms significantly increased

R^2.

To assess how varying pollinator density affects the relative importance of different life stages to

the persistence and growth of clover populations, I conducted elasticity analyses of all projection matrices.

Elasticities allow for comparison of the relative contribution of each vital rate to λ, even rates that are

measured on different scales such as survival and fecundity (de Kroon et al. 2000).   Following methods

similar to Silvertown et al. (1993, 1996), matrix cells of transitions not directly affected by pollinators were

partitioned into biologically relevant groups to facilitate elasticity comparisons; i.e. seedling and juvenile

elasticities were summed to represent the contribution of the "pre-adult" life phase, and elasticities of

flowering and vegetative adults were summed to represent the contribution of the "adult" life phase. Over

bumblebee addition levels, I compared the relative elasticities of: fecundity leading to immediate

recruitment the next year, fecundity leading to recruitment from the seed bank, pre-adult life stages, and

adult life stages.

RESULTS

Bumblebee addition experiment

In early flowering patches, increasing *B. balteatus* density tended to affect flower visitation differently in *T. dasyphyllum* and *T. parryi* (Fig. 2A, Table 2; BEES*SPECIES $F_{1,18}$=3.05, P=.098). For *T. parryi*, visitation showed a pattern of diminishing returns, increasing sharply up to an intermediate bumblebee density then saturating (BEES^2 $F_{1,9}$=8.37, P=.018). Including a quadratic term in the regression model significantly increased the amount of variance explained by bee density for *T. parryi* but not *T. dasyphyllum* (respectfully, R^2 increased from .63 to .81; $F_{2,9}$=8.37, P=.009; and from .57 to .61; $F_{2,9}$=.949, P=.42). *T. dasyphyllum* experienced a more gradual linear increase in flower visitation with bumblebee addition (BEES $F_{1,10}$=13.24, P=.0045).

In early flowering patches, the relationship between bumblebee visitation and pollen tubes per style also differed between species (VISITS*SPECIES $F_{1,106}$ = 4.89, P=.029; Fig. 2B, Table 3). The number of pollen tubes per style increased with visits to *T. parryi* ($F_{1,23.3}$=31.83, P<.0001), but not *T. dasyphyllum* ($F_{1,42}$=0.50, P=.483). Increases in pollen tubes per style enhanced seed set for both clovers, suggesting that fecundity in both species was pollination limited (Fig 2C, Table 4; $F_{1,100}$=12.89, P=.0005). However, seed set of hand-pollinated plants near each patch explained a significant amount of variation in average seed set of experimental inflorescences ($F_{1,24.5}$=9.55, P=.005), indicating that among patch variation in resources also affected fecundity.

B. balteatus abundance had similar positive impacts on visitation rate to *T. parryi* inflorescences early and late in the flowering season (Fig 2D, Table 2; BEES $F_{1,10}$=8.55, P=.015). However, the relationship between *B. balteatus* visitation rate and pollen tubes per pistil disappeared late in the season after worker bees emerged and non-*Bombus* visitors increased (VISITS*TIME $F_{1,21.3}$=15.52, P=.0007; Fig. 2E, Table 3). Similarly, the relationship between pollination and fecundity for *T. parryi* declined in importance over the season (POLLINATION*TIME $F_{1,65.3}$=4.62, P=.035; Fig 2F, Table 4). Late in the season only among-patch variation in resources (represented by the seed set of the adjacent hand-pollinated individuals) explained a significant proportion of the variance in seed set for *T. parryi* (HP SEEDS $F_{1,2.45}$=18.5, P=.034; Table 4).

Impact of resources and co-pollinators on seed set

The impact of resources and co-pollinators on seed set (Fig. 3, Table 5) differed over the flowering season (POLL*TIME $F_{2,91}$ =5.21, P=.007) for both species (POLL*TIME*PLANTSP $F_{2,91}$ =.28, P=.733). Resource limitation of seed set increased over time; Early in the season, hand-pollinated plants had greater fecundity than open-pollinated plants (a priori comparison, P=.0007), but late in the season seed set did not differ between hand-pollinated and open-pollinated plants (a priori comparison, P=.332).

The contribution of co-pollinators to seed set also increased over the flowering season. For early flowering plants (prior to the emergence of worker bumblebees), co-pollinators contributed to seed set of bumblebee-excluded *T. dasyphyllum* plants, but not for *T. parryi,* which essentially set no seeds when bumblebees were excluded (Mean seed set of caged plants + 95% CI = .112 $\pm$ .06 and .021 $\pm$ .03, respectively for *T. dasyphyllum* and *T. parryi*). However, both clovers set significantly more seed when bumblebees (queens only at this time) were allowed access to flowers (a priori comparison, bumblebee-excluded vs. open-pollinated, P=.0066). Conversely, late in the season (after emergence of worker bumblebees) both species set higher numbers of seeds with or without excluded bumblebees (cages excluded all *Bombus* queens and *B. balteatus* workers; a priori comparison, P=.296).

Recruitment

Seed germination depended on bumblebee addition in both clovers. For *T. dasyphyllum* germination was lowest in patches augmented with three bees (Planned contrast, $F_{1,24}$=52.89 , P<.0001; Fig. 4B, Table 6). *T. parryi* seeds from patches augmented with *B. balteatus* exhibited higher germination than seeds from control patches (Planned contrast $F_{1,24}$=62.01, P<.0001; Fig. 4A, Table 6).

B. balteatus addition tended to affect seed germination schedules in a species-specific manner, (RMANOVA, TIME*PLANT SPECIES*BEES $F_{3,32}$=2.46, P=.081; Table 7). For *T. dasyphyllum* (Fig. 4C) seeds from most patches were equally likely to germinate in 2008 or 2009 (paired t-tests comparing germination each year for 0, 2, and 3 bees added, respectively: T_4=-.595, T_4=.426, T_3=0; P>.50 for all). However seeds from patches receiving one individual of *B. balteatus* delayed germination until the second year after planting (T_3=5.00, P=.015). For *T. parryi* (Fig. 4D), seeds from bee addition patches tended to germinate faster (in 2008, the first year after planting: For 1, 2, and 3 bees added, respectively, T_3=-2.33, P=.10; T_4=-

5.11, P=.0069; T_3=-3.00, P=.058), whereas seeds from control patches were equally likely to germinate in either year (T_3=1.21, P=.31). There were no differences in seedling survival among groups ($F_{19,23}$=.83, P=.658; Table 8).

Matrix model

  B. balteatus density had no consistent impact on *T. dasyphyllum* population growth dynamics (Table 9). Instead, λ oscillated in a non-linear manner with bee density (BEES[3] $F_{1,7996}$=1571.09, P<.0001; Figure 5A). For *T. parryi,* population growth rate increased modestly but significantly with *B. balteatus* density then leveled off (BEES[3] $F_{1,7996}$=149.84, P<.0001; Figure 5B). Adding the quadratic and cubic terms for bee abundance significantly improved R^2 for *T. dasyphyllum* and *T. parryi* models (Respectively, from .19 to .34, $F_{3,7996}$=1570, P<.0001 and .03 to .10, $F_{3,7996}$=579, P<.0001). High among-patch variance in vital rates, seen in the wide confidence intervals surrounding the means of the bootstrapped estimates of λ for each treatment, reduced the explanatory power of *B. balteatus* density, especially for *T. parryi*. For all pollinator addition treatments including controls, the confidence intervals surrounding λ for *T. dasyphyllum* included 1.0, indicating population persistence is not sensitive to bumblebee density (Fig. 5A and C). In contrast, for *T. parryi*, only with bumblebee addition was extinction not a certainty (Fig. 5D).

  For both clovers, the overall importance of fecundity and recruitment was little affected by pollinator density; however, population growth for *T. dasyphyllum* depended more on adult stasis at the highest density of *B. balteatus* (Fig. 6). Fecundity and recruitment contribute about twice as much to λ for *T. dasyphyllum* as for *T. parryi,* regardless of bumblebee density. For both clovers, as bee density increases, impact of recruitment from dormant seeds in the seed bank is inversely proportional to that of recruitment from seeds germinating the first season after dispersal. Interestingly, the effects of *B. balteatus* density on the importance of seedlings and juveniles (pre-adults) mimic patterns seen for seed production and recruitment the first year after dispersal: reduced importance for fecundity and recruitment under the one and three bee addition treatments for *T. dasyphyllum* and a pattern of diminishing returns at the highest bee density for *T. parryi*. This trend suggests that bumblebees have congruent impacts on seed set and seed quality in these clover species, and that these effects carry on through the pre-adult years.

DISCUSSION

I found that *Trifolium parryi* and *T. dasyphyllum* differ in functional and demographic responses to changes in abundance of *Bombus balteatus*, a shared primary pollinator. For early-flowering *T. parryi,* benefits of bumblebee addition were manifest in flower visitation, pollination, and fecundity. In contrast, for *T. dasyphyllum*, bumblebee addition enhanced visitation, but attenuated in impact on sequential transitions. Moreover, the shape of the benefits curve differed between species, with diminishing returns in visitation for *T. parryi* and gradual linear returns for *T. dasyphyllum.* At the population level, the contrast between species was most dramatic, with *T. parryi* populations forecast to go extinct under the current density of *B. balteatus.* Conversely, for *T. dasyphyllum,* my model predicts that population dynamics should be largely independent of *B. balteatus* abundance. As explained below, some but not all of these differences aligned with expectations based on pollination niche breadth.

Individual-level effects

The cascade of effects from increasing pollinator abundance to seed set varied with interspecific and intra-seasonal differences in pollination niche breadth between *T. parryi* and *T. dasyphyllum*. Specifically, for the more specialized host plant, *T. parryi*, *B. balteatus* visitation peaked rapidly and satiated at an intermediate density, supporting a model of diminishing returns and concurring with predictions for benefit accrual curves in obligate pair-wise interactions (Bronstein 2001) and recently extended to "typical" pollination mutualisms (Morris et al. 2010). For the generalist, *T. dasyphyllum*, visitation accrued gradually with increased bee density and a satiation threshold was not reached. Mutualism theory predicts increases in individual benefits as partners increase followed by satiating or diminishing returns at the highest partner density as other biotic and abiotic factors limit individuals' ability to respond (Stanton 2003). Notably, I likely would not have seen this trend had I estimated benefit response curves from single species patches. As bee density increased and nectar resources were exhausted in *T. parryi*, individual foragers shifted their diet to include a greater proportion of flower visits to *T. dasyphyllum* (Fig. 7). The propensity for bees to expand their diet breadth in a density dependent manner has been reported in other studies (Fontaine et al. 2008), and is not surprising given their capacity to discriminate among flowers based on visitation history (Heinrich 1979). My results suggest that species

switching at high pollinator density is likely to be a common mechanism for diminishing returns in flower visitation by facultative mutualists, especially in the mixed species assemblages frequented by bumblebees. Young and Young (1992) proposed a number of other mechanisms promoting diminishing returns in single species stands including pollen tube crowding, pollen removal or stigma damage from excess visitation, and deposition of inviable pollen.

I predicted that the relationship between visitation of *B. balteatus* and pollen deposition/ovule fertilization rates in both clover species would vary with co-pollinator abundance. Hand-pollinations early in the season enhanced seed set, demonstrating pollination limitation. However, after co-pollinators emerged seed set in both clovers was likely resource-limited since it was unrelated to pollen tube density and unaffected by pollen supplementation. Flies, solitary bees, and potentially worker bees of *Bombus balteatus and B. sylvicola* effectively pollinated *T. dasyphyllum* throughout the summer. Accordingly, the modest density dependent increase in flower visitation by *B. balteatus* attenuated with little fitness impact. Similarly, for plants of *T. parryi* flowering late in the summer, loss of sensitivity to *B. balteatus* density is consistent with a co-pollinator effect. However, this conclusion would have been strengthened had I increased sample size for late-flowering mixed clover patches, which were, unfortunately, in limited numbers at my site. That this and other studies (Bosch et al. 2009; Holland and Fleming 2002; Miller 2007) have documented temporal changes in partner specialization within mutualisms (pollination and others) emphasizes the importance of observing interaction webs throughout the season and over multiple years before drawing conclusions about the importance of any one partner species or species guild.

Effects of pollinator density at stages linking individual fitness and population growth

Morris et al. (2010) make predictions about recruitment rates in three typical pollination mutualisms based on plant fitness responses to pollinator density. However, my results revealed novel linkages between pollinator density and recruitment. Specifically, bumblebee density not only affected offspring quantity but unexpectedly altered seed fate after dispersal. Pollinator addition increased *T. parryi* recruitment via enhanced germination rate and advanced germination timing. Seeds produced from patches with two or more *B. balteatus* germinated more often and earlier than seeds from open-pollinated plants.

49

Conversely, for *T. dasyphyllum* bumblebee addition was costly, reducing seed germination when three bees were added and delaying germination with one bee added.

Pollinator effects on seed germination and dormancy likely represent paternal effects, or maternal-by paternal interaction. Seed germination can be impacted by genetic compatibility of the pollen and ovules (Sobrevila 1988; Waser and Price 1989), by the environment conditions of the pollen parent (Galloway 2001), or by deposition of heterospecific pollen (Brock 2004; Murphy 2000). Although my study was not designed to reveal underlying mechanisms, positive impacts on *T. parryi* germination may be due to outcross advantage or increased opportunities for mate choice (Marshall and Folsom 1991). However, preference of *B. balteatus* for *T. parryi* may increase costs to *T. dasyphyllum* from heterospecific pollen transfer, such as mentor effects or stigma clogging that reduces opportunities for mate choice. Brock (2004), for example, showed that deposition of congeneric pollen on stigmas of *Taraxicum ceratophorum*, broke down its ability to recognize and prevent fertilization by self pollen (mentor effect). In highly outcrossing species, selfed seeds should exhibit poor fitness due to inbreeding depression. It is interesting that in patches receiving two *B. balteatus* queens, bumblebees rarely visited *T. dasyphyllum* (Fig. 7); *T. dasyphyllum* seeds in these patches likely resulted from co-pollinators, reducing or eliminating the offspring quality costs observed under the one- and three-bee addition levels (Fig. 4A,C). Similar mechanisms may explain effects of bee addition on seed dormancy. Embryos of different quality may vary in requirements for breaking dormancy. Pollen parent effects on optimal seed stratification regimes (García-Gusano et al. 2005) as well as maternal by paternal interaction effects on germination timing (Snow 1990) are known from other species and consistent with density mediated opportunities for mate choice in this system. Dormancy in some clover species is sensitive to embryo genetics (e.g. *T. subterraneum* L., Morley 1958). Other studies have documented effects of pollinator density on seed weight a correlate of seed quality, (Steffan-Dewenter 2003), but to my knowledge this is the first to document the effects of pollinator abundance on seed germination and dormancy. Although further studies are needed to ascertain underlying mechanisms, it seems likely that these kinds of effects are widespread in facultative pollination mutualisms, since the key features of my experiments (mixed species stands, pollinator sharing, and patchy spatial distributions) are commonplace in nature.

Population effects

Do benefits to individual plants from increased pollinator abundance translate to benefits at the population level? My results suggest that whether such linkages arise in facultative pollination mutualisms, depends largely on co-pollinator abundances. For plants that exploit broad assemblages of co-pollinators, population persistence is unlikely to depend on density of any one species. *T. dasyphyllum* fits this scenario. However, in more specialized *T. parryi*, the pattern of diminishing returns with increased *B. balteatus* density cascades from individual benefits to population growth rates. In addition, likelihood of persistence for the population I studied depends on abundance of its bumblebee partner. As many bumblebee populations continue to decline worldwide (Goulson et al. 2008), declines in the ranges of linked specialist plants like *T. parryi* are likely to ensue, a pattern supported by other studies (Biesmeijer et al. 2006). Further studies of the population-level consequences of variation in pollinator abundance are desperately needed in a wider array of species and habitats to inform predictions about pollinator losses and efforts to restore pollination services to affected plants.

Alpine clovers, true to the nature of long-lived plants, depend most on adult plant survival to maintain their populations (Fig. 6). Under normal conditions, the stages depending most on pollinator density, fecundity and recruitment, contribute relatively little to λ. This suggests at first that for *Trifolium*, on an ecological time scale, interactions with pollinators are of greater importance to the success of individuals rather than populations. Interactions (positive and negative) that impact life stages with greater contributions to λ may be more important at the population level, e.g. disturbance, herbivory, or mycorrhizal associations. The few studies that have compared demographic impacts on plants from multiple ecological interactions support this. Knight (2004), for example, found that plants of the long lived understory forest perennial, *Trillium grandiflorum*, produced fewer seeds as a result of pollen limitation, but that pollen limitation had no effect on population growth; instead, herbivory of adults was a primary factor. However, my study shows that increasing pollinator density can impact the relative importance of fecundity and recruitment in a plant's life history. Here, for *T. parryi*, the contribution of fecundity and recruitment to λ peaked at the intermediate density of bees. Even more interesting, though I measured no direct effects of pollination regime on seedling survival, increased fecundity and recruitment in the two bee treatments also magnified the importance of the pre-adult classes in *T. parryi* life history.

51

Together these results indicate that in some cases increased pollination services could minimize dependence on long-lived adults for maintaining plant populations, a factor which could be important in highly disturbed habitats. In the alpine habitats of these clovers, for example, disturbance (e.g. frost heave, burrowing mammals, anthropogenic) is a primary threat to long-lived adult plants, and recruitment is the primary mechanism for re-populating disturbed sites (Chambers 1995). As outcross pollination also promotes genetic diversity among offspring in a population, the effects of increased pollinator density may be extremely important on an evolutionary time scale.

Theory suggests that mutualisms should evolve in such a way that benefits to any one species from increasing partners are limited in order to ensure the stability of the interaction (Holland et al. 2004). Reduction of benefits and/or increased costs at high partner abundances have been observed in pair-wise obligate mutualisms (Anderson and Midgley 2007; Herre and West 1997; Pellmyr and Huth 1994). A few studies have also demonstrated patterns of diminishing returns in facultative interactions. A review of mycorrhizal colonization and benefits to plants showed benefits maximizing at an intermediate colonization rate (Gange and Ayres 1999). A study of a barrel cactus-ant mutualism (Ness et al. 2006) observed saturation in benefits at highest partner density. Morris et al. (2010) show saturating or diminished pollination and seed set at high pollinator visitation in facultative pollination mutualism. Here I have demonstrated reduced benefits and increased costs during multiple life history stages at high partner density.

I have attempted to be thorough in tracing the effects of partner density through every transition linking individual benefits and population growth, yet there are plenty of stones left unturned: How does male fitness respond to varying pollinator density in a multi-species system? Would the trends I have observed hold for multiple years, for other pollination webs, or for other mutualisms? By definition, mutualisms benefit partners on both sides of the interaction; are there feedback loops as populations increase on each side? What roles do other positive and negative interactions play in limiting or facilitating these responses? I hope that my study will inspire future research addressing these questions and more.

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Table 1. Projection matrices for alpine clovers. *f(P)* indicates vital rates that vary as a function of pollinator density. SB=dormant seeds in the seed bank, SL=seedling, JV=juvenile, AF=flowering adult, AV=vegetative adult.

Stage i year t+1	Stage j year t				
	SB	SL	JV	AF	AV
T. dasyphyllum					
SB	0	0	0	*f(P)*	0
SL	*f(P)*	0	0	*f(P)*	0
JV	0	0.40	0.68	0	0
AF	0	0	0.09	0.14	0.39
AV	0	0	0	0.64	0.39
T. parryi					
SB	0	0	0	*f(P)*	0
SL	*f(P)*	0	0	*f(P)*	0
JV	0	0.40	0.64	0	0
AF	0	0	0.06	0.61	0.43
AV	0	0	0	0.24	0.43

Table 2. ANCOVA testing the relationship between *B. balteatus* density and visitation rates for A) early-flowering plants of each clover species and B) for early and late-flowering *T. parryi*. Bees = bee density treatment (0, 1, 2, 3 bees added); time of season = early or late (before or after emergence of worker bees, respectively).

Source	Num DF	Den DF	F value	Pr > F
A) Type III tests of fixed effects: Interspecies differences				
Whole model				
bees	1	18	20.15	0.0003
bees^2	1	18	7.66	0.0127
plant species	1	18	0.01	0.9361
bees*plant species	1	18	3.05	0.0977
bees^2*plant species	1	18	1.98	0.1768
T. dasyphyllum				
bees	1	10	13.24	0.0045
T. parryi				
bees	1	9	18.69	0.0019
bees^2	1	9	8.37	0.0178
B) Type III tests of fixed effects: Interseason differences				
Whole model				
bees	1	10	8.55	0.0152
bees^2	1	10	3.06	0.1109
time of season	1	10	0.14	0.7152
bees*time of season	1	10	0.65	0.4377
bees^2*time of season	1	10	0.56	0.4712

Table 3. ANCOVA testing the relationship between *B. balteatus* visits and clover pollination A) early-flowering plants of each clover species and B) for early and late-flowering *T. parryi*.

Source	Num DF	Den DF	F value	Pr > F
A) Type III tests of fixed effects: Interspecies differences				
Whole model				
visits	1	69.8	12.66	0.0007
plant species	1	103.0	4.76	0.0315
visits*plant species	1	106.0	4.89	0.0291
T. dasyphyllum				
visits	1	42.0	0.50	0.4832
T. parryi				
visits	1	23.3	31.83	<.0001
B) Type III tests of fixed effects: Intraseason differences				
Whole model				
visits	1	21.3	0.01	0.9144
time of season	1	15.7	55.74	<.0001
visits*time of season	1	21.3	15.52	0.0007
T. parryi Early				
visits	1	23.3	31.83	<.0001
T. parryi Late				
visits	1	5.3	0.82	0.4030

Table 4. ANCOVA testing the relationship between clover pollination and seed set for A) early-flowering plants of each clover species and B) for early and late-flowering *T. parryi*. Pollination = stylar pollen tubes, HP seed set = seed set of hand-pollinated plant near each patch.

Source	Num DF	Den DF	F value	Pr > F
A) Type III tests of fixed effects: Interspecies differences				
Whole model				
pollination	1	100.0	12.89	0.0005
plant species	1	89.0	3.81	0.0541
pollination*plant species	1	101.0	0.51	0.4748
HP seed set	1	24.4	9.55	0.0049
HP seed set*plant species	1	59.8	0.64	0.4273
B) Type III tests of fixed effects: Intraseason differences				
Whole model				
pollination	1	65.3	1.93	0.1698
time of season	1	22.5	0.16	0.6931
pollination*time of season	1	65.3	4.62	0.0354
HP seed set	1	14.6	18.74	0.0006
HP seed set*time of season	1	14.6	3.03	0.1029
T. parryi early				
pollination	1	44.4	11.20	0.0017
HP seed set	1	12.7	4.14	0.0634
T. parryi late				
pollination	1	6.86	0.12	0.7346
HP seed set	1	2.45	18.5	0.0345

Table 5. ANOVA testing how clover seed set responded to pollination treatments (+P, -
B. balteatus, natural pollination). +P = saturated with pollen by hand, - *B. balteatus* = *B.
balteatus* queens and workers excluded from plants.

Source	DF	Type III SS	F value	Pr > F
pollination treatment	2	1.053	4.50	0.0137
time of season	1	1.976	16.88	<.0001
plant species	1	0.031	0.26	0.6083
pollination treatment*time of season	2	1.221	5.21	0.0072
pollination treatment*plant species	2	0.093	0.40	0.6733
time of season*plant species	1	1.022	8.73	0.0040
pollination treatment*time of season*plant species	2	0.073	0.31	0.7334
error	91	10.652		

Table 6. ANOVA testing the effects of bumblebee addition of total seed germination two years after planting. Bees = bee density treatment (0, 1, 2, 3 bees added).

Source	DF	Type III SS	F Value	Pr > F
A) Whole model				
block	4	0.201	3.28	0.0280
plant species	1	0.006	0.38	0.5433
bees	3	0.081	1.77	0.1804
plant species*bees	3	0.092	2.00	0.1412
block*plant species	4	0.112	1.83	0.1564
error	24	0.368		
B) Planned contrasts				
T. parryi 0 vs. 1,2,3	1	0.951	62.01	<.0001
T. dasyphyllum 3 vs. 0,1,2	1	0.811	52.89	<.0001

Table 7. Repeated-measures ANOVA testing for the effects of bumblebee addition on cumulative germination in 2008 and 2009

Source	DF	Type III SS	F Value	Pr > F
Tests of hypotheses for between subjects effects				
plant species	1	0.092	2.91	0.0979
bees	2	0.194	2.05	0.1270
plant species*bees	2	0.267	2.81	0.0549
error	32	1.011		
Tests of hypotheses for within subjects effects				
time	1	0.231	43.34	<.0001
time*plant species	1	0.039	7.28	0.0110
time*bees	2	0.031	1.92	0.1464
time*plant species*bees	2	0.039	2.46	0.0807
error(time)	32	0.171		

Table 8. ANOVA testing the effects of bumblebee addition on clover seedling survival. Bees = bee density treatment (0, 1, 2, 3 bees added).

Source	DF	Type III SS	F Value	Pr > F
block	4	3.129	1.70	0.1838
plant species	1	0.942	2.05	0.1656
bees	3	0.372	0.20	0.9344
plant species*bees	3	1.231	0.54	0.7471
block*plant species	4	0.502	0.22	0.9509
error	23	10.570		

Table 9. ANCOVA testing the relationship between *B. balteatus* density and clover population growth rate (λ). Bees = bee density treatment (0, 1, 2, 3 bees added).

Source	DF	Type III SS	F Value	Pr > F
A) Whole model				
bees	1	3.036	821.62	<.0001
bees^2	1	4.482	1212.98	<.0001
bees^3	1	5.687	1539.05	<.0001
plant species	1	48.007	12990.9	<.0001
bees*plant species	1	2.763	747.59	<.0001
bees^2	1	2.162	584.94	<.0001
bees^3	1	2.273	615.21	<.0001
error	15992	59.182		
B) By plant species				
T. dasyphyllum				
bees	1	5.796	1201.89	<.0001
bees^2	1	6.435	1334.39	<.0001
bees^3	1	7.576	1571.09	<.0001
error	7996	38.560		
T. parryi				
bees	1	0.003	1.26	0.2623
bees^2	1	0.209	81.48	<.0001
bees^3	1	0.384	149.75	<.0001
error	7996	20.790		

FIGURE LEGENDS

Figure 1. Alpine clover life history. A) Loop diagram of life history stages and transitions between them (arrows). Bold arrows represent stages through which I traced the effects of increasing pollinator abundance on vital rates. For species-specific vital rates (S_{ij}, survival probabilities and F_{ij}, fecundity) see Table 1. B) Hypothesized sequence of effects through which pollinator density contributes to transitions noted by bolded arrows in the loop diagram.

Figure 2. Comparisons of pair-wise relationships between A) bee density and visitation, B) visitation and pollen tube density, and C) pollen tube density and seed set in early-flowering *Trifolium dasyphyllum* and *T. parryi*. D), E), and F) Comparisons of the same relationships between early-flowering and late-flowering *T. parryi*. Squares with solid lines = *T. parryi*; filled = early-flowering plants, open = late-flowering plants. Diamonds and dashed lines = early *T. dasyphyllum*. Curvilinear lines represent significant polynomial regression. Non-significant regression lines are not shown.

Figure 3. Seed set for *Trifolium* plants receiving supplemental outcross pollen by hand (black bars), screened from all *B. balteatus* (white bars), or naturally pollinated controls (gray bars). The species are pooled because differences between them were not statistically significant. Within each time of season, cohort means for treatments sharing superscripts are not significantly different at p<.05. Error bars represent standard error.

Figure 4. The effects of *B. balteatus* abundance on seed germination rate and timing. Comparisons of total germination rate (pooled over years) among treatment groups for (A) *T. dasyphyllum* and (B) *T. parryi*. Means for treatments sharing superscripts are not significantly different at p<.05. Within each treatment group, black and gray sections show relative contribution to total germination from 2008 and 2009, respectively. * indicates a trend for differences between years at p<.10, and ** indicates significant differences between years at p<.05. Error bars represent standard error.

Figure 5. Mean population growth rates (λ) with 95% confidence intervals for A) *T. dasyphyllum* and B) *T. parryi* and probability of population persistence ($\lambda \geq 1.0$) for C) *T. dasyphyllum* and D) *T. parryi* over the range of bumblebee addition levels. Note difference in y-axis scales between (C) and (D).

Figure 6. Relative contribution of *Trifolium* life stage groups to λ (elasticities) under *B. balteatus* addition treatments for A) *T. dasyphyllum and* B) *T. parryi*. Patterned areas denote the following: black =

66

fecundity + recruitment year 1, striped = fecundity + recruitment year 2, white = seedling + juvenile stages,

bubbles = adult stages.

Figure 7. Mean flower visits (+ SE) to *T. dasyphyllum* and *T. parryi* under each level of *B.*

balteatus density. Black bars denote *T. parryi*; white bars denote *T. dasyphyllum*.

Figure 1.
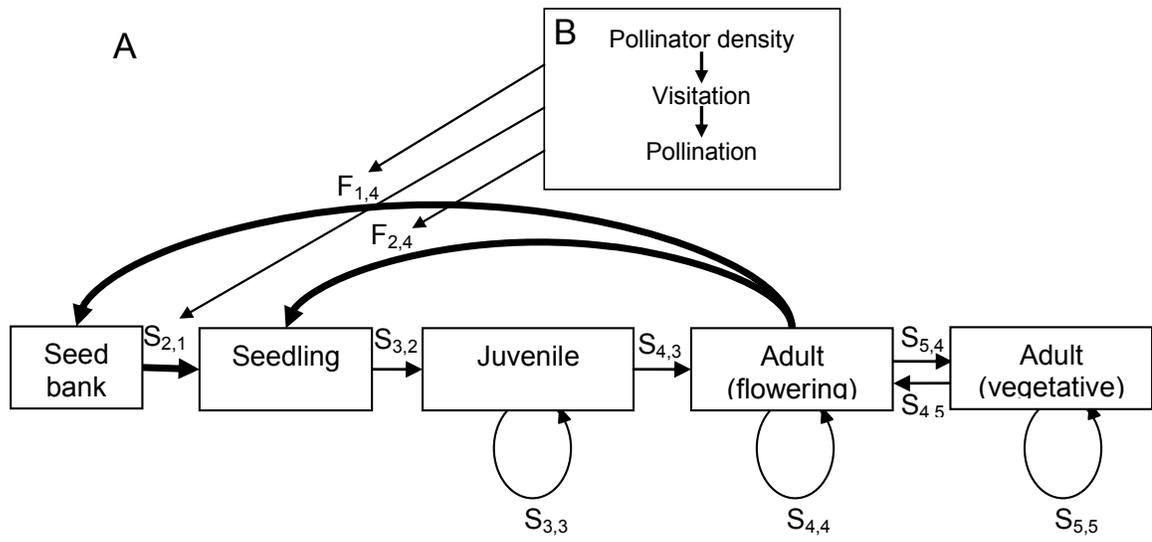
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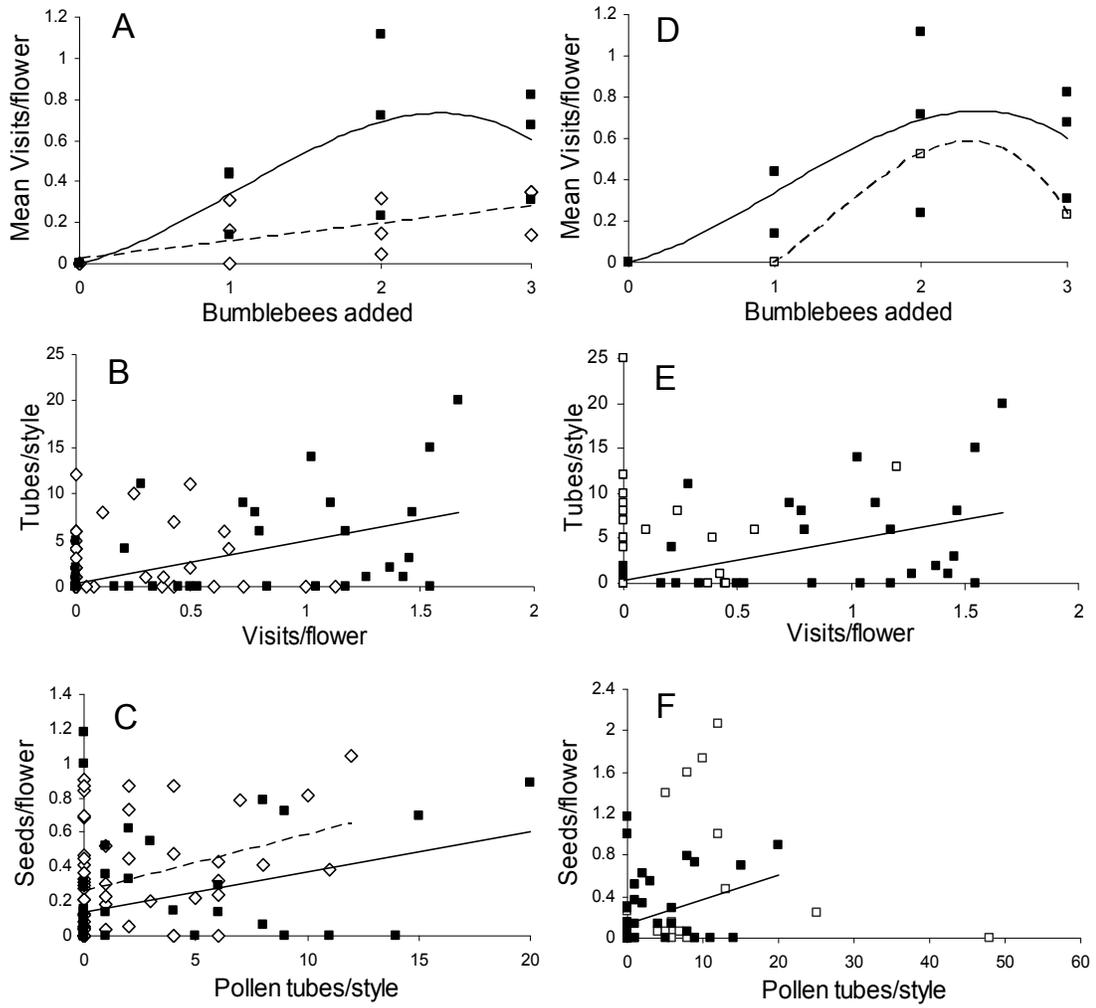
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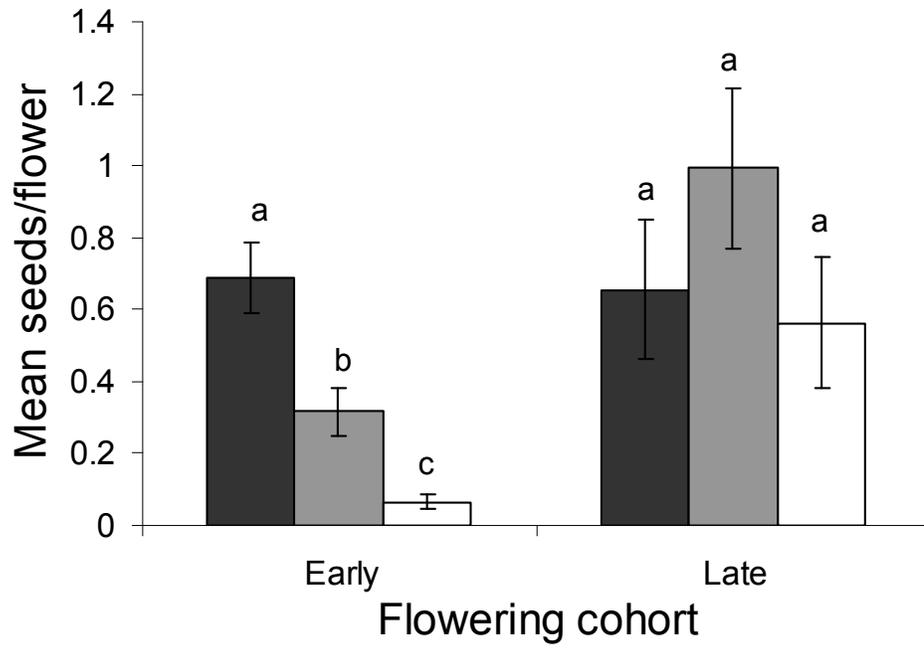
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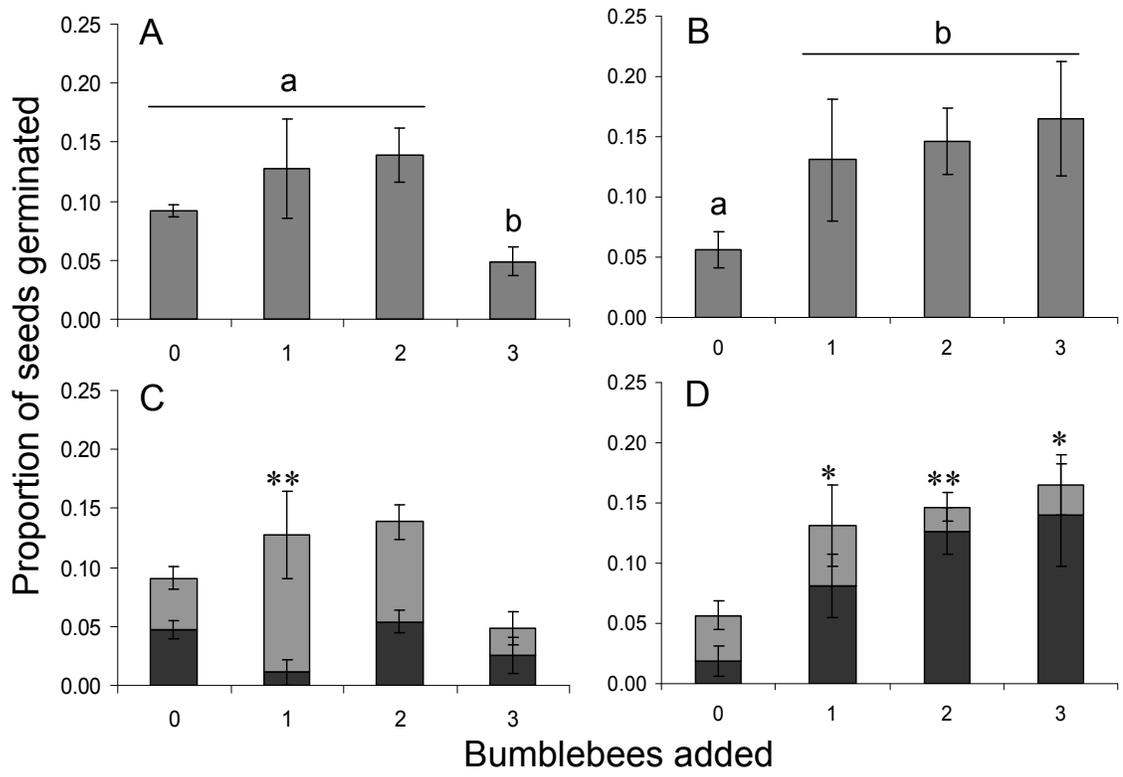
Figure 5.
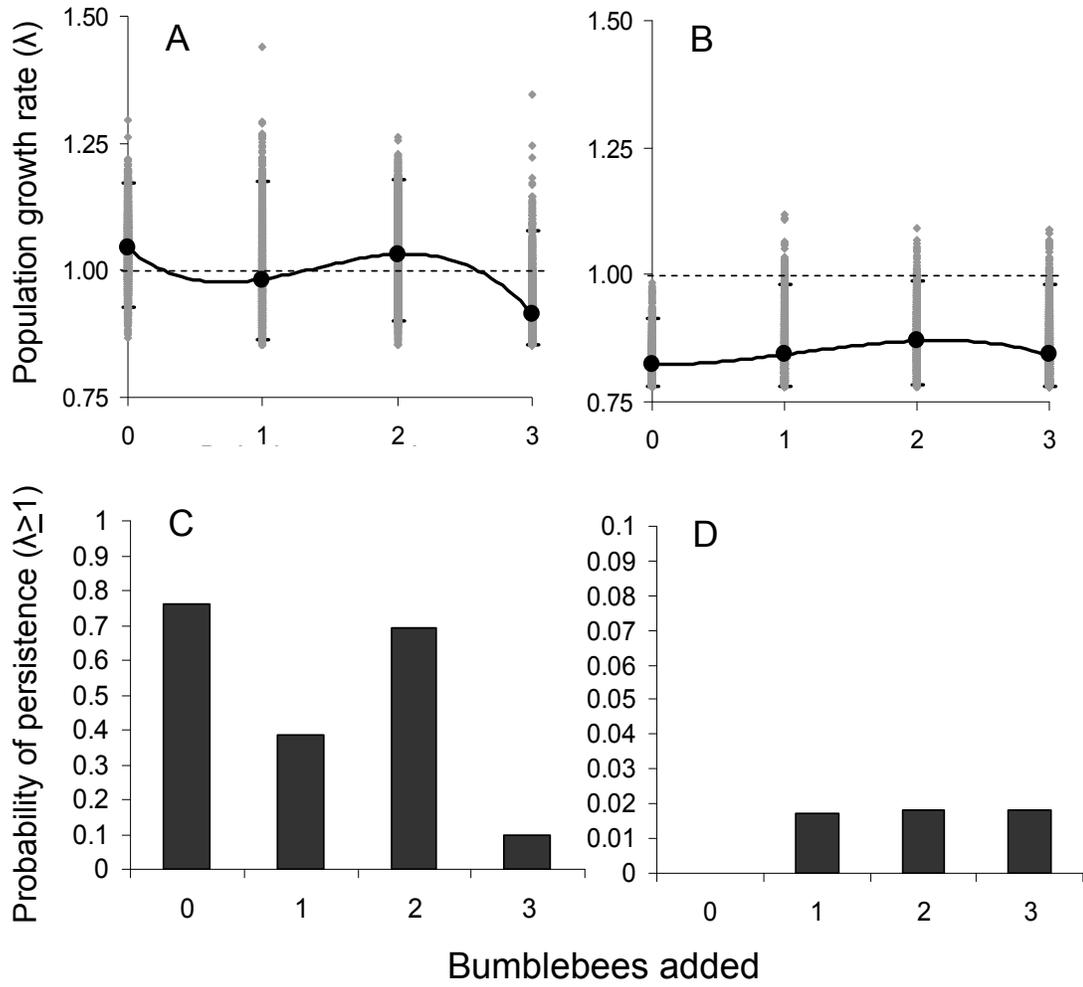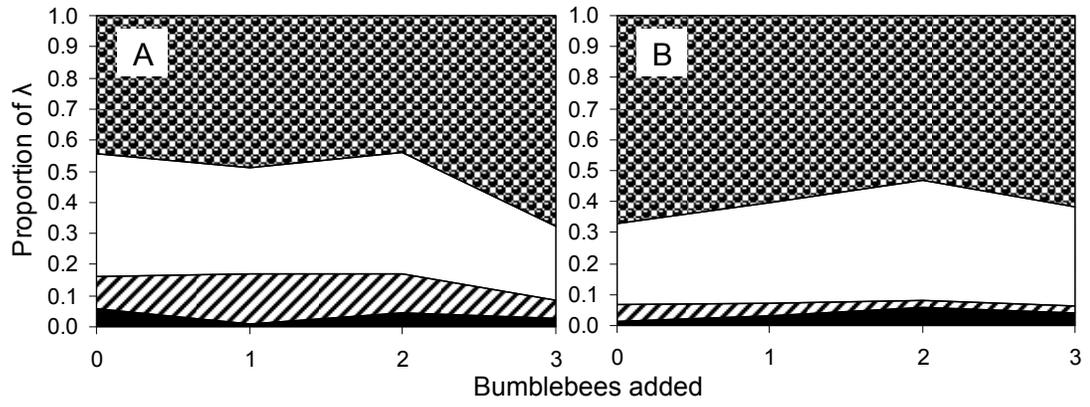
Figure 6.

Figure 7.
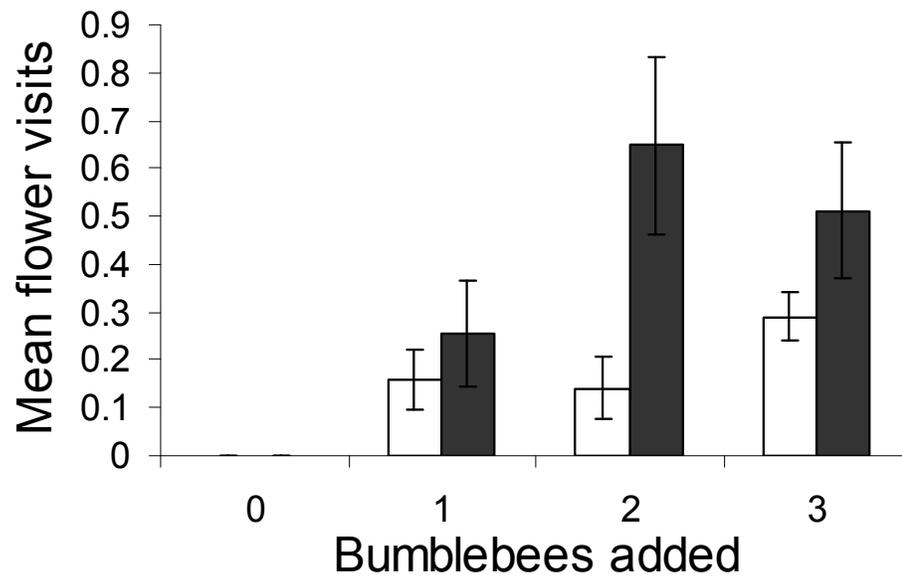
CHAPTER 4

HABITAT-SCALE RELATIONSHIPS BETWEEN NEST ABUNDANCE OF BUMBLEBEES AND REPRODUCTIVE SUCCESS OF THEIR HOST PLANTS

Jennifer C. Geib

ABSTRACT

Recent reports on the status of pollinators in North America and documented declines of multiple pollinating species have highlighted the need for improved monitoring of pollinator populations and better understanding of consequences of pollinator population sizes for linked plants. I used highly variable microsatellites to assess sibships among sampled worker bees of four native North American *Bombus* species, *B. balteatus*, *B. flavifrons*, *B. bifarius*, and *B. sylvicola*, and estimated the number of nests of each species in alpine habitats in the central Rocky Mountains, Colorado. I estimated foraging ranges for each species from nestmate distributions and evaluated habitat features that may function as foraging or dispersal barriers. I also examined relationships between habitat-level nest abundance and reproductive rates of two bumblebee-dependent host plants, *Trifolium dasyphyllum* and *T. parryi*.

Nest abundance varied among the four *Bombus* species, and between high and low alpine habitats. Effective population sizes of the four species ranged from 18.1 colonies to 77.8 colonies per .01 km^2

habitat, with the long-tongued species *B. balteatus* most abundant, especially high above treeline. *B. balteatus* nestmates were distributed among sites while *B. flavifrons* nestmates were primarily found within sites. Snowbeds and elevational gradients did not restrict nestmate distributions. However, isolation was associated with interpeak valleys and/or dense willow canopy. Foraging ranges of both subalpine and alpine *Bombus* species were generally smaller than those published for lowland species, with means ranging from 25-110 m. Fruit set of a specialized host plant, *Trifolium parryi,* increased with effective population size of its bumblebee partner, *B. balteatus*. However, the congeneric generalist species, *T. dasyphyllum*, showed little variance in fruit set with the number of *Bombus* nests. There was a nearly 1:1 correspondence of abundance of foragers of each species and their nest numbers within habitats. Relationships of plant reproductive rates with forager abundance were similar to that with nest abundance, except that total number of *Bombus* foragers was the only significant predictor for seed production of either clover. These results suggest that for natural resource managers, forager abundance is an appropriate measure for monitoring the status of pollinator services.

Key words: mutualism, facultative, density dependence, pollination, pollination niche, specialization, *Trifolium dasyphyllum*, *Trifolium parryi*, alpine clover, *Bombus balteatus*

INTRODUCTION

Recent declines of pollinating species in Europe and North America have prompted calls for greater monitoring of pollinator populations and greater understanding of relationships between pollinators and plants (NRC 2007). Pollinator forager densities correlate with those of linked plant species across continents (Biesmeijer et al. 2006) and at the individual and population scales (Chapters 1 and 2). Yet, populations are integrated via dispersal and colonization at the habitat scale, and key ecological processes play out in this domain. From the pollinator perspective, data suggest that habitat- and landscape-level distributions of floral resources impact the abundance, diversity, and richness of individual foragers, and that these relationships exist at different spatial scales (habitat vs. regional landscape) depending on pollinator taxa (Westphal et al. 2006). Less clear is whether plant reproductive success correlates with habitat-scale variation in pollinator abundance. While yield of agro-forestry resources (e.g., coffee, Klein et

al. 2002, 2003) often correlates with pollinator density and diversity in surrounding forest ecosystems, it is unclear whether plants in natural communities show similar dependencies.

One challenge in evaluating relationships between pollinator abundance and plant reproduction at the scale of habitat or landscape has been accurate assessment of pollinator population abundance. Individual forager counts provide estimates of effective population size only for solitary pollinator species; assessing effective population size of eusocial species, including bumblebees and honeybees, requires estimating colony numbers as individual foragers are not reproductive. Until recently, robust estimates of natural colony abundance for such eusocial species remained elusive due to difficulties in locating nests and lack of adaptable technologies. Methods employed to locate and map natural bumblebee colonies include systematic searching of habitats (Cumber 1953, Harder 1986, Osborne et al. 2008b) and use of canines to sniff out colonies (Scott 2008). More recently, development of algorithms that reconstruct genetic relationships among sampled individuals of social pollinating species (Goodnight and Queller 1999, Konovalov et al. 2004, Wang 2004, Konovalov et al. 2005, Kokuvo et al. 2007a, Kokuvo et al. 2007b) have allowed estimation of effective population sizes in different habitats. This method has been used frequently for *Bombus* species in Britain and recently in Japan (Chapman et al. 2003, Darvill et al. 2004, Knight et al. 2005, Kokuvo et al. 2008, Knight et al. 2009). To date however, no published studies of effective population size for social pollinators exist for North America, despite the importance of such species for numerous plant taxa (NRC 2007). Here I employed molecular techniques to ascertain habitat-level effective population sizes of bumblebee pollinators servicing different habitats within a North American alpine tundra ecosystem. I then assessed habitat-scale relationships between the number of nests in these areas and the reproductive success of outcrossing plants serviced by these pollinators. I also evaluated the relative use of nest counts vs. individual forager counts in predicting plant reproductive rates.

Whether and at what spatial scales host plant fitness correlates with pollinator nest density should depend on the propensity for individual workers to forage near to or far from their central nest location. Reproductive success of plants visited by pollinators with large average foraging ranges are more likely to be averaged across habitats, whereas for plants visited by pollinators with relatively short foraging ranges, fecundity should vary with habitat-level colony abundance. Predictions of appropriate spatial scale are difficult because mark-recapture methods underestimate the tendency to forage far from nests (Knight et al.

2005) while use of harmonic radar, to track trajectories of individual foragers is not practical in structurally heterogeneous vegetation, and at best has a maximum detection range of 700 m (see Osborne et al. 1999, Riley et al. 1999). Molecular methods of characterizing large scale foraging patterns are most promising. Moreover, estimation of the scale over which pollinators move has primarily targeted agricultural habitats where homogeneous vegetation ensures optimal detection. Findings suggest that foraging range varies among eusocial pollinating species with most maximum foraging distances between 500 m and 1000 m (Osborne et al. 1999, Knight et al. 2005) and a few species foraging 1500 m or more away from the nest (Walther-Hellwig and Frankl 2000, Osborne et al. 2008a). Foraging range varies with body size (Westphal et al. 2006, Greenleaf et al. 2007), suggesting that plants dependent on larger pollinators may have interbreeding populations at more vast scales (but this depends on pollen carryover; see Waser 1982). Body size likely affects the energy balance of foraging in ways that impact foraging range. Yet in most other respects, we have little knowledge of how different pollinating species experience a landscape, and to what extent they perceive different natural habitat features as foraging boundaries (but see Galen and Plowright 1985, Kreyer et al. 2004).

In alpine habitats, bumblebees are keystone pollinators, yet their numbers are thought to have declined markedly in recent years (Goulson 2008). Foraging ranges for alpine *Bombus* species have not been estimated, though a recent subalpine mark-recapture study (Elliott 2009) suggests ranges may be small relative to estimates from *Bombus* in agricultural habitats. Alpine habitats change sharply over a small spatial scale due to elevation gradients in biotic and abiotic environmental factors. Temperature, air density, and oxygen partial pressure, for example, decline with altitude (Dillon et al. 2006), impacting bees' physiology, metabolic foraging costs, and potentially brood rearing success (Lundberg 1980, Bergman et al. 1996, Dillon et al. 2006). In addition alpine landscape features may be perceived as foraging and dispersal barriers including snowbeds, forest-tundra transitions, and inter-peak valleys.

Here I use molecular methods to examine habitat-scale effective population sizes and foraging patterns of *Bombus* species servicing alpine plants. I also assess whether reproductive rates of their host plants correlate with bumblebee colony density at the habitat scale. Findings are relevant to our fundamental knowledge of pollination mutualisms and perhaps more importantly, key to managing and conserving both bumblebee and plant biodiversity above tree-line. My specific aims were to 1) assess the

number of bumblebee colonies servicing local alpine habitats ("effective population size"), 2) estimate foraging ranges of alpine bumblebee workers and assess their propensity to traverse natural habitat "boundaries", and 3) test for correlations between *Bombus* species' nest abundance and reproductive rates in a sub-sample of their host plants with contrasting pollination niche breadth.

MATERIALS AND METHODS

Study system

This study was conducted on Pennsylvania Mountain (Park County, Colorado; USA). Pennsylvania Mountain is divided by the headwaters of an east flowing stream, Pennsylvania Creek. The southern portion of the mountain comprises a series of relatively gentle east-facing slopes, each topped by ridges or plateaus, extending from treeline ("low alpine"; 3530 m) to the summit ("high alpine"; 4000 m). I selected two low alpine and two high alpine sites (Fig. 1), all of which support a rich assemblage of bee-pollinated species (Byron 1980). Sites were separated by natural landscape features providing potential foraging barriers for bumblebee pollinators (e.g. ridges, large permanent snowbeds and inter-peak valleys; Table 1). At the center of each site, I placed a marker and delineated a 50 m radius circle around it. Linear distance between the center points of the sites varied from 255 m to 784 m, as measured by GPS (Garmin 60CSx, Garmin Ltd.) (Table 1).

Prior studies on Pennsylvania Mountain identified two permanent native *Bombus* resident species above treeline, a long-tongued species, *B. balteatus*, and a short-tongued species, *B. sylvicola*; these two species comprised 99% of all observed foraging individuals in both low and high alpine habitats (Byron 1980). However, in 2008, large numbers of a short-tongued subalpine species, *B. bifarius*, and a medium-tongued subalpine species, *B. flavifrons*, were also observed. Thus, I chose to include all four of these species in assessments of colony abundance.

To assess habitat scale relationships between *Bombus* colony abundance and reproductive success of bumblebee-dependent alpine plants I focused on two native alpine clovers, *Trifolium dasyphyllum* and *T. parryi*. Both species are abundant from treeline upward in the central Rocky Mountains and depend to varying degrees on bumblebee pollinators. *T. parryi* is a *B. balteatus* specialist as evidenced by variation in

individual fitness and population growth rate with *B. balteatus* density (Chapter 3). *T. dasyphyllum* has a more generalized pollination niche (Chapter 3) receiving visits from multiple bumblebee species (Macior 1974). Reproductive success in *T. dasyphyllum* is not known to vary strongly with abundance of any one *Bombus* species.

Worker bee sample collection

I systematically sampled foraging workers bees within each of the four alpine sites on Pennsylvania Mountain. All workers were sampled near the end of the flowering season, August 1-August 15, to avoid possible negative effects of worker removal on brood rearing. Bees were sampled by walking a spiral pattern from the site center and capturing all observed *Bombus* foragers (Table 1). Upon capture, bees were cooled to torpor, placed in vials containing 95% ethanol, and stored in a -20 C freezer until analysis. The four most abundant species, *B. balteatus*, *B. flavifrons*, *B. sylvicola*, and *B. bifarius* were included in subsequent analyses.

Microsatellite genotyping of individual bees

DNA extraction, amplification and PCR were conducted at the USDA Bee Lab in Logan, Utah, in February 2009. Bees were removed from vials, then dried and pinned to verify species identities. A metathoracic leg was taken from each bee, cut into 4-6 pieces, and placed into a separate well of a 96-well plate. DNA was extracted using a modified Chelex protocol (Walsh et al. 1991, Erler and Lattorff 2010): 5 µl of (10 mg/ml) Proteinase K in dH_2O and 150 µl of 5% Chelex 100 in dH_2O were added to each well, followed by incubation at 55°C for 1 hour, 99°C for 15 minutes, 37°C for 1 minute, and 99°C for 15 minutes. DNA (1 µl) was amplified at ten microsatellite loci (B10, B96, B116, B119, B124, BL11, BL13, BT10, BT28, BTERN01; Estoup et al. 1995, 1996, Funk et al. 2006), five primers per reaction, using FAM-, VIC-, PET-, or NED-labeled forward primers. Amplified PCR products were sized on an ABI3730 (Applied Biosystems) sequencer with GeneScan LIZ 500 internal size standard (Applied Biosystems). Alleles were scored using GeneMapper 4.0 software (Applied Biosystems). Samples which lacked data or were ambiguous at more than one locus were re-processed from the original extracted DNA. Despite reprocessing, some individuals failed to yield genetic data. Genotyping success was generally high (85-92%), with the exception of samples from site 3 which had only a 50% success rate (Table 1). The

underlying cause for low data from site 3 is unclear, but tissue deterioration or a problem with the
extraction process may have contributed.

Nest distribution and effective population size

For each bumblebee species, the number of nests represented at each site is estimated from the
number of full sister groups found among genotyped worker bees within each sample. To assess
sisterships, I used the sibship reconstruction software COLONY 1.2, which uses a maximum likelihood
algorithm (Wang 2004) that currently provides the most accurate reconstruction of actual relatedness
patterns in bumblebee species (Lepais et al. 2010). Eight to nine loci were included in the sibship analysis
for each species, and only individuals with no missing data for all loci were used. The maximum
likelihood algorithm assumes single queen matings, and queens of one focal species, *B. bifarius,* have
occasionally been observed to mate with >1 male. However, *B. bifarius* parental genotype reconstruction
probabilities here were very high (near 1.0 for each locus), thus queens were likely monoandrous. To
ensure convergence of results in the sibship reconstructions, each species' dataset was analyzed three times,
each with a different random seed number. To account for variation in genotyping success, the number of
nest groups per site for each species determined by COLONY software ("raw" number of nests, N_{nr}) was
divided by number of successfully genotyped individuals (N_g) in each sample and multiplied by the actual
number of caught individuals (N_i) to get a "standardized" nest number (N_{ns}).

Despite intensive searching for bees at each site, it is likely that many nests were missed during
the sampling process. Many prior studies have dealt with this problem by assuming that bumblebee nests
are randomly distributed, and thus best described by a Poisson distribution. The frequency distribution of
nests represented by one, two, three… individuals within the sample is plotted and an iterative "trial and
error" process is used to estimate the value of the "0" (unsampled) category that provides the best fit
(Chapman et al. 2003, Darvill et al. 2004, Knight et al. 2005). However, small nest numbers preclude the
use of this process for site-level data. In addition, bumblebee nests may be aggregated rather than
randomly distributed (e.g. Harder 1986, Osborne et al. 2008b), making the assumption of a Poisson
distribution untenable. Therefore, for each site, I instead calculated effective population size (N_e) as $N_e =$
1.5 x standardized number of nests, N_{ns} (sensu Charman et al. 2010) based on N_e of social haplodiploid

species characterized by monogyny and monoandry: $N_e=(4.5Nnm)/(1+2m)$ where N=number of colonies, m=mating frequency, and n= number of queens per colony (Crozier 1979).

Here, the importance of calculating N_e is not to assess the size of the breeding population, but to assess relative *Bombus* abundance. N_e could not be statistically compared among sites within each species, due to lack of replication within sites. However, comparisons of N_e were conducted using ANOVA (SAS Proc GLM) in three ways. First, to assess whether overall mean *Bombus* colony abundance differed among alpine sites or whether overall mean colony abundance per site differed among *Bombus* species, I used an ANOVA and included site and species as fixed effects. Second, I assessed whether relative species abundances differed among high and low alpine sites by analyzing N_e with species, elevation (high and low), and their interaction as fixed effects. Third, as tongue length is predicted to impact bee associations with specific flower species and/or types and thus floral resource partitioning, I assessed whether relative abundances of short, medium, and long tongue *Bombus* differed among high and low alpine sites; I analyzed N_e with tongue length group, elevation (high or low), and their interaction as fixed effects. For the last two analyses, planned pair-wise comparisons were conducted using Least Squares Means. N_e was square-root transformed for all analyses.

To assess whether the number of caught foraging individuals per species in a particular site is representative of the number of nests of that species estimated by sibship reconstruction (N_{nr}), I used ANCOVA (SAS: Proc GLM) to test whether the relationship between N_i and N_{nr} differed among species. N_i was a continuous predictor variable and species was a categorical fixed effect in the model for N_{nr}. Because of the poor genotyping success at site 3, data from this site were not included in the analysis.

Estimates of bumblebee foraging range and isolation barriers

Foraging ranges of workers of each *Bombus* species were estimated from the distribution of sisters among the four sites (Darvill et al. 2004, Knight et al. 2005). For siblings found in circular sites with radius r and centers separated by a distance x, the most conservative estimate of foraging range from the nest was assumed: that the nest was located at the midpoint of the line connecting the site centers. The distance between the nest and the edge of each site was calculated as $x/2 - r$. This is a conservative estimate of foraging range as it is unlikely that all siblings' nests are perfectly centered between sites (Knight et al.

2005). The upper foraging limit for each species was estimated from the greatest separation observed between members of sister pairs.

Differences in mean sister separation and foraging distances among species were analyzed with ANOVA (SAS; proc GLM) with *Bombus* species as a fixed factor in the model. Pair-wise comparisons among species were conducted with Least Squares Means.

Impact of nest abundance on host plant fecundity

To assess whether clover fruit and seed set correlated with habitat-scale effective population sizes of bumblebees, I randomly collected infructescences of *T. dasyphyllum* and *T. parryi* within each site (N = 30-60 infructescences per site for each species). Large patches were selected for ease of collecting. I assessed per-flower fruit production, seeds per fruit, and seeds/flower for each species and conducted ANCOVA (SAS proc MIXED) to individually assess relationships between each dependent variable and (1) site-level nest abundance of *T. parryi's* primary pollinator, *B. balteatus,* (2) summed site-level nest abundance of all *Bombus* species, (3) site-level forager abundance of *B. balteatus*, and (4) summed forager abundance of all *Bombus* species. To assess whether relationships differed between the clovers, the categorical variable "plant species" was included in all models. All fecundity measures were square-root transformed for analysis.

Study system

RESULTS

Nests distribution and effective population size

The estimated number of nests per site (effective population size, N_e) spanned a wide range among and within species, ranging from 18.1-71.6 nests (Table 2). There were no overall differences in mean N_e among sites (species pooled) (Table 3A), but mean nests per site differed by species ($F_{3,9}$=3.97, P=.0468; Table 3A). *B. balteatus* colonies per site were overall more numerous than *B. bifarius* or *B. sylvicola* nests (pair-wise comparisons P<.05; Fig. 3). High alpine sites were serviced by significantly greater nest numbers of *B. balteatus* than of the other three species, and high alpine sites had more *B. balteatus* nests

than low alpine sites (planned pair-wise comparisons, P<.05; Fig. 4A).When analyzed with *Bombus* species grouped by tongue length, long tongue bee species' nests were more abundant than nests of medium and short-tongued bees, and there were more long tongue bee nests at the highest sites (planned pair-wise comparisons P<.05; Fig. 4B); however, both of these results are due to *B. balteatus* as it is the only long tongue species. Interestingly, for three species, *B. balteatus*, *B. bifarius,* and *B. flavifrons*, the difference in N_e between the two low alpine habitats was very high, with the site 2 being serviced by 19.4 fewer *B. bifarius,* 36.4 fewer *B. flavifrons,* and 16.4 more *B. balteatus* nests than site 1 (Table 2). Regardless of species or elevation, there was a strong relationship between the number of individual foragers caught (N_i) and the number of estimated nests (N_{nr}) servicing each site ($F_{1,7}$=6.70, P=0.002, R^2=.86; Fig. 5).

Estimates of bumblebee foraging range and isolation barriers

For all species, putative sisters were identified between sites separated by elevational gradients (Table 4). With the exception of *B. bifarius*, putative sisters were also identified between sites separated by a large snowbeds (ranging from 255-681 m apart). No sister pairs were identified for any species between the lower alpine sites separated by a wide inter-peak valley and spanning the greatest Euclidian separation distance (784 m). *B. balteatus* workers often foraged between multiple sites; 87% of all sister pairs identified were site-separated, whereas for other species, site-separated sister pairs ranged from 20% (*B. flavifrons*) to 40 or 50% (*B. bifarius* and *B. sylvicola*, respectively; Table 4).

Mean sister separation and foraging distance (Fig. 6) differed among species (respectively, SPECIES $F_{3,54}$= 4.86, P=.0046 and $F_{3,54}$= 4.20, P=.0096; Table 6), with *B. flavifrons* having a significantly lower separation and foraging distance than two of the three other species (planned pair-wise comparisons P<.05). Estimates of the upper limits of foraging ranges also varied among species (Table 6). *B. flavifrons* again exhibited the narrowest foraging zone ($\leq$ 202.5 m) while *B. sylvicola* foraged over the broadest area ($\leq$290.5 m).

Impact of nest abundance on host plant fecundity

Relationships between *B. balteatus* nest abundance ("effective population size", N_e) and per-flower fruit set varied between *T. dasyphyllum* and *T. parryi* (N_e*PLANT SPECIES $F_{1,321}$ =7.51, P=.0065; Fig. 7A, Table 7A). Fruit set per flower of *T. parryi*, the *B. balteatus* specialist, increased significantly with

habitat-level nest abundance of the long-tongue bee, *B. balteatus*, although the small portion of variance explained suggests numerous other factors affect fruiting level in this species ($F_{1,165}$=15.76, P=.0001; R^2= .088). Conversely, *T. dasyphyllum* fruit production was independent of *B. balteatus* N_e ($F_{1,156}$=0.06, P>.80). The number of *B. balteatus* foragers sampled was an equally good predictor for fruit set as nest abundance (Fig. 7B, Table 8A). There was no relationship between any measure of *B. balteatus* abundance and seeds per fruit or seeds per flower (Table 7B and C, Table 8B and C). Interestingly, fruit set of both clovers was also correlated with ΣN_e and ΣN_i for all *Bombus* species in each habitat (ΣN_e $F_{1,321}$ = 31.73, P<.0001; R^2=.14, Fig. 7C, Table 7D; ΣN_i $F_{1,321}$ = 25.15, P<.0001; R^2=.13, Table 8D) though the slopes of the relationship with ΣN_i differed between the clovers (ΣN_i*PLANT SPECIES $F_{1,321}$ = 4.22, P=.041; Table 8D). Seed set per flower for both clovers was significantly correlated with the summer forager abundance of all *Bombus* species (ΣN_i $F_{1,321}$ = 4.61, P=033; R^2=.02; Fig. 7D, Table 8F).

DISCUSSION

Results from this study in a North American alpine ecosystem show that habitat-scale colony abundance and foraging patterns differ among *Bombus* species. Colonies of long-tongued bees, which potentially have broader ranges of available resources, were overall more abundant than bees of other tongue lengths. By species, nests of the relatively large long-tongued alpine bumblebee *B. balteatus* were more abundant than nests of shorter tongued species, *B. bifarius* and *B. sylvicola*, especially far above treeline. Nestmates of *B. balteatus* were most often distributed among multiple habitats whereas *B. flavifrons* nestmates were primarily found within the same habitat. *B. balteatus* foraging range was also greater than that of *B. flavifrons*. Foraging ranges for alpine and subalpine *Bombus* species overall appeared to be smaller than their counterparts in lowland agricultural habitats. Landscape features were also identified that may function as dispersal barriers for foragers, and had consistent impacts for all *Bombus* species. Fruit set of plants specialized within the genus *Bombus* for pollination correlated with habitat-scale nest abundance of the partner species. For specialist and generalist clovers, fruit production also correlated with summed effective population size for all *Bombus* species. Forager abundance was as good a predictor of fruit production as nest abundance and was the only predictor of seed set, a result with implications for monitoring pollinator populations.

Effective population size of bumblebees

Numerous studies have investigated effective population sizes of *Bombus* species, though primarily in agricultural habitats. Estimates for the mean number of nests servicing individual sites of .01 km^2 range from 20.4-150 nests among species (Darvill et al. 2004, Knight et al. 2005, Osborne et al. 2008b, Knight et al. 2009). My results fit these data closely, with nests per species ranging from 18.1-71.6 within .01 km^2 site. However, predicted patterns of relative nest abundances among species as determined by past studies of forager distributions and nesting patterns in alpine habitats were only partially supported. In two past extensive surveys of *Bombus* forager abundance in the Colorado Rockies including a survey thirty years ago on Pennsylvania Mountain, *B. bifarius* comprised only 1-10% of short tongued species above treeline, at elevations ranging from 3400-4000 meters (Macior 1974, Byron 1980, Pyke 1982). In addition, the alpine bumblebee species *B. balteatus* and *B. sylvicola* were the only permanent residents constructing nests above treeline on Pennsylvania Mountain (Byron 1980). It was expected, therefore, that nest abundances of *B. balteatus* and *B. sylvicola* should greatly exceed those of the subalpine species *B. bifarius* and *B. flavifrons* (Macior 1974, Byron 1980, Pyke 1982), especially high above treeline. Instead, my results suggest that the subalpine species, *B. bifarius,* comprises 40-74% of short tongued individuals, and that only the long-tongued bumblebee *B. balteatus* was more abundant, even at the highest sites (>3700 m). This trend is surprising, but consistent with predictions of altitudinal range expansions under climate change for diverse species including social insects (Parmesan and Yohe 2003, Kirilenko and Hanley 2007, Jepson et al. 2010). These data, along with mounting evidence for declines in North American pollinator species, threats to alpine habits from anthropogenic disturbances (Ricketts et al. 1999), and predictions of an increasingly warmer more arid climate in the future (Saunders et al. 2008), suggest that it is critical to continue to monitor further changes in the status of alpine pollinator populations.

Bombus foraging ranges and isolation barriers

Flight distances vary among Apidae pollinators, with ranges extending up to many kilometers: e.g. up to 5 km, 10 km, and 24 km for *Xylocopa* spp., honeybees, and euglossine bees, respectively (Kapil and Daliwahl 1969, Janzen 1971, Visscher and Seeley 1982 in Cresswell et al. 2000). For bumblebees, energetic models suggest that individuals should rarely forage more than 1000 m from their central nest

location unless nearby resources are scarce or distant resources are so great that they outweigh metabolic costs (Cresswell et al. 2000). Species in lowland agricultural habitats forage from 275-800 m on average (Osborne et al. 1999, Knight et al. 2005, Westphal et al. 2006, Osborne et al. 2008a). Foraging ranges reported in this study are likely underestimates since sisters caught in the same site were assigned a separation distance of 0 m but may have been separated by as much as 100 m. In addition, small worker sample sizes reduce the likelihood of capturing sisters at separate habitats, and the maximum foraging distance detectable was limited by the maximum distance between the sites Nonetheless, my findings along with two subalpine studies (Bowers 1985, Elliott 2009) suggest that higher elevation bumblebees may have shorter foraging ranges than lowland counterparts. Though nestmates of one species, *B. balteatus*, were often observed foraging at sites separated by broad physical barriers, average foraging distances were modest, ranging from 25-100 m. Elliot (2009) suggested that the shorter blooming season and reduced time available for nest construction in high elevation habitats may force bumblebees to forage closer to their nests to maximize energetic return.

There is conflicting evidence for the idea that landscape features act as foraging and dispersal barriers for pollinators (Plowright and Galen 1985, Bhattacharya et al. 2003, Kreyer et al. 2004, Zurbuchen et al. 2010). Here, results contribute to this debate. Nestmates of all four *Bombus* species were found in sites separated by permanent snowbeds and elevational gradients. Long flat plateaus between slopes (e.g., between sites 1 and 3 as well as 2 and 4) did not appear to limit nestmate distributions in any species. Interestingly, no nestmates of any species were found in sites that would require a direct crossing of the dense willow canopy situated in the wide interpeak valley (Fig. 1), even though bumblebees nest in such canopies (Byron 1980). That the species composition of one low alpine site (site 2; Fig. 1) was more similar in character to the high alpine sites (see Table 2 and Fig. 2) may also have been a consequence of features of the surrounding habitat. High numbers of nestmates of the alpine species *B. balteatus* were observed between site 2 and the high alpine site 4, suggesting facilitation of forager exchange by a lack of foraging barriers, while the dense willow canopy below site 2 may limit the influx of subalpine foragers. Alternatively, heterogeneity of snow pack in the krummholz and low alpine region, a critical factor for overwintering success of arctic and alpine insects (Danks 2004), could lead to patchier species distributions in the lower alpine sites.

Relationships between clover reproduction and habitat-level Bombus nests

For specialized host plants, individual fitness and population growth rates vary with the abundance of their primary pollinators (Pellmyr and Huth 1994 and Chapter 3). Accordingly, similar relationships might be expected for specialists at a habitat scale. In part, results from this study agree with this prediction: for *T. parryi,* a specialist on *B. balteatus*, fruit production varied with colony abundance. Mean foraging range for *B. balteatus* was relatively small, 85.4 m promoting covariation between plant fecundity and pollinator density. Reproductive rates of plants that are pollination generalists are less likely to vary with abundance of any one pollinating species, as co-pollinators disrupt the relationship (Holland and Fleming 2002 and Chapter 3). *T. dasyphyllum* is visited by multiple *Bombus* species and is a broad generalist for pollination throughout its blooming period (Chapter 3). As with its individual fitness and population growth rate (Chapter 3), here fruit set was independent of *B. balteatus* abundance, and instead correlated with summed nests of all *Bombus* species.

While neither species' habitat-scale seed production correlated with bumblebee nest abundance, both species seed set correlated with total number s of foraging *Bombus* individuals, suggesting that, in part, reproduction in clovers may be maintained despite year to year fluctuations in any one species, if balanced by influx of another. Never-the-less, the model explained little variation in seed set (only 2%), likely due to the fact that heterogeneity in resources is pronounced in alpine habitats and seed set for plants of both clovers is partially resource limited (Chapter 3). However, total offspring production may still be highly dependent on pollinator density because (as seen in Chapter 3) offspring quality can vary with pollinator abundance as plants have more opportunities for mate choice.

Findings from this study have implications for pollinator conservation and management. Here I show some of the first estimates of habitat-level nest abundance for important social pollinators in a North American ecosystem, and find evidence that species distributions have shifted upward, a pattern consistent with prediction of the effects of global warming. In addition, the assessment of the strong relationship between nest abundance and forager abundance of each species, as well as their similar predictive abilities for the habitat-scale reproduction of host plants has implications for continued monitoring of the status of individual social pollinator species. Results indicate that forager counts, while time consuming, may be more cost efficient, less detrimental to bumblebee populations and equally accurate to use of molecular

genetic methods. Given the importance of bumblebees in alpine habitats and their sensitivity to rapidly

changing climate dimensions, long term monitoring to build on these data is highly advisable. Findings

suggest that such monitoring efforts may be relatively inexpensive and straightforward.

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Table 1. Distance between sites and habitat features separating them (see Fig. 1 for site locations).

Sites		Separation distance (m)	Habitat features separating sites
1	1	0	
1	2	784	willow canopy and interpeak valley
1	3	541	elevation gradient, flat between slopes
1	4	681	elevation gradient, snowbed, interpeak valley
2	2	0	
2	3	505	elevation gradient, snowbed, flat between slopes
2	4	255	elevation gradient, snowbed
3	3	0	
3	4	266	meadows and fellfield
4	4	0	

Table 2. *Bombus* workers sampled (N_i), successfully genotyped individuals (N_g), raw nest numbers (N_{nr}), standardized nest numbers (N_{ns}), and effective population size (N_e) for sites on Pennsylvania Mountain. For site locations see Fig. 1.

Site	Habitat	*B.balteatus*					*B. flavifrons*					*B. bifarius*					*B. sylvicola*				
		N_i	N_g	N_{nr}	N_{ns}	N_e	N_i	N_g	N_n	N_{ns}	N_e	N_i	N_g	N_n	N_{ns}	N_e	N_i	N_g	N_n	N_{ns}	N_e
1	low alpine	18	16	15	16.9	25.3	44	40	33	36.3	54.5	28	27	25	25.9	38.9	9	9	9	9	13.5
2	low alpine	29	24	23	27.8	41.7	29	24	10	12.1	18.1	13	12	12	13	19.5	11	9	8	9.78	14.7
3	high alpine	46	25	25	46	69	35	26	20	26.9	40.4	18	9	8	16	24.0	23	10	9	20.7	31.1
4	high alpine	51	47	44	47.7	71.6	24	22	21	22.9	34.4	17	13	13	17	25.5	15	12	11	13.8	20.6

Table 3. ANOVA testing for differences in effective population size (N_e) of *Bombus* species on Pennsylvania Mountain, CO. A) test for variation in N_e among sites and among *Bombus* species B) test for variation in N_e among *Bombus* species and elevations. C) Differences in N_e among *Bombus* tongue length groups and elevations.

	Source	DF	SS	F Ratio	Prob > F
A)	site	3	5.37	1.37	.3135
	species	3	15.58	3.97	.0468
	error	9	11.77		
B)	species	3	15.58	5.03	.0302
	elevation	1	3.77	3.65	.0924
	species*elevation	3	5.11	1.65	.2541
	error	8	8.26		
C)	tongue	2	14.47	6.74	.0140
	elevation	1	4.65	4.33	.0642
	tongue*elevation	2	3.74	1.74	.2247
	error	10	10.74		

Table 4. Distribution of sister pairs for each *Bombus* species. Numbers in each cell are the number of sister pairs per species found between sites.

Sites where sisters were identified		*B. balteatus*	*B. flavifrons*	*B. bifarius*	*B. sylvicola*
Sister 1	Sister 2				
1	1	1	9	3	0
1	2	0	0	0	0
1	3	1	0	4	0
1	4	0	0	0	1
2	2	1	5	0	1
2	3	1	2	0	0
2	4	10	4	0	0
3	3	0	9	1	1
3	4	1	0	0	1
4	4	0	1	0	1

Table 5. ANOVA for variation in A) mean separation distance between sisters of each *Bombus* species and B) mean foraging distance among *Bombus* species.

Source	DF	SS	F Ratio	Prob > F
A) Mean separation distance between sisters of each *Bombus* species				
species	3	498941	4.86	0.0046
error	54	1847680		
B) Mean foraging distance among *Bombus* species				
species	3	69299	4.20	0.0096
error	54	296797		

Table 6. Total sister pairs, total site-separated sister pairs (with percent in parentheses), maximum sister separation distance (m), conservative estimate of foraging range upper limit (m), and mean foraging range for each species (m) with standard error in parentheses.

Species	Total sister pairs	Total site-separated sister pairs (%)	Max. sister separation (m)	Min. range upper limit (m)	Mean foraging range (m) (+SE)
B. balteatus	15	13 (86.7%)	541	220.5	85.4 (15.0)
B. flavifrons	30	6 (20%)	505	202.5	23.8 (10.1)
B. bifarius	8	4 (50%)	541	220.5	110.25 (41.7)
B. sylvicola	5	2 (40%)	681	290.5	74.7 (56.3)

Table 7. Relationships between (A-C) clover reproductive rates and habitat-level nest abundance (N_e) of *B. balteatus,* and (D-F) clover reproductive rates and habitat-level summed N_e for all *Bombus* species.

Source	DF	Type III SS	F Ratio	Prob > F	Source	DF	Type III SS	F Ratio	Prob > F
A) Mean fruits/flower and *B.balteatus* N_e					D) Mean fruits/flower and ΣN_e				
Whole model					Whole model				
N_e	1	0.684	9.48	0.0023	ΣN_e	1	2.182	31.73	<.0001
plant species	1	0.043	0.60	0.4404	plant species	1	0.037	0.55	0.4607
N_e * plant species	1	0.542	7.51	0.0065	ΣN_e * plant species	1	0.171	2.48	0.1162
error	321	23.17			error	321	22.08		
T. dasyphyllum									
N_e	1	0.004	0.06	0.8021					
error	156	9.968							
T. parryi									
N_e	1	1.260	15.76	0.0001					
error	165	13.21							
B) Mean seeds/fruit and *B.balteatus* N_e					E) Mean seeds/fruit and ΣN_e				
Whole model					Whole model				
N_e	1	0.218	1.35	0.2462	ΣN_e	1	0.002	0.01	0.9139
plant species	1	0.377	2.34	0.1273	plant species	1	0.250	1.54	0.2149
N_e * plant species	1	0.011	0.07	0.7984	ΣN_e * plant species	1	0.066	0.41	0.5229
error	321	51.79			error	321	51.95		
C) Mean seeds/flower and *B.balteatus* N_e					F) Mean seeds/flower and ΣN_e				
Whole model					Whole model				
N_e	1	0.233	2.91	0.0889	ΣN_e	1	0.192	2.40	0.1221
plant species	1	0.097	1.21	0.2722	plant species	1	0.073	0.91	0.3409
N_e * plant species	1	0.074	0.92	0.3380	ΣN_e * plant species	1	0.058	0.73	0.3944
error	321	25.65			error	321	25.71		

Table 8. ANCOVA testing for relationships between clover reproductive rates and (A-C) habitat-level forager abundance (N_i) of *B. balteatus*, and (D-F) habitat-level summed N_i for all *Bombus* species.

Source	DF	Type III SS	F Ratio	Prob > F	Source	DF	Type III SS	F Ratio	Prob > F
A) Mean fruits/flower and *B.balteatus* N_i					D) Mean fruits/flower and ΣN_i				
Whole model					Whole model				
N_i	1	0.709	9.81	0.0019	ΣN_i	1	1.750	25.15	<.0001
plant species	1	0.036	0.50	0.4812	plant species	1	0.157	2.25	0.1343
N_i * plant species	1	0.500	6.91	0.0090	ΣN_i * plant species	1	0.294	4.22	0.0407
error	321	23.20			error	321	22.34		
T. dasyphyllum					*T. dasyphyllum*				
N_i	1	0.009	0.14	0.7087	ΣN_i	1	0.295	4.76	0.0307
error	156	9.963			error	156	9.677		
T. parryi					*T. parryi*				
N_i	1	1.230	15.28	0.0001	ΣN_i	1	1.800	23.45	<.0001
error	165	13.24			error	165	12.67		
B) Mean seeds/fruit and *B.balteatus* N_i					E) Mean seeds/fruit and ΣN_i				
Whole model					Whole model				
N_i	1	0.149	0.92	0.3378	ΣN_i	1	0.135	0.84	0.3603
plant species	1	0.376	2.33	0.1281	plant species	1	0.143	0.89	0.3465
N_i * plant species	1	0.012	0.08	0.7829	ΣN_i * plant species	1	0.046	0.28	0.5954
error	321	51.86			error	321	51.83		
C) Mean seeds/flower and *B.balteatus* N_i					F) Mean seeds/flower and ΣN_i				
Whole model					Whole model				
N_i	1	0.187	2.33	0.1275	ΣN_i	1	0.366	4.61	0.0326
plant species	1	0.086	1.07	0.3023	plant species	1	0.109	1.37	0.2424
N_i * plant species	1	0.063	0.79	0.3748	ΣN_i * plant species	1	0.096	1.21	0.2727
error	321	25.71			error	321	25.49		

FIGURE LEGENDS

Figure 1. Pennsylvania Mountain study sites. See descriptions in Table 1.

Figure 2. Species composition of worker bee samples collected at each of the four study sites sites.

Figure 3. Mean nest abundance per site (N_e) among *Bombus* species. Different superscripts indicate significant differences between groups at $p<.05$.

Figure 4. A) Comparisons of effective population size (N_e) between low and high alpine habitats and among *Bombus* species. B) Comparisons of effective population size (N_e) between low and high alpine habitats and among *Bombus* of different tongue lengths. Filled squares = *B. balteatus* (long tongue), open circles = *B. flavifrons* (medium tongue), open triangles = *B. bifarius* (short tongue), filled diamonds = *B. sylvicola* (short tongue), filled triangles = pooled N_e of short tongue species. * indicates that, within species, differences in N_e between low and high alpine sites are significant at $P<.05$. Within low and high alpine sites, different letters indicate significant differences in N_e among species at $p<.05$.

Figure 5. The relationship between number of individuals caught per species at each site (N_i) and effective population size (N_e).

Figure 6. Mean separation distance between worker nest mates and worker foraging distances for four *Bombus* species foraging in alpine habitats on Pennsylvania Mountain. Groups with different lower case letters differ significantly ($p<.05$) for separation distance between worker nest mates (black bars) and mean worker foraging distances (white bars).

Figure 7. The relationship between habitat-scale pollinator abundance and per-flower fruit and seed production for *T. dasyphyllum* and *T. parryi*. In each graph pollinator abundance is represented by A) *B. balteatus* nest abundance per site (N_e), B) summed N_e for all *Bombus* species per site (ΣN_e), C) *B. balteatus* individuals caught per site (N_i), and D) summed *Bombus* individuals caught per site (ΣN_i). Triangles with solid lines are *T. parryi*. Circles with dashed lines are *T. dasyphyllum.* Closed symbols represent mean fruits/flower, and open represent mean seeds/flower. Error bars show standard error.

Figure 1

Figure 2.

Site 3

Site 4

Site 1

Site 2

□ balteatus
▨ flavirons
▤ frigidus
⊞ mixtus
■ sylvicola
▒ bifarius
▧ centralis

103
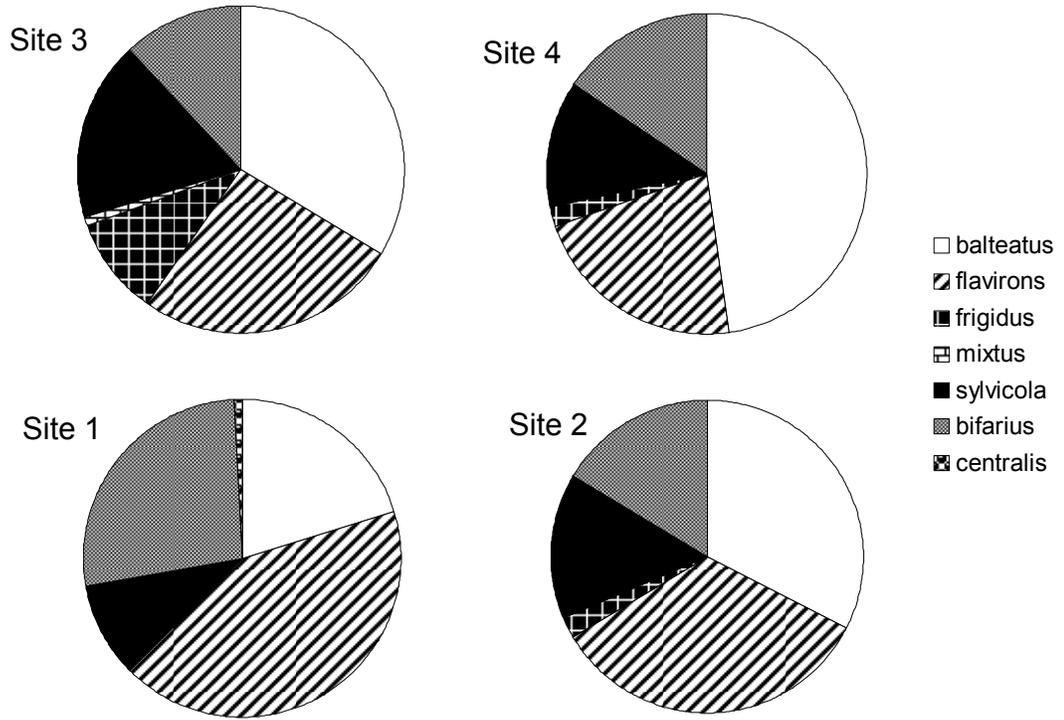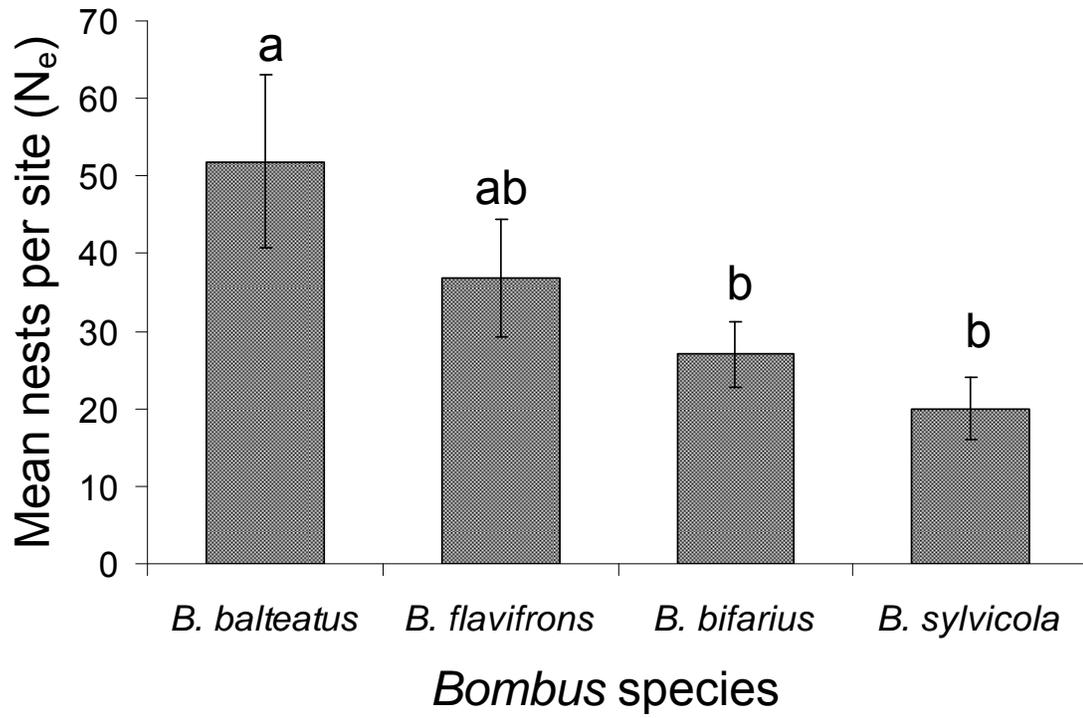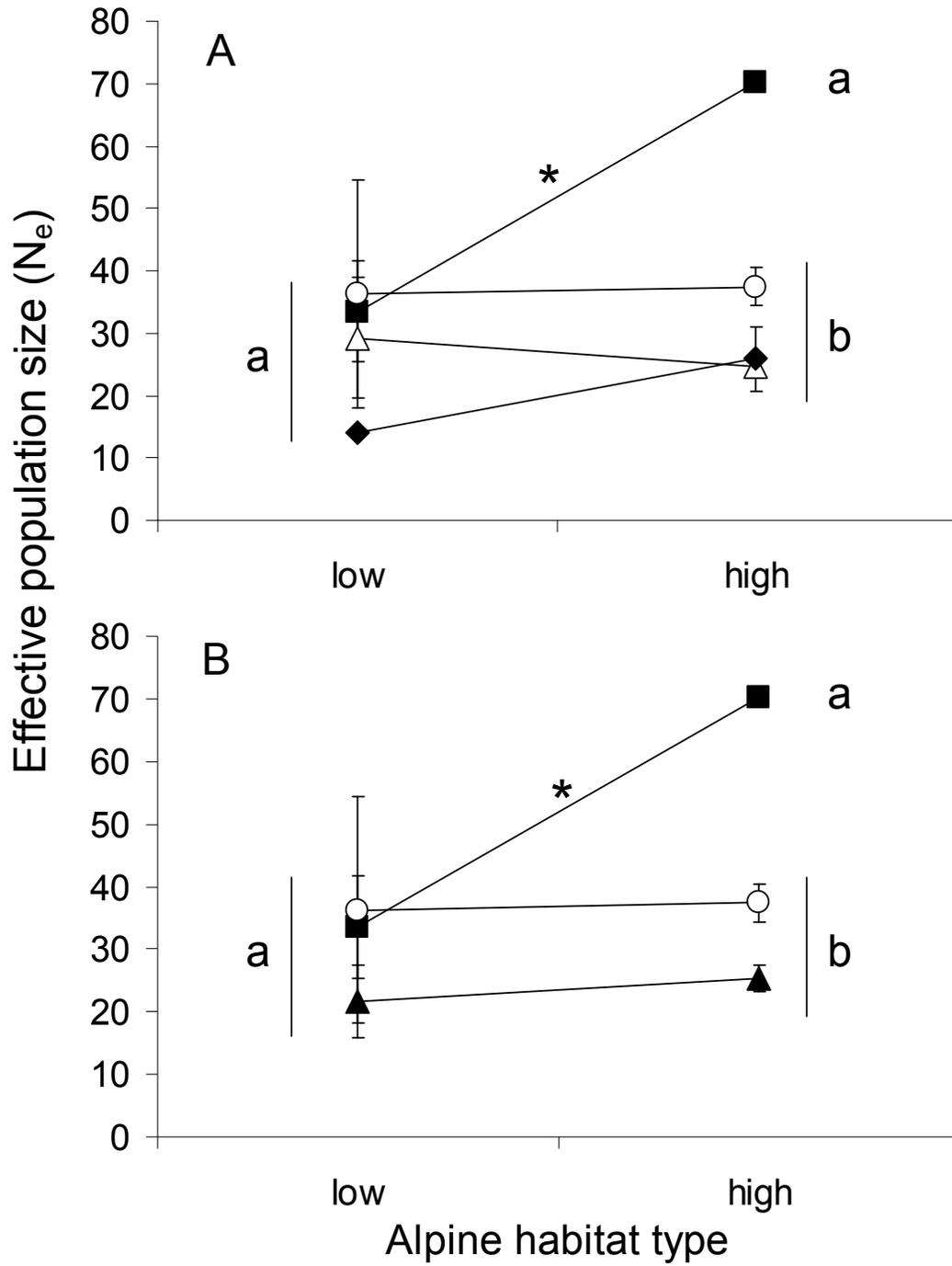
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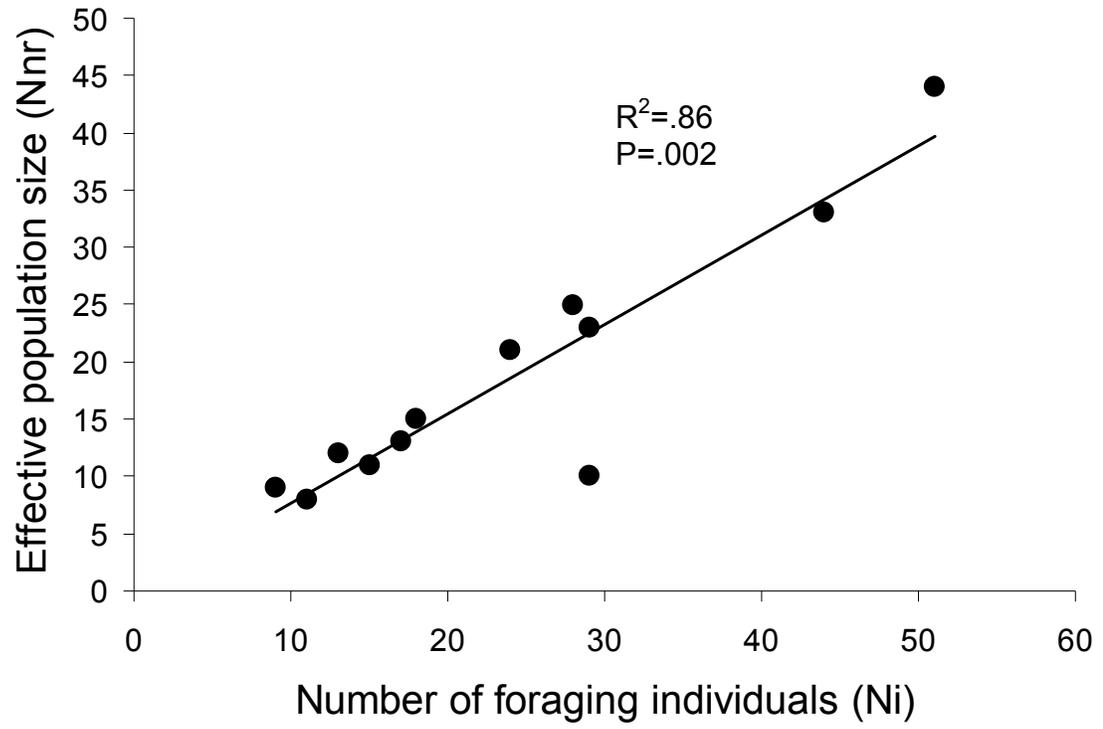
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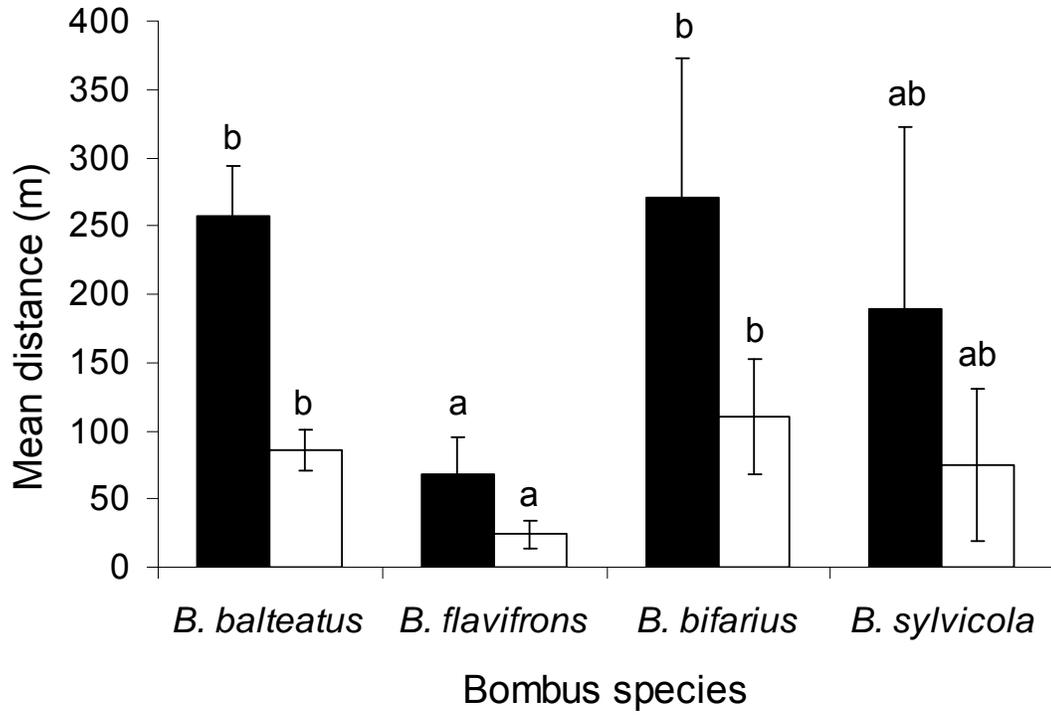
Figure 5.

Figure 6.

Figure 7
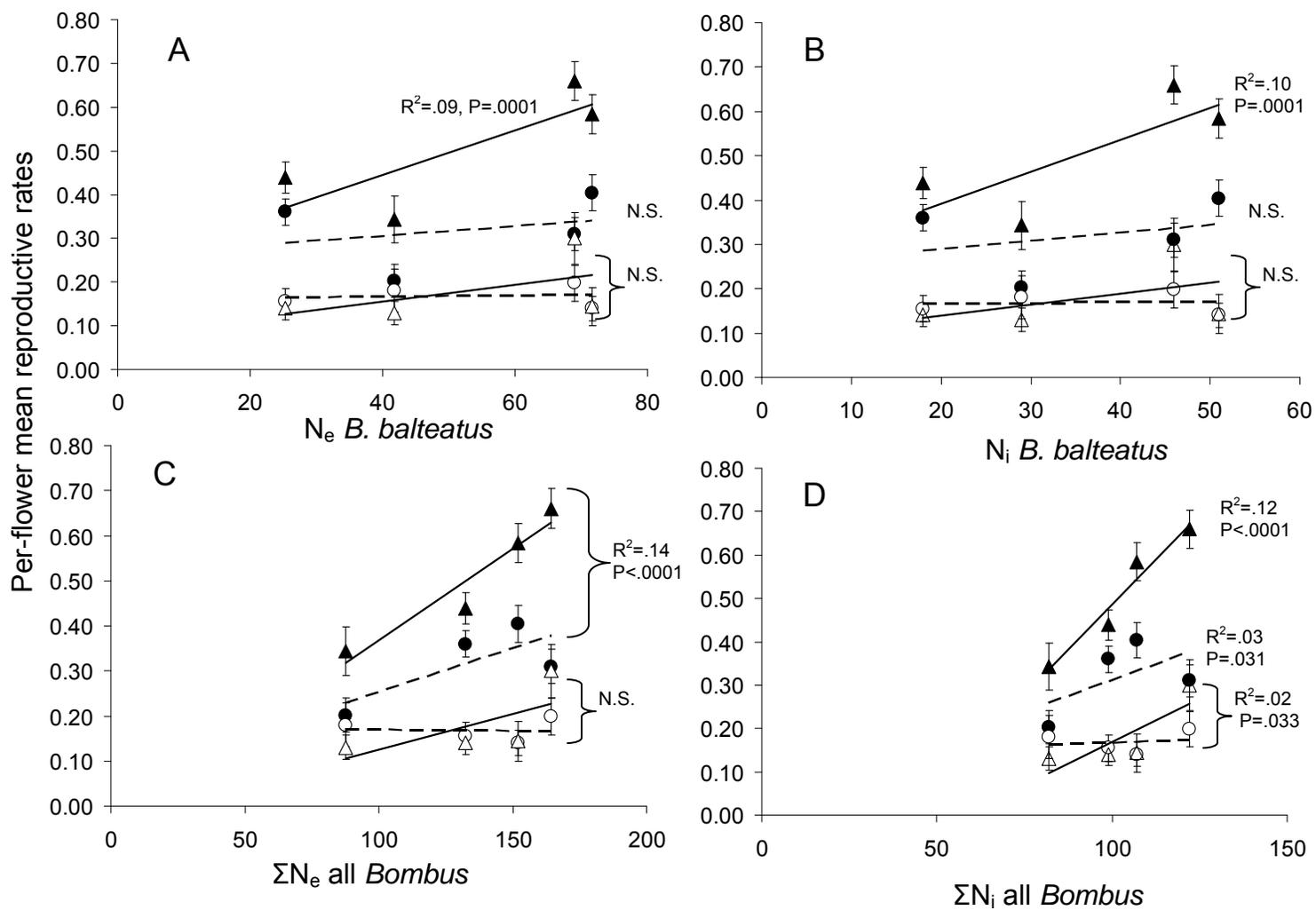
CHAPTER 5

A CASE STUDY OF NSF BROADER IMPACTS IN A K-12

SETTING: TEACHER ORIENTATIONS

Jennifer C. Geib

INTRODUCTION

In 1997, the National Science Foundation (NSF) revised its merit criteria for scientific grant proposals, for the first time asking scientists to consider not just the intellectual merit of their proposed research, but also the broader impacts of their research for society (NAPA 2001). NSF considers broader impacts to be an important part of their mission to promote the progress of science and advance the national "health, prosperity, and welfare" (Public Law 81-507 1950). Practically, though, scientists' efforts to address the broader impacts criteria have been lacking in quality (Frodeman and Holbrook 2007). NSF itself acknowledges that "many proposers have difficulty understanding how to frame the broader impacts of the activities they propose to undertake" (NSF 2007), which is an understatement of criticisms of broader impacts from some scientists (e.g. APS 2007), who as a group remain divided on the validity and worth of including broader impacts as a merit review criteria (Holbrook 2005).

To aid scientists' development of broader impacts in their proposals, a supplement to the NSF grant proposal guide (NSF 2007, 2009a) outlines the five components of the broader impacts criterion as defined by NSF, and representative activities that fall within each component. Here I detail a pollinator gardens outreach program, based on a pollination research project funded by NSF, which addresses two

components of Broader Impacts: "Broaden participation of underrepresented groups" and "Advance discovery and understanding while promoting teaching, training and learning". The first component and a portion of the second are met through involving diverse students in inquiry-based learning and investigative research related to plants and pollinators. However, the primary aim of the outreach program is to provide for teachers a model for effective incorporation of research in K-12 science education (Table 1).

What factors mediate the implementation and outcome of outreach intended to function as teacher professional development? First, scientists, as program developers, have a vision of the program they hope to implement and goals they hope to accomplish. To scientists this might seem like a simple linear process. However, the participating teacher is the gatekeeper to the very entry of the scientist and curriculum into the classroom (Fig. 1). He/she is a trained professional with opinions about what should occur for their students in the outreach program. These opinions reflect the teacher's "knowledge of the guiding purposes and frameworks for teaching science" in a certain context, defined as teaching "orientations" (Magnusson et al. 1999, Abell 2007), and may mediate the implementation of the outreach program.

The primary objectives of my study were to describe the implementation of the pollinator gardens outreach program and examine the how the science teaching orientations of the participant teacher may have mediated progression toward program goals. Because the program also aimed to promote future use of the outreach curriculum and teaching methods, another objective was to examine the orientations of the participating teacher following outreach and the prospects for the teacher's future use of the outreach curriculum and teaching methods. I examined teacher orientations and their role in mediating outreach activities within a bounded case study (Creswell 1998) of the pollinator garden outreach project. It is important to note that my dual roles as outreach curriculum developer/implementer and primary investigator made me a "participant observer" during the study (Adler and Adler 1994, Mack et al. 2005).

The specific questions addressed in this study were:

1) Prior to the implementation of the pollinator outreach program

    a. What are the science teaching orientation(s) of the teacher, especially related to use of the pollinator gardens?

    b. How does the teacher's orientation to the pollinator garden compare to his orientation during an (indoor) environmental science course?

  c. How does the teacher's orientation compare to that of the scientist, as outlined in the curriculum of

   the outreach program?

2) How do the participant teacher's orientations interact with implementation of the outreach program?

3) Following the implementation of the outreach program activities,

  a. Is there any change in the teachers' orientations related to use of the pollinator gardens?

  b. What is the prognosis for the teacher's future use of the curriculum materials and pedagogy

   developed by the scientist?

My aim in examining these questions is to provide a model for self-assessment of broader impacts activities

by other principal investigators.

THE POLLINATOR GARDENS OUTREACH PROGRAM

My vision: Orientations of the pollinator garden outreach program

  The pollinator outreach project that functioned as an "intervention" during this study of teacher

orientations utilized activities and materials based on my research of the impacts of pollinator abundance

on benefits from pollination mutualisms (Chapters 2-4). Planned curriculum and activities are listed in

Table 2.

  To elicit my own orientation toward use of the pollinator gardens, I developed an orientations

heuristic based on ideas about orientations and curricular emphases from published literature as well as my

own thoughts about the guiding purposes and frameworks for teaching science (Table 3). Existing

orientations classification schemes (e.g. Roberts 1988, Magnusson et al. 1999, Cheung and Ng 2000)

seemed to confound the *purposes of science teaching* with the *approach toward instruction* and/or *the focus

of action (teacher vs. student)*. Thus, in my own heuristic I delineated these three categories (see Table 3).

This heuristic was used only to clarify my own orientations in developing the pollinator garden curriculum.

  I developed the outreach garden curriculum with five goals in mind (in order of importance):

Students will 1) Display positive attitudes about pollinator garden experiences and activities; 2) Describe

pollination as relevant to their lives; 3) Exhibit curiosity about nature, and feel empowered to act upon it; 4)

View pollinators as important for society; 5) Describe basic understandings of pollination-related concepts.

In my heuristic (Table 3), this would correspond with primary orientations toward *everyday coping*, *science as inquiry*, and *science/technology/society*, with learning *academic* concepts as a "peripheral" orientation (Friedrichsen and Dana 2005).

Underlying the activities and teaching methods I planned for the outreach program were the views that 1) students have prior knowledge about gardens, pollinators, plants, and ecological interactions, and 2) students develop their own understandings about concepts internally through active experiences and reflection upon those experiences. I therefore planned instruction (Orientation II) reflecting a *conceptual change/constructivist* and *reflectivist* philosophy. This included activities to actively involve students (physically and cognitively) in learning with the instructor functioning as facilitator of students' experiences, thinking, reflections, and conceptual development. The planned activities loosely followed a 5E learning cycle (Bybee et al. 1989, Bybee et al. 2006; Table 2) where I would engage students' minds and allow them to explore concepts prior to introducing specific terms, definitions, or explanations. During activities I asked planned questions to access students' prior knowledge, elicit discussion and reflection, and scaffold student learning about pollination. However, I also allowed students' own interests and questions to change the direction of the lessons, reflecting a *student-centrism (*Orientation III). Concurrent with the topical lessons in the outreach program, I planned ongoing student-driven investigation in the gardens (Table 2), where students would develop research questions and design and implement experiments to answer them. I envisioned dissemination of students' findings on a student-developed website.

Vision vs. reality: Implementation of the pollinator gardens program

I conducted the outreach program at an urban Midwestern alternative high school. 2008 data for the school indicated that its 177 students were comprised of 48% African American, 50% white, and 2% Hispanic students; 69% of students received free or reduced lunch. This met the requirements of a Title 1 school as defined by the Elementary and Secondary Education Act (1970). The participant teacher, Joe Smith, was an experienced, white male teacher who was my former colleague in the same public school system from 1997-2000. Mr. Smith was selected as the collaborating teacher in this project for a number of reasons. He had a history of establishing wildflower and vegetable gardens at schools in the district. He

had an established after-school club that worked with the gardens and an environmental science course in which the topics of plant reproduction and plant-pollinator interactions were relevant. Though multiple teachers were contacted about participation in the outreach program, Mr. Smith was the only respondent who followed through. In addition, the school itself provided an ideal setting; though populated with students considered "challenging" due to behavior issues that prevent them from succeeding in a classic school setting, these same students seemed highly likely to benefit from active participation in the outdoor gardens. The outreach curriculum and pedagogy were designed to be used with wildflower gardens that Mr. Smith's students planted in 2008 and vegetable gardens planted in 2009.

I had originally thought that this school, teacher and setting would be perfect for the outreach program. I thought that my pollinator garden projects would fit naturally with Joe's instincts to take the student outdoors. I anticipated that Joe would give me free reign with the students, in part because this removed the burden of teaching from him. However, the realized outreach program was very different from my expectations, even from the planning stages. Joe Smith and I met first in February 2009, to discuss plans for the outreach program. I presented my lesson plans, and Joe was very agreeable. However, he also interjected ideas for multiple field trips for the students related to pollination: One to a local vegetable farm, one to a bee keeper, one to community gardens, and nature walks along the creek and at a state park. He didn't reject any of the activity ideas I proposed, but would only nail down specific dates for the first outreach lesson.

The first week of May, 2009, I conducted the first outreach lesson. My original agreement with Joe was to conduct the lessons with his after-school club, but the day before the lesson was to take place he requested that I conduct it with his Environmental Science class instead. Because I had requested permission from the district science coordinator to do lessons only with the club, I had to quickly email a request for permission to work with the class. Although the coordinator acquiesced, she was, ironically, scheduled to observe Mr. Smith's class (and evaluate his teaching) on that same day. I suspected that Mr. Smith's impulse to invite me to his class during the coordinator's visit was driven by what he perceived as a trade-off that would work in his favor: The coordinator would have positive feelings about the planned activities, even if she was irked that Mr. Smith himself was not the facilitator. This put me in an uncomfortable situation, but I proceeded due to the time constraints of needing to complete the program

113

before the end of the public school semester.

I started out the lesson with questions to assess students' prior knowledge about flowers and floral anatomy; holding a beautiful red tulip, I asked, "What is this? What is it for?" I had purposefully selected a tulip with a very prominent pistil. I asked students, "Is this one male or female?" One girl responded, "Oh that's male!" Another student shouted, "No! It's both; it has anthers and a stigma!" Because of the original plan to work with the after school club rather than the class, I was under the impression that "pollination" would be a separate topic from the students' curriculum. I was therefore surprised by this student's answer, and said, "You must have studied flowers before!" Joe then interjected, "I wanted the students to be ready for your activities, so I had them do worksheets on pollination last week!" Never-the-less, the first lesson went extremely well. After the introduction, students dissected numerous flowers to identify and compare floral anatomy. Students were excited and very engaged in the lesson, and almost everyone participated fully. I facilitated the lesson with the whole class, and both Mr. Smith and I worked with students individually. At the end of the dissections I showed students slides of different types of unusual flowers and had students predict who the pollinators might be. At the end of the lesson, Mr. Smith commented, "I had no idea that they would be so engaged." The next week I conducted a second lesson in which students were equally engaged, about fruits and the importance of pollination for agriculture.

After such a great start I had high hopes for the rest of the program. I kept telling Joe that I was ready to implement the investigation piece and get students into the gardens. However, he kept putting it off, instead asking me daily to join the Environmental Science class for nature walks along creeks, and field trips to state parks, community gardens, a flower nursery, and kayaking on a lake. He always told me how they could address issues related to pollination on these trips. I was amenable to this, and even wrote up ideas for tasks students could do. However, instead of letting me give students tasks during these expeditions, Mr. Smith himself took full control, leading students along with a constant narrative of what they were observing. For example, during a nature walk along a creek:

> I call this the poison ivy tree – three leaves, red stem – Box Elder. These three leaves look just like poison ivy, but it's not.
> Hey is this the one that taste like lemon? It is! Who wants to try this? Concord grape.
> French 1700s grape stock got root rot…now they graft their plants onto the root of this one from Missouri, well from the US.
> Little bugs plant their eggs on it, and the trees wrap around it. It's a gall. Open it and you should see some little insects. Yep, there are some little wormy guys.

It started to seem like Joe just wanted me along on these trips to have another adult present. When I finally

brought up the garden investigation again, Joe vetoed it. He just didn't think it would work for these

students. However, my perception was that Joe felt reluctant to turn control of the students over to me. To

understand what happened during the outreach program I turned to what I learned about Joe's orientations

toward teaching science.

GROUNDED THEORY: ORIENTATIONS AS A FACTOR MEDIATING OUTREACH

Paradigm

I approached this study of science teaching orientations and their impact on outreach as a

constructivist qualitative researcher (Guba and Lincoln 2005). This perspective describes knowledge as a

human construction and implies that the meaning assigned to knowledge is different for each individual.

Substantive theory

The study framework was based on assumptions of both cognitive and behavioral theory.

Cognitive theory assumes that subjects' thoughts may be accessed by listening to what they say. Cognitive

theory is an ideational theory, since it leads researchers to "view the human world through the perspective

of its mental origins" (Fetterman 1998). This is in contrast to materialist theories, which lead researchers to

examine subjects' observable behavior patterns to understand their world. However, my understandings of

Joe Smith's orientations in this study are derived from both his words and actions.

Data collection and analysis methods

To elicit Joe's science teaching orientations, especially toward use of the pollinator gardens, I used

grounded theory methodology (Glaser and Strauss 1967) because orientations arise from each individual's

unique prior experiences and may not match either published orientations categories or the categories I

developed in my own heuristic. I collected data through participant observation (Adler and Adler 1994, Mack et al. 2005). Though qualitative methods such as these may be unfamiliar to scientists, they have been long established in the social sciences. Lincoln and Guba (1985) outlined the means for operationalizing the criteria for trustworthiness in qualitative studies. Their recommendations served as a guideline here for addressing the issues of data credibility, transferability, and dependability. Prior to entering the setting, I gained the informed consent of the participant, Mr. Smith, and all relevant administrators. I collected data before, during, and after the outreach program using multiple methods (field notes, audio tapes; Table 4) and checked each datum with multiple sources to increase data credibility through triangulation (Lincoln and Guba 1985; Table 4). Sources included observations of Mr. Smith's teaching during his environmental science course and after school club, conversations, interviews, and a card-sort task (based on Friedrichsen and Dana 2003) designed explicitly to elicit Joe's orientations toward teaching science (Table 4 and Appendix 4). As previously stated, I allowed categories of data analysis and the interpretation of the relationships between the data to emerge as the study progressed. This involved identifying recurring events and themes in the transcripts and field notes that became my categories of focus (constant comparison sensu Glaser 1978; Table 4). I coded all data products, and as I continually amassed related incidents I analyzed the relationships among them. I noted daily my perceptions of what occurred and the questions that arose for future clarification. As these analyses progressed, I checked the accuracy of my perceptions and reconstructions with Mr. Smith.

RESULTS

Joe Smith's orientations to teaching science

Based on my observations, conversations, and interviews with participant teacher, Joe Smith, I identified three orientations toward teaching science: two primary and one peripheral, as defined by Friedrichsen and Dana (2005).

Primary orientation 1: Develop student's appreciation of nature/ease fear of

nature

One of Joe's primary goals in working with students is to give them experiences in nature they would never seek out on their own in order to reduce their fear of and discomfort with nature. In many schools this would be a difficult goal to accomplish given the limited funds available for student transportation. However, Joe's classes and club uniquely have access to transportation that enables them to leave the school campus at will, which they do regularly; during the seven observations I conducted of Joe's classes, five of those were spent off school grounds. Joe reports that he starts with experiences (e.g. paved paths or parks) that ease students into natural settings, and graduates toward greater immersion in nature that pushes students' boundaries of comfort. Some of these later experiences that I observed included off-road walks along a creek, hiking in a state park, trips to a community garden and plant nursery, and kayaking on a lake.

In the classroom and off campus Joe often makes attempts to connect students' everyday lives to the natural world. I frequently observed Joe endeavor to convey to students that nature provides the very food that sustains them. Joe meets daily with a student lunch club that partakes in foods he prepares from his own and the school gardens, especially fresh colorful salads with a variety of vegetables. Joe's environmental science class grew potatoes, which they harvested, prepared, and ate for extra credit. Additionally, during three separate observations off campus Joe offered extra credit to students to eat plants they encountered outdoors, after he tasted them first. Even on our first nature walk Joe said, "I try to get kids eating plants." My feeling was that helping students establish this nature-self connection was an integral part of reducing the psychological separation between students and nature, and consequentially easing their fear of nature.

Many students took their nature experiences in stride, but not all; I observed one student vocalizing his discomfort with nature on multiple occasions. For instance, he was very afraid of the height of a boardwalk above a creek during an excursion to a state park, a surprisingly short distance from the ground for such a strong reaction. This student also suggested that it was unnatural for people of his race to be in nature, saying once, for example, that "Black people don't kayak!" Never-the-less, Joe continued to ask this student along for the adventures of both the environmental science class and Explorer's Club, and

usually the student acquiesced. The progression of the dialog between this student and Mr. Smith followed a regular routine, escalating in both insistence (Joe) and resistance (student). Joe himself reported that it often took cajoling for him to win the "battle of wills", and that the student acquiesced primarily to save face in front of his peers. Interestingly, I witnessed a phone call between Mr. Smith and the student's father, who appeared to be highly supportive of his son's participation in the outdoor activities.

What Mr. Smith wanted students to get out of the nature experiences was left unclear to both the students and me; there were no guiding questions to focus students' attention to any particular aspect of their surroundings. The effect these experiences had on students was also unknown. Joe never inquired about students' conceptions and/or perceptions of their experiences, and there was no facilitated discussion or required reflection.

I asked Joe about his rationale behind the desire to increase students' comfort with nature, which was difficult for him to articulate.

> 09/17/09 Well, you know, take away the building and the door and the windows and air conditioners and it's you and nature. You are nature. So it harms their relationship with themselves in a way. … I guess human nature and nature are so interconnected to me…you separate them from their own human nature that's somehow stunting their growth.
> Just exposing – at least getting kids comfortable – so they become adults that are comfortable with nature that, in turn, try to bring it into their lives and keep it around them. Politically, they participate in the political system to keep it around them, and they enjoy the fruits of it…
> I guess you're eternally young in nature because there's so many things you don't know. You're always surprised. I guess if you're always sitting in an artificial environment you come to expect things to stay the same or be predictable and control it, where there's always a randomness and surprise in nature that keep your mind stimulated. And from what I've heard, humans are unique in that their brains are in a child-like state almost stuck in an eternal immaturity which is a adaptive strategy that keeps us able to adapt to change to new environments and situations which is a good thing.

Despite Joe's inability to clearly articulate a rationale for this orientation, it was clear that it was long-held and transcended the context of this particular school. Joe's outdoor club was well established when I met him in 1997 as a middle school science teacher, often heading out on hikes and cleaning up natural areas. His past club and classes also planted native flower beds and vegetable gardens which remain at those schools to this day. My feeling was that this orientation arose from Joe's own deep connection to nature. As I talked with Joe I learned that he spent much of his own free time in outdoor activities. He showed me photos of canoeing with his pals and talked about kayaking with his wife. His tone of voice became more

positive and enthusiastic when he spoke of being outdoors. I suspected Joe wanted to give students the

opportunity to experience nature as he did, and meanwhile serve his own desire to be outdoors where he

was happiest and most at ease.

Primary orientation 2: Help prepare students to become healthy adult community members

Another of Joe's primary motivations in working with students outdoors is to help develop their

potential to be healthy, successful adults and contributing members of society. Joe sometimes calls this

purpose in his teaching the "hidden curriculum."

> 5/4/09 What do they say, that 60% of what kids learn has nothing to do with the topic.
> They call it the "hidden curriculum". I think I've heard of that. … I do try to hook it up
> with the curriculum, but I'm also very comfortable knowing that I'm taking charge of
> over 50% of what kids learn in the classroom…, and that is, how they get along with each
> other, how they get along with adults, and …getting them comfortable with healthy
> activities like going out into nature.

Joe's definition of hidden curriculum is relevant to but broader than the generally accepted connotation

which usually refers to unintended, sometimes negative, messages conveyed in schools about social norms,

social status, and culture (Jackson 1968, Nieto 2002, Horn 2003). Joe wants to consciously take charge of

these messages by modeling and teaching social norms that are the standard in working and middle class

society, particularly related to interpersonal interactions. He views the ability to function within these

norms as skills needed to be successful in life and work, and thus helping students foster these skills is one

part of this orientation. However he also is concerned about students' internal perceptions of themselves,

which have been impacted by the hidden messages pervasive in their own home environments, as well as

schools and all other aspects of society.

A) Help students develop skills for the future, including social skills and responsibility

Developing students' skills in interacting with others was a primary goal Mr. Smith had during

outdoor activities, including the pollinator gardens. Mr. Smith often described this as giving students

opportunities to interact in a "different way" than the ways they interacted in class. Based on my

observations, he was often successful in changing students' interactions by going outside. Indoors, though

students seemed to really like and care for Mr. Smith, they knew it was possible to get him to elicit strong

emotional responses. They would test him. The outcome of these tests varied greatly from day to day. On days when Joe was in a good mood, he handled situations with humor. Other times he would snap and become clearly angry, defeated, and vindictive. Students would retaliate with hurtful comments. Students also became increasingly antagonistic with each other if the mood of the class deteriorated. Given this often combative nature of Joe's classroom climate, it was easy to see why he preferred interacting with students in an outdoor setting where everyone was usually in a better mood and there were fewer shifts in power dynamics. Outdoors, whether in the gardens or on fieldtrips, students acquiesced to most requests Mr. Smith made of them, with only small amounts of protest if any. For example, they would tend vegetable and flower beds upon request or would follow him along paths, at least pretending to examine things he pointed out along the way. They would even help each other out with jobs, though usually after being prompted. On the students' part, there were no intellectual expectations of them outdoors, so often (though not always) they preferred outdoor activities to indoor lessons.

Mr. Smith also provided students many opportunities to interact with other adults in the community. For example, students worked with university students in community gardens, helped on river clean-up teams led by local volunteers, and participated in service learning projects (planting pollinator gardens) for local nursing homes and a Head Start program. These activities were designed to help students develop skills in networking and making connections for volunteer or job opportunities later. However, they were also meant to give students actual physical experiences that could be used to gain employment, in fields such as landscaping, carpentry, or working with young children. At least some students followed up on these opportunities, as I observed Mr. Smith discussing with a student a day care job she had acquired from volunteering there during club activities.

Though helping students develop interpersonal and other life skills was most mentioned by Mr. Smith, giving students opportunities for developing responsibility was also important. For example, he subtly encouraged students to take on regular garden duties, such as composting or watering the plants. It was clear that these jobs were very important to some students; if another student did their job, there was an obvious sense of betrayal. Mr. Smith provided opportunities for responsibility indoors, as well as out, as I often observed students eagerly caring for indoor plants and class pets.

B) Help students develop positive self-perceptions

As well as the social and other life skills that Mr. Smith wanted to foster in students, there were, concurrently, other internal changes he wanted to bring about, such as changing students' perceptions of themselves. To Joe, changing students' self-perceptions was intertwined with students developing their capacity for positive social interactions and skills that they could use as future working adults:

> e.g. 5/4/09 …see themselves as contributing to the community and puts them in a positive position, where they view themselves in a more positive manner. They interact with other adults in the community. That puts them in a different social relationship besides student to student or student to teacher. It's student to community member, which, I feel is a really healthy thing to prepare them to develop their more adult behaviors, and it puts them in more of a working context that would relate to the real working experience.

By involving students in service learning experiences, for example, such as the aforementioned stream teams, river relief, and gardens for local organizations, Mr. Smith hoped that students would see their potential to have a positive effect on others and be contributing members of society. Mr. Smith's enthusiasm for one of my outreach activities also seemed to be derived, not from what students would learn about pollination, but from the potential to impact students' visions for their futures by connecting them to the college environment. For example, when we brought his students to the university lab for one of my outreach lessons, Mr. Smith was most interested in students touring campus to see what college looks like. Additionally, Joe was excited about my connections to undergraduates and the possibility of having minority students that had participated in ecological research give presentations to his class. He felt that these would be great opportunities for his students because "None of them see themselves going to college." Yet even though Joe wanted students to see college as a potential option, again, he did nothing to facilitate students' awareness that they were at "college" or facilitate their thinking about what it might be like to be a college student.

Never-the-less, Mr. Smith created many opportunities to address students' social skills, life skills, and healthy habits of mind during both clubs and scheduled class time; environmental science students would often work on the school gardens and spend time outside the school with other community members. In many ways, Joe seemed to be torn between the job he knew he was expected to do in the classroom (teach science content) and his desire to address these other issues:

> 5/1/09 Sometimes the principal gets a little bug…you know it's like, "We've gotta raise our kids scores." The rest of us are like, "What?!" You know it's like …give me a kid that comes three out of five days a week…and it's just…attendance is crazy…much less all of the other issues.

5/4/09 My gut [feeling] is, being at an at-risk school, the social interactions are more important than the science curriculum…how they view themselves, how they interact with each other, how they interact with adults…but at the same time I would really give them equal value. But given the amount of my ability to like juggle five things at once, I guess I focus a little bit more on the ...I guess I feel that…developing, you know, healthy adults that have healthy social interactions, self awareness is real important.

3. Peripheral orientation: Learn academic concepts

Though at the gut level Joe felt that addressing the hidden curriculum was more important than these at-risk students developing an understanding of what he termed "core" academic concepts, he never-the-less identified learning academic concepts as one of his goals in teaching. The "backbone" of his academic curriculum in all classes is AIMS worksheet packets, because of students' irregular attendance and what he felt was students' (and, I would argue, his own) need for predictable routines. Joe recognized that he may be sacrificing something of the quality of students' educational experiences in his effort to find a practical solution to the contextual problems of an at-risk school.

> 5/4/09 Really in all of this is the underlying, you know, give them some text and some questions that go with the text. Have 'em look up definitions for terms. To me it's a low level, but – and the kids, they get tired of these worksheets – but then as soon as I start doing a hands-on activity, or start lecturing, or taking notes, or anything, they're like, "Give me a worksheet! Shut up Mr. Smith!" So it's like what do you do? When it comes down to it, attendance is so low that to get a kid to make up an activity, I really need a bread and butter something that I can just give them to do and take home. Unfortunately, I feel that really lowers the quality of the educational experience, and I feel like if I was a better teacher I would have …fewer worksheets… than I do right now, with more hands-on activities. But…I'm not that good. I'll just say it.

Almost all of Joe's active teaching of science concepts was didactic, with students as passive recipients of his teaching. Joe asked some lower level thinking questions, but they were never planned, and he never used wait time. Even Joe's language reflected his view of teaching and learning. He repeatedly used phrases like "talk about" and "tell" in reference to his teaching. Often times the "science concepts" that Joe addressed were really just isolated facts, as often observed during his narration during students' nature walks.

> Joe does, however, recognize that most people expect classroom science to be more experiential:
>
> 5/1/09 …and I'm teaching physics…two days… I'm a biologist, but you know… somehow I got elected…and then my principal is a former physics teacher. So he'll check in and go, "What's happening, Joe?" So I make sure I've got a bunch of stuff on the tables that looks 'physics-y'.

However, Joe feels that that teaching a cohesive curriculum with active lessons that build upon each other

is unrealistic for the setting at his school, where student attendance is irregular and unpredictable.

Occasionally, even in the classroom, Joe attempts to make science learning both experiential and

relevant to student's experiences.

> e.g. 5/19/09
> Mr. Smith: "We're harvesting potatoes today."
>
> Jasmine: "So we're not doing a packet today?"
>
> There were two potato plants for the class. Jasmine and Tobias dumped their potatoes in the tub and dug through the dirt to find the potatoes. The other students helped them look for potatoes. While students were harvesting potatoes, Mr. Smith said, "These potatoes didn't come from pollinators. No plant sex was involved."
>
> Jordan: "What does this have to do with pollinators?"
>
> Mr. Smith: "Hey Jordan give me your finger! If I cut off Jordan's finger will it grow into a new Jordan?"
>
> Students: "No!"
>
> Mr. Smith: "Thank goodness! Well potatoes are clones of the one we planted."
>
> Jasmine found the empty potato skin of the mother plant. She asked Mr. Smith what it was. Mr. Smith showed the class and said, "Here's the mother potato – all the others in Jasmine's tub are clones of this one!"
>
> Jasmine: "I'd like to have a clone!"

Interestingly, Joe has not recognized that by changing his teaching methods he could also use

outdoor experiences to accomplish his academic goals for students, increase students' active participation

in their learning, and reduce students' boredom and acting out (Skinner and Belmont 1993), with potential

positive consequences on his own stress and fatigue (Kyriacou 2001).

Interaction between Joe's orientations and the outreach program

How did Joe's orientations impact the implementation of the outreach program? First, there was a

clear conflict between Joe's orientations and mine. I wanted to demonstrate through the outreach

curriculum and teaching methods that students could be physically active, mentally engaged, and learn

about science concepts in both indoor and outdoor settings. This conflict resulted in reducing a two week

lesson plan to just two lessons. I generally thought I would facilitate all outreach activities, but Joe rarely

relinquished being "in charge" of the students. In addition, one of my primary goals during outreach was to get students actually doing science: developing questions for investigation in the gardens, designing studies, and collecting data outdoors that students could share with each other. The most significant and surprising impact of the orientations conflict between Joe and I was Joe's elimination of this student-driven investigation in the pollinator gardens. In general, due to the conflict in our orientations, Joe hindered my ability to carry out the objectives I had for the outreach program.

Post-outreach program: Prognosis for future use of materials and pedagogy

At the end of the study, I conducted a post-program interview of Joe. I asked for feedback about the two activities and Joe's overall impressions. It was interesting that the first thing he said to me was that he would cut off students' explorations of floral anatomy using the microscopes sooner, even though he acknowledged in the same conversation that students were really "into" it:

> Joe: Yeah, I don't think I would do it as extensively. I would give them the flowers with the really obvious sexual parts like the pistil and the stamen like a tulip. I think that was well worth it to get the kids to identify the parts. And then I would pretty much take them out of the room and go out.
>
> Me: So maybe less with the microscopes?
>
> Joe: Yeah…maybe so. Yeah. Yeah, yeah…and then…but boy they enjoy it…hmm…I think it's good to use the microscopes for part of the time, though, to catch that real detail, the bristly, just the texture. It really does focus their interest, you know, literally, and well just literally it focuses them…

In addition, the day after my second outreach lesson about fruits, I observed Joe leading a lesson with students. He sat on table at the front of the room, and posed a question to the class:

> Joe: "What's a fruit?"
>
> Student: "Mr. Smith!" (groaning)
>
> Joe was clearly annoyed, but recovered and tried again: "Which comes first the seed or the flower?"

A few students whined about the question. Jasmine engaged in the discussion (although with a bit of an argumentative attitude), and a few other students contributed ideas. However, Joe didn't notice this. He became visibly agitated, and said to me, "You see what happens? Does this remind you of anything? It's like *(local school omitted)* if you take away everyone but the trouble makers." He said this in front of the

students, even though some were actually engaged in the question and discussion. They were actually responding despite of complaining! Clearly frustrated, Joe assigned student to do worksheet packets instead, with no explanation of what they were about. Never-the-less, I felt that I had two pieces of evidence of a disturbance in Joe's thinking about teaching. I found this to be exciting because a state of "cognitive dissonance" is often a necessary precursor to a change in practice (Ball and Cohen 1999, Loucks-Horsley and Matsumoto 1999).

The primary issue Joe had with my two outreach lessons was that they were conducted indoors. He felt that we should have extended the fieldtrips conducted with the class, using opportunities along the local trails or students to find plants in both flowering and fruiting stages to "get them to have those experiential interactions". I found it interesting that Joe thought of the fieldtrips as giving students experiential interactions with the plants, yet the students were always passive on these excursions. Fieldtrips universally consisted of Joe telling students facts about what they were seeing, and occasionally asking rhetorical questions. Joe never asked students to observe, think about, touch, manipulate, or discuss anything in their environment – with the exception of occasionally asking them to eat something for extra credit. Regardless, I felt that the suggestion to go outdoors and have students select their own flowers was a great suggestion, and perfectly aligned with my own teaching orientation. Had the flowers in the school gardens been further along, I would actually have asked students to do that for their lesson on floral anatomy

On a more positive note, during my time with Joe, we certainly formed a positive relationship with potential for future collaboration. Before fall of the next school year he emailed me with ideas for the semester. He thought we could write grants to expand the gardens, and he invited me back to work with students, which I did. However, as for future use of the activities and teaching methods by Joe himself, at this point, I would say probably not.

DISCUSSION

The objective of this case study was to examine the interactions between a science teacher's orientations and the implementation and outcomes of an outreach program curriculum, developed by an outside entity, for use in that teacher's classroom. I found that the orientations of the classroom teacher

mediate the vision of the curriculum developer to determine what actually occurs within the classroom. In addition, future use of the curriculum materials by the teacher is unlikely to occur if the teacher's orientations conflict with those of the curriculum developer as represented by the outreach program lessons and pedagogy.

Implications for entry of curricula into classrooms

In general, teachers function as the gatekeepers to entry of any program developed by an outside entity into K-12 classrooms, regardless of whether the target audience is the students, or as in the case of this study, the teacher. In the case of outreach programs developed by scientists to meet the broader impacts merit criteria of their grant proposals, scientists, though experts in their content fields and experienced in post-secondary education, may lack knowledge and skills relevant to working with K-12 students and teachers. Even if the scientist has such expertise, it is reasonable that teachers, also trained professionals possessing a body of knowledge and skills in their fields, may view scientists as "outsiders". However, scientists can play the role of outreach "ambassadors". To ensure the program enters the classroom and progresses as planned, it is important to figure out who the gatekeepers are and involve them in the planning process. Even if the goal is to promote a certain curriculum or pedagogy, it is important to honor the gatekeepers' orientations.

In this study, conflicts between the orientations of the participant teacher toward science teaching, particularly toward use of the pollinator gardens, and my orientations as program developer, represented by the outreach curriculum, resulted in reduction and/or elimination of many planned objectives and activities. However, despite necessary compromises to "let go" of some planned objectives and activities, such as the student-driven investigation, I was able to promote my own orientations through modeling inquiry based teaching during the lessons I personally facilitated. Regardless, even if the outreach program had been developed through collaboration and consensus from the beginning, many things wouldn't go exactly as planned, so the ability to be flexible and adapt to unexpected challenges is a necessity.

Teacher Orientations

The scope of this project was limited to examining the orientations of one science teacher within his particular context, rather than making broad generalizations about science teaching orientations overall.

I conducted the outreach program with this school and teacher because I perceived that this was a best-case scenario. The school was known for flexible approaches to dealing with students and curriculum, and I perceived the teacher as amenable to involving students in learning in the pollinator gardens. As such I did not consider this setting/participant as representative of the typical context for such an outreach program, but rather as a "critical" case (Flyvbjerg 2006) which had particular relevancy to my study questions about the impact of teacher orientations on outreach. Though I am unable to make generalizations about specific teaching orientations or orientations held by most teachers with outdoor classrooms, I do feel this study supports already existing generalities about orientations from others' research. In particular this study supports that orientations are tenaciously held part of teachers' pedagogical content knowledge (Friedrichsen and Dana 2005, Abell 2007). However, I do feel that this study makes a strong general case for considering teacher orientations in planning any program that brings outsiders' perspectives into the classroom – whether this involves the entry of the actual person or the curriculum that represents the perspectives of the outsider. Teachers' attitudes and beliefs about teaching and learning impact their use and implementation of curriculum materials (and likely pedagogy) developed by any outside source (Roberts 1988).

Implications for teacher professional development

In this study the pollinator outreach program was designed originally to meet NSF Broader Impacts merit review component, specifically targeting the goal to "Advance discovery and understanding while promoting teacher training and learning." In addressing this component the outreach program functioned as teacher professional development, modeling effective incorporation of inquiry and student-driven investigation into K-12 science education. However, orientations held by the participant teacher (the "target audience") prior to the professional development intervention are likely to impact the teacher's adoption of the curriculum and/or pedagogy promoted during the professional development. If there is a conflict between the teacher's prior orientations and the philosophical underpinnings of the "new" curriculum and methods, future use of the curriculum materials and pedagogy may require a change in the teacher's orientation. It is unclear what factors may promote this change, however, because orientations are both tacit and tenaciously held suggesting that change may be difficult.

It was beyond the scope of this study to examine any change in the teacher's orientations as a result of the outreach program; however, I was interested in the prognosis for the teacher's future use of the curriculum materials and teaching methods. Though all but two planned outreach activities were eventually eliminated during my time at the school, I did see evidence of cognitive dissonance in the participant teacher's thinking about effective teaching, reflected by his statements and practices. However, the teacher did not plan to adopt the curricular activities for regular use with students in the pollinator gardens, nor was there evidence of systematic adoption of the pedagogy reflected in the outreach lessons. Convincing the teacher to integrate inquiry-based teaching methods and student-driven investigations into his daily teaching would represent real transformative change in his orientations, and would almost certainly require a longer term commitment than even the full version of the originally planned pollinator gardens outreach program.

Many K-12 science teachers have little or no science research experience. Without these experiences their understanding of both the importance and nature of scientists work can be, at best, academic. In addition, any negative attitudes teachers have as a result of misconceptions about the nature of science research (e.g. that it rigid, "boring", solitary work) are likely to impact teachers' willingness to facilitate their own students "doing science" (Brickhouse 1990). Here, the participant teacher had unexpected negative attitudes toward intellectual exploration of science concepts and the natural world. It is unknown how participating in the research projects that preceded the development of this outreach program would have influenced his orientations. However, for some teachers, personally engaging in science research may influence their understanding of the nature of science, attitudes toward science, and their orientations toward science teaching. Organizations promoting scientists work in K-12 classrooms have begun to understand this connection, and are beginning to encourage and/or require reciprocal participation of teachers in scientists' work during such collaborations (e.g. NSF Graduate Stem Fellows in K-12 Education (GK-12); NSF 2009b). If I were to conduct this program in the future I would certainly incorporate prior participant teacher involvement in research as an essential feature.

Upon reflection of the choice of classroom setting for this outreach, I might choose next time to involve elementary rather than secondary teachers and students. Since one of my goals is for students to develop a love of science, it makes sense to target early school ages before the well-documented decline in

students' interest in science that occurs around middle school (Osborne et al. 2003). In addition, research has shown secondary educators to be more resistant to reform than educators at lower grades (National Research Council 1996), and thus there may be more chance of adoption of the pollinator gardens activities and/or student-driven garden investigations by working with an elementary level teacher.

Perhaps the most important outcome of this study was the realization that my role as investigator kept me engaged and excited for the duration of my immersion in the school. If it hadn't been for that, I likely would have been very disappointed in the derailing of the plans I had for the outreach program. As with any action research, perhaps as well as focusing on what we have done for our target audience, we should also focus on what we are learning from the outreach.

ACKNOWLEDGMENTS

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130

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Table 1. NSF broader impact objective addressed by the pollinator outreach program and representatives activities (as defined by NSF) that would meet the objective. Activities in italics were part of the planned outreach program pedagogy and curriculum.

<u>Advance Discovery and Understanding While Promoting Teaching, Training and Learning</u>

Representative Activities:

Integrate research activities into the teaching of science, math and engineering at all educational levels (e.g., K-12, undergraduate science majors, non-science majors, and graduate students).

Include students (e.g., K-12, undergraduate science majors, non-science majors, and /or graduate students) as participants in the proposed activities as appropriate.
Participate in the recruitment, training, and/or professional development of K-12 science and math teachers.

Develop research-based educational materials or contribute to databases useful in teaching (e.g., K-16 digital library).

Partner with researchers and educators to develop effective means of incorporating research into learning and education.

Encourage student participation at meetings and activities of professional societies.

Establish special mentoring programs for high school students, undergraduates, graduate students, and technicians conducting research.

Involve graduate and post-doctoral researchers in undergraduate teaching activities.

Develop, adopt, adapt or disseminate effective models and pedagogic approaches to science, mathematics and engineering teaching.

Table 2. Schedule of activities for the pollinator gardens outreach program

Curricular topics		Activities
Lesson 1 (1 class)	Plant reproduction Floral anatomy Pollination and pollinators Co-evolution in plants and pollinators	Engage: What is a flower? What is it for? How can you tell if it is a boy or a girl?
		Explore: Floral dissection of flowers that differ in morphology; Identification and comparison/contrast of floral anatomy
		Explain: Facilitated discussion about floral reproduction, ways to move pollen between plants, and pollinators
		Elaborate/Evaluate: PowerPoint of interesting diverse flowers; students predict what pollinator matches with each.
Lesson 2 (1 class)	Importance of pollinators to natural and agricultural ecosystems Ecosystem services Interconnectedness of ecological communities	Engage: How many of you depend on pollinators for survival?
		Explore: Fieldtrip to community garden. Show fruits and other items (e.g. honey), set up as stations. Have students predict which are dependent on pollinators.
		Explain: Show how all are dependent. Show examples of fruits/veggies that didn't receive enough pollination services. Have students examine pollen and pollen tubes on plant pistils using microscopes.
		Elaborate/Evaluate: Students discuss: What are other examples of ecosystem/services? How could we find out if pollinators are important to particular plants?
Lesson 3 (2 classes)	Pollinator decline Conservation of pollinators Effects of land use on pollinators Habitat fragmentation, corridors, stepping stones Pollinator restoration Keystone species	Engage: Show global maps of pollinator decline; discuss honeybee colony collapse. Watch documentary "Colony".
		Explore: Given limited financial resources, students use Ecobeaker computer simulation to design reserves to conserve the endangered Fenders Blue Butterfly
		Explain: Compare results of reserve design simulations: Which worked best to maintain butterfly populations? Why? Which were the most cost-effective strategies?
		Elaborate/Evaluate: Students discuss: Can we restore lost pollinators? Would all plants be affected similarly? Which pollinators should we restore?
Ongoing lesson	The nature of science Methods of science Experimental design	Students design and implement investigations of their own choosing related to pollination and/or other ecological interactions using the gardens they planted.

Table 3. Heuristic used to elicit my orientations toward teaching science

Orientation A: Purpose		Orientation B: Approach		Orientation C: Centrism
Academic	Science is discipline knowledge Develop foundation for future learning	**Activity-Driven**	Hands-on activities relating to concepts, but with no follow-up discussion or structured thinking	A continuum describing the locus of authority and decision-making, often correlating with the level of physical and/or cognitive activity. It ranges anywhere from:
Everyday Coping	Students utilize science to improve their lives; curriculum organized around student needs and interests	**Conceptual Change/ Constructivist**	Prior ideas about concepts accessed, followed by hands-on experiences related to concepts and structured	
Nature of Science	What is science? What can science do?	**Didactic**	Teacher: Transmit content via lecture, book, video Student: "Tabla rasa", memorize content	Completely student-centered
Science as inquiry	Investigation of science concepts Development of scientific skills	**Discovery**	Investigating science concepts solely through exploration, without guidance	
Science, technology, society	Prepare future citizens, voters, decision-makers; curriculum organized around issues	**Guided Inquiry**	Investigating science concepts with learning facilitated by the teacher	
		Project-Based	Researching a topic, culminating with the production of a tangible product	
		Reflectivist	Students are asked to examine and articulate how their ideas about concepts have changed over time; metacognitive thinking	Completely teacher-centered

Note: I developed this heuristic after examining published classification schemes of orientations toward teaching science. Published orientations did not accord with my own understanding of the guiding purposes and frameworks toward teaching science. In addition, many schemes seem to confuse the purpose of teaching science with the pedagogical approach. Thus I delineated these aspects in my own heuristic. In addition I added a third aspect, which describes the teacher- vs. student-centrism of the classroom. Centrism is really a second aspect of approach. However, it must be delineated separately since one can, for example, use a project-based approach where the locus of decision making is entirely with the teacher, entirely with the student, or somewhere in between.

Table 4. Data collection sources and analysis methods

Data collection		Data analysis	
Sources	**Products**	**Methods**	**Products**
Observations of teaching practices	Audiotapes	Coding data	Theoretical notes
(2 club sessions, 7 classes)	Field notes	Identifying patterns	Interpretations
Conversations	Transcripts	Developing analysis categories	Reconstructions
(3 prior to outreach program)		Developing explanations	
Card-sort task, Interview		Constant Comparative Method	
(Pre- and post- program)			

FIGURE LEGENDS

Figure 1. Hypothesized pathway for orientations toward teaching science to impact the implementation and outcome of outreach activities conducted in K-12 classrooms that are designed to model effective teaching for the classroom teacher.

Figure 1.

Scientist → Outreach program → Teacher
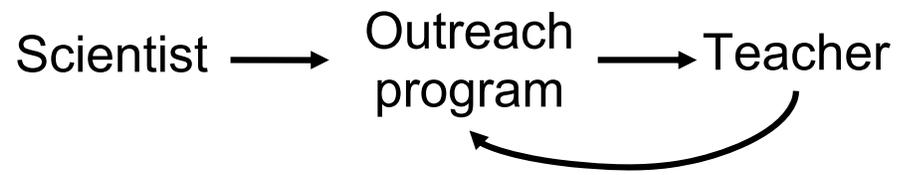
CHAPTER 6

GENERAL DISCUSSION

Jennifer C. Geib

Ecosystems are complex entities (Storch and Gaston 2004, Montoya et al. 2006, McNamara and Houston 2009). Within them, every interaction between species is situated in a broader biotic and abiotic context, making the outcome of interactions for either partner conditional (Bronstein 2001, Gruner 2004). Those of us who strive to attain greater understanding of interactions between species and guilds of species must deal with this complexity and tease out general patterns from what seems like an incomprehensible tangle of potential interacting variables. One way of dealing with this complexity in studying ecological processes is to "articulate their interplay across multiple scales" (Holt *public communication*), for example, traversing the organizational hierarchy of ecological systems, across space, and through time (e.g. Holt and Lawton 1994, Holt and Barfield 2003, Storch and Gaston 2004, Holt 2009). Here I have attempted to examine benefits from increased partner abundance in facultative multispecies mutualisms from multiple perspectives over multiple scales.

In the preceding chapters I have reported on the propensity for the long-tongue alpine bumblebee, *Bombus balteatus*, while foraging among mixed patches of alpine clovers, to prefer the architecturally stronger *Trifolium parryi* over the weaker *T. dasyphyllum*, likely due to differences in metabolic costs of foraging (Chapter 2). Because of *B. balteatus'* preference for *T. parryi* and because *T. parryi* has fewer alternative pollinator species, under conditions of increasing *B. balteatus* density, benefits to *T. parryi* are greater than those to *T. dasyphyllum* for individual plants, at life stages beyond fecundity, and at the

population level (Chapter 3). Because *B. balteatus* has a relatively small foraging range, habitat-scale fruit production of the specialist clover also correlates with local abundance of both *B. balteatus* nests and individual foragers (Chapter 4).

By looking at the interaction between *B. balteatus* and its clover host plants from multiple perspectives and scales there are rich conclusions that can be drawn. For example, by elucidating both the mechanisms behind pollinator behavior and the consequences of the behavior, I have linked individual traits, such as plant architecture, pollinator morphology, and pollinator behavior with host plant population trajectories (Chapters 2 and 3). The efficient movement of these bees, mediated by matching of morphological traits between bee and host plant, is a crucial driver of population dynamics for at least *T. parryi*.

At the most basic level, my results suggest that specialists benefit more than generalists from increased abundance of one mutualist partner. That this pattern is repeated at every scale – individual plants, life stages beyond fecundity, host plant population growth, and fruit production rates within habitats (Chapters 3 and 4) – makes this an even more robust conclusion than had I had just one data set alone.

Results at different temporal scales suggest that even for specialists the outcome of mutualism is not guaranteed. Biotic and abiotic conditions change 1) within seasons, as demonstrated by the change in co-pollinator contribution to both clovers' seed set from June to July of the same year (Chapter 3) and 2) over years or decades, as shown by the surprising changes in the species composition of the bumblebee guild between past and current surveys of the same research site (Chapter 4 and Byron 1980). At even larger temporal scales, evolutionary and ecological processes may interact to strengthen or weaken the relationships. Here, there are really more questions rather than answers: For example, evolution of traits driving the interactions between mutualists – e.g. plant architecture or pollinator mass – may be impacted by pressures outside the mutualism and could be a game changer for this whole scenario.   Environmental variation, such as changes in pollinator guilds or predicted environmental impacts of climate change in these regions, could also affect the evolution and or ecology of the interaction. Though today I can't provide answers as to how these scenarios might play out, my dissertation work has resulted in a model that could be used to explore those possibilities.

Increasingly, the role of scientists in society is evolving beyond the generation of pure science knowledge. There are calls for scientists to take active roles in advising policy makers and stakeholders on issues, as well as in effectively communicating research findings with members of the general public (Jordan et al. 2009, Groffman et al. 2010, Meyer et al. 2010, Whitmer et al. 2010). In Chapter 5 I reported on one of my own efforts to interface with secondary students and their science teacher with the aim to "advance discovery and understanding" of plant-pollinator interactions and the nature of scientists' work while "promoting teaching, training, and learning" about effective incorporation of student-driven science investigations in classrooms (Chapter 5). However, the multiple perspectives from which I investigated the role of pollinator abundance in facultative pollination mutualisms lend themselves particularly well to other audiences, as well. Investigations of pollinator behaviors and preferences among host plants (Chapter 2) would be appropriate for elementary age school groups, as well as informal venues of all ages where there is readily access to pollinator gardens. Activities might vary from public seminars to garden clubs, elder hostels, etc. (if targeting older audiences) to involving amateur groups in the monitoring of local pollinator species and abundances (for example, see the Great Sunflower Project; LeBuhn et al. *public communication*). In fact amassing these armies of volunteers could be crucial in assessing the current and continuing status of pollinators in the face of concern about the declines of some species (NRC 2010). Individual, population, and habitat-scale relationships between host plant reproduction and pollinator density (Chapters 3 and 4) are topics of particular interest to land managers and conservation agencies, particularly due to both increased awareness of the need to include pollinators in land management regimes and a dearth of relevant information about how such regimes might impact target plant species (Corbet 2000).

My future work will again strive to blend both worlds, that of pure science research and that at the interface of science and society. Planned future studies will involve pre-service teachers, undergraduates, and K-12 students and teachers in conducting ecological investigations in local pollinator gardens. In turn I will examine how this work impacts ecological literacy among participants and their attitudes and beliefs about science and the environment. Just as bridging spatial and temporal scales informs our understanding of ecological interactions, bridging across the boundaries that separate scientists from the public at large

leads to greater understanding and progress on environmental issues shaping our world. Scientists after all,

no more than the organisms they study, can scarce afford isolation.

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APPENDIX 1: ALPINE CLOVER SUPPLEMENTARY DATA

NECTAR STANDING CROP SURVEY

In July 2007, I surveyed the nectar standing crop of the clovers to assess relative rewards available to pollinators. I collected one haphazardly chosen floret from randomly selected *T. dasyphyllum* and *T. parryi* inflorescences (N=25 per species) along a south-facing ridge on Pennsylvania Mountain. Each floret was collected in the field and placed into a vial with damp paper towel for transport to the lab. There, I rinsed nectar out of each floret using a wash bottle with pipette tip attachment. As the floret was rinsed, rinsate was collected below into a 1 mL microcentrifuge tube using a small funnel made of a cut plastic pipette. Samples were frozen for later analysis. In September 2007, I analyzed the total carbohydrate content of each clover floret using the anthrone method as described in McKenna and Thompson (1988) on a Tecan "Sunrise" microplate absorbance reader (Tecan Group Ltd., Switzerland). Prior to analysis, nectar samples were thawed and diluted to 2 mL. A t-test showed that mean available sugar per floret did not differ between the clovers (t_{42}= 2.018, P=0.899; Fig. 1).

FLORETS/INFLORESCENCE

In July 2006 and 2007 I surveyed *T. dasyphyllum* and *T. parryi* inflorescences for floret display. Florets per inflorescence values were square-root transformed for analysis. ANOVA (SAS 9.2: Proc GLM) showed that pooled over the two years there was no difference in the number of florets per head between the species (PLANT SPECIES $F_{1,364}$=.22, P=.64; Table 1).

CLOVER FLORET LENGTH KEEL STRENGTH

In addition to scape and pedicel strength (Chapter 2), I tested for additional floral trait differences among *T. dasyphyllum* and *T. parryi* in surveys conducted in the clover sympatric zone on the south-facing ridge on Pennsylvania Mountain and at Kite Lake near Alma (Park County), Colorado, elevation 3666 m. Surveys were conducted from June-July, 2008. Kite Lake populations were allopatric, but less than 500 m apart. At both sites I ran a 50 m transect through each clover population and randomly selected 20 experimental plants which had at least one head containing $\geq$ 10 florets. For plants displaying more than one flowering head, I haphazardly selected among those with $\geq$ 10 florets to standardize trait comparisons.

For the uppermost floret on each experimental inflorescence I measured the total length of the floret (equivalent to the length of the banner petal) using digital calipers and the resistance of the keel to depression and triggering of the pollen release mechanism. Keel resistance was measured by looping a small wire of known mass over the tip of the keel and adding small washers and beads until the keel deformed and the pollen release mechanism was "tripped" (sprayed pollen). To standardize the keel test all scapes were held perfectly upright. Washers and beads were collected in individual plastic bags and weighed indoors using an Acculab V-200 electronic balance (Acculab, Newtown, PA). Keel strength was calculated in Newtons (N): total mass (kg) x 9.8 m/s^2, the acceleration due to gravity.

Comparisons of clover floret length and keel strength were conducted using ANOVA (Proc GLM: SAS 9.2) with site, species, and site by species interaction as fixed effects. Results showed that *T. parryi* floret length and keel strength were significantly greater than *T. dasyphyllum* (Table 2; Floret length, PLANT SPECIES $F_{1,75}$= 345.8, P<.0001; Keel strength, PLANT SPECIES $F_{1,76}$=57.6, P<.0001).

RELATIVE INFLORESCENCE DISPLAY OF ALPINE CLOVERS IN SYMPATRIC ZONES

Data were collected in June-August 2007, from mixed patches of alpine clover along a south-facing ridge in the krummholz of Pennsylvania Mountain. In this area I located all possible 2 m x 2 m patches that had at least five plants each of *T. dasyphyllum* and *T. parryi*. However, *T. parryi* was always the limiting factor, and thus only sixteen suitable patches were found. The natural floral display of each

experimental plant was left untouched, but inflorescences from any excess plants were removed. All

excess plants were of *T. dasyphyllum*. Data were collected when all plants in the patch were in full flower.

I counted the number of flowering heads on each *T. dasyphyllum* and *T. parryi* plant and calculated means

for each species. Per-plant means were multiplied by five to calculate mean inflorescences per patch.

Relative inflorescence abundance was then calculated as the patch mean for each species divided by the

sum of means for both species x 100. Some *T. dasyphyllum* plants did produce additional inflorescences

later in the season, however all *T. parryi* and nearly all *T. dasyphyllum* inflorescences within the patch

bloomed concurrently during this primary flowering period.

Relative inflorescence abundance for each species was compared with a t-test. Results showed that

T. dasyphyllum averaged 8.1 flowering heads per plant and comprised a significantly greater proportion of

the inflorescences per patch (86%) compared to *T. parryi*, which averaged 1.2 inflorescences per plant and

14% of inflorescences per patch (t_{30}=2.04, P<.0001; Fig. 2).

The relative inflorescence abundance of the 16 patches was assumed to be representative of the

relative inflorescence abundance of the broader clover sympatric zone. If anything, estimates from patches

would underestimate *T. dasyphyllum* in the sympatric zone since 1) *T. parryi* plants were always the

limiting factor in locating suitable 2 m x 2 m mixed patches and 2) only *T. dasyphyllum* plants were ever in

excess within the 2 m x 2 m mixed patches.

NATURAL VISITORS TO ALPINE CLOVERS IN THE CLOVER

SYMPATRIC ZONE

To assess the guild of pollinators available to alpine clovers, I documented visitors to the 16

naturally occurring mixed patches of *T. dasyphyllum* and *T. parryi* described above. Observations on each

patch were conducted during two fifteen minute blocks (1 each AM and PM) twice during flowering (1

hour total per patch). Observations were conducted under clear to partly cloudy conditions. All insects

observed landing on inflorescence were tallied and assigned to one of nine categories: queen *B. balteatus*,

queen *B. sylvicola*, worker *B. balteatus*, worker *B. sylvicola*, solitary bee, Syrphid fly, Anthomyiid fly,

Lepidopteran, and other.

No queen bees, and only one worker and solitary bee each were observed during experimental observation periods despite many informal observations of these events in multiple years; thus I conducted natural visitor analyses on the 'all visitor' category rather than on separate categories. I used ANOVA (SAS Proc MIXED) to assess the contribution of plant species, time of season (early and late), and their interaction to variation in natural visitation rates. I also included patch (within time of season) as a random effect. "Early" and "late" time of season referred to before and after the emergency of worker bumblebees on July 6.

T. dasyphyllum received significantly more visitors than *T. parryi* throughout their growing seasons (PLANT SPECIES, $F_{1,58}$=35.12, P<.0001) and both species received significantly more visitors late in the growing season compared to early (TIME, $F_{1,14}$=21.66, P=.0004) (Fig. 3; Table 3). Flies comprised 40-80% of all visitors throughout the season (Fig.4). I observed almost no bees on clovers during scheduled observations (though I observed and filmed many solitary bees and bumblebees casually).

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Table 1. ANOVA testing differences in the number of florets per clover inflorescence

Source	DF	Type III SS	F Value	Prob>F
plant species	1	5.46	0.2233	0.6368
year	1	1052.27	43.0609	<.0001
plant species*year	1	186.00	7.6114	0.0061
error	364	8894.97		

Table 2. ANOVA testing for differences between A) corolla length and B) keel strength of alpine clovers

Source	DF	Type III SS	F Value	Prob>F
A) Corolla length				
site	1	0.03	0.0127	0.9105
plant species	1	715.52	345.83	<.0001
site*plant species	1	1.49	0.7216	0.3983
error	75	155.18		
B) Keel strength				
site	1	15.73	65.1	<.0001
plant species	1	13.91	57.6	<.0001
site*plant species	1	0.05	0.194	0.6609
error	76	18.37		

Table 3. ANCOVA testing for differences in natural visitors to alpine clovers

Source	Num DF	Den DF	F Value	Pr>F
Type III tests of fixed effects				
plant species	1	58	35.12	<.0001
time of season	1	14	21.66	0.0004
plant species * time of season	1	58	7.12	0.0099

FIGURE LEGENDS

Figure 1. Mean nectar standing crop (+SE) for *T. dasyphyllum* and *T. parryi* measured in μg sugar per floret.

Figure 2 Relative inflorescence abundance of *T. dasyphyllum* and *T. parryi* in mixed patches. Error bars represent standard error.

Figure 3 Mean visits to inflorescences of each clover species during observations periods. Lower case letters indicate significant differences among groups.

Figure 4 Mean proportion of visits by type of visitor during observation periods. A) *T. dasyphyllum* early B) *T. dasyphyllum* late C) *T. parryi* early D) *T. parryi* late FLY=Anth. fly, LEP=Lepidopteran, QBK=*B. balteatus* queen, SOLB=solitary bee, SYR=Syriphid fly, UB=Unidentified bee, WBK= *B. balteatus* worker, OTHER=other visitors including ants and beetles. Only bumblebees, solitary bees, and a few flies were actually observed entering the clover florets.

Figure 1. Mean nectar standing crop (+SE) for T. dasyphyllum and T. parryi measured in μg sugar per floret
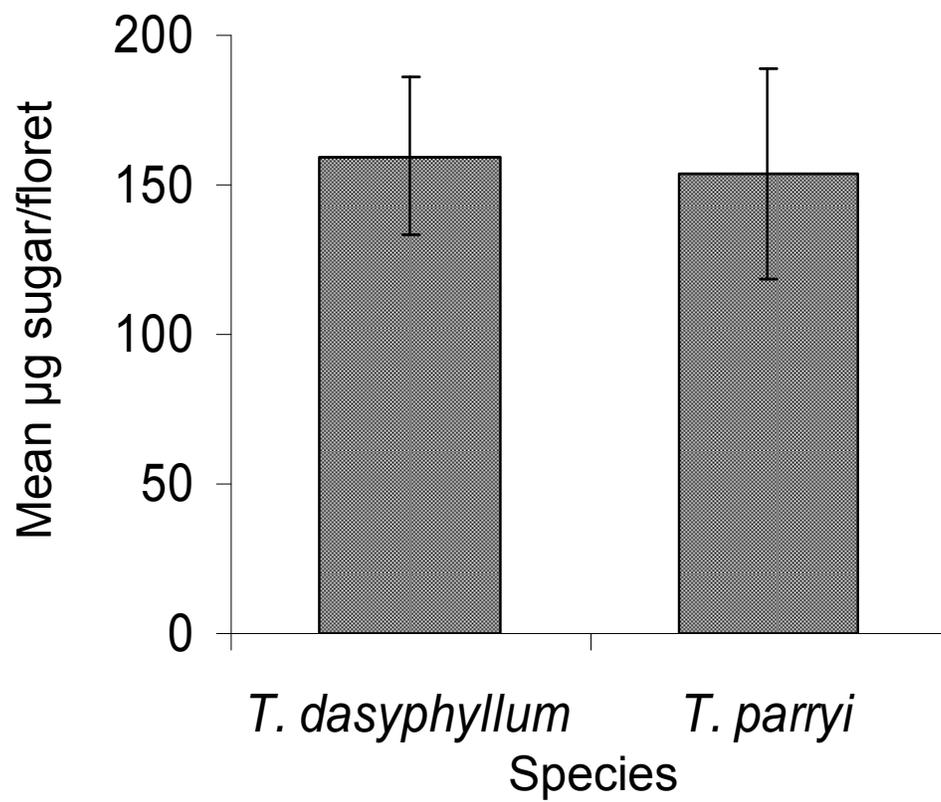
Figure 2 Relative inflorescence abundance of *T. dasyphyllum* and *T. parryi* in mixed patches. Error bars represent standard error.
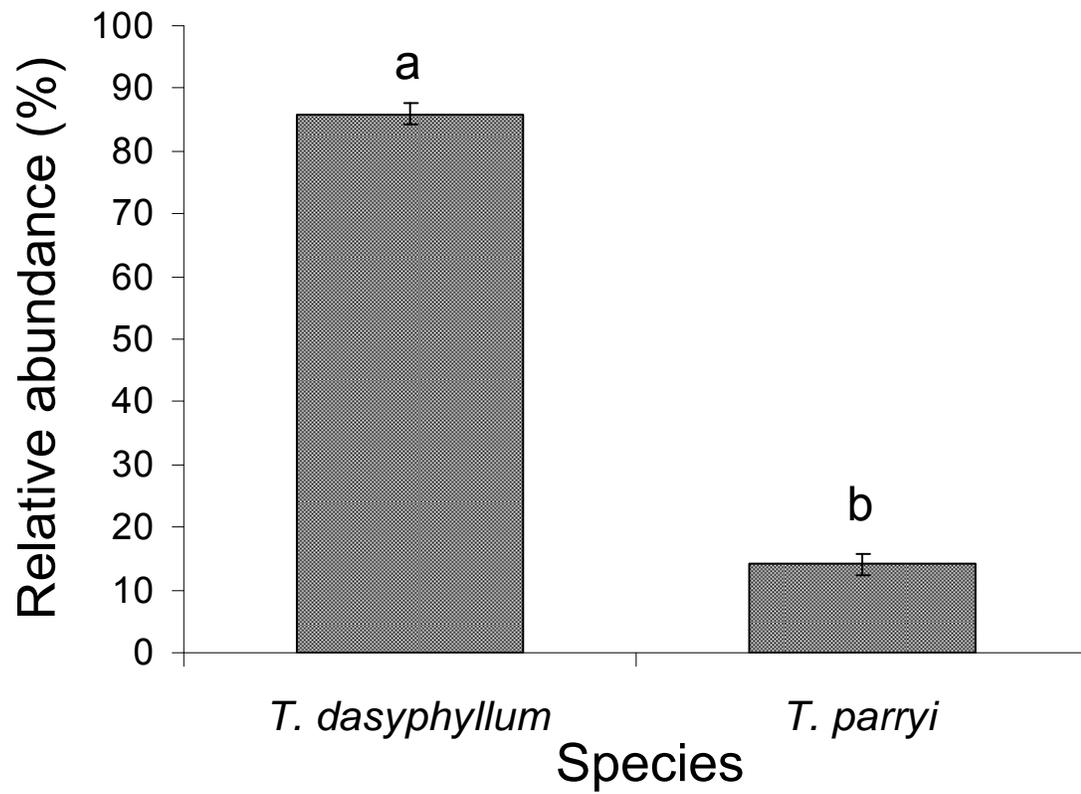
Figure 3 Mean visits to inflorescences of each clover species during observations periods. Lower case letters indicate significant differences among groups.
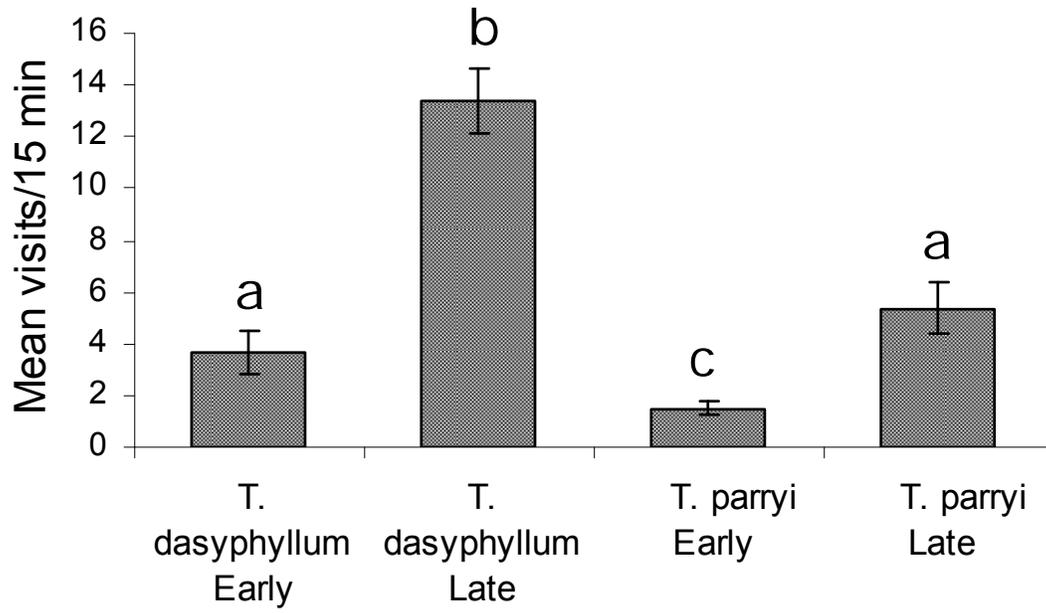
Figure 4. Mean proportion of visits by type of visitor during observation periods
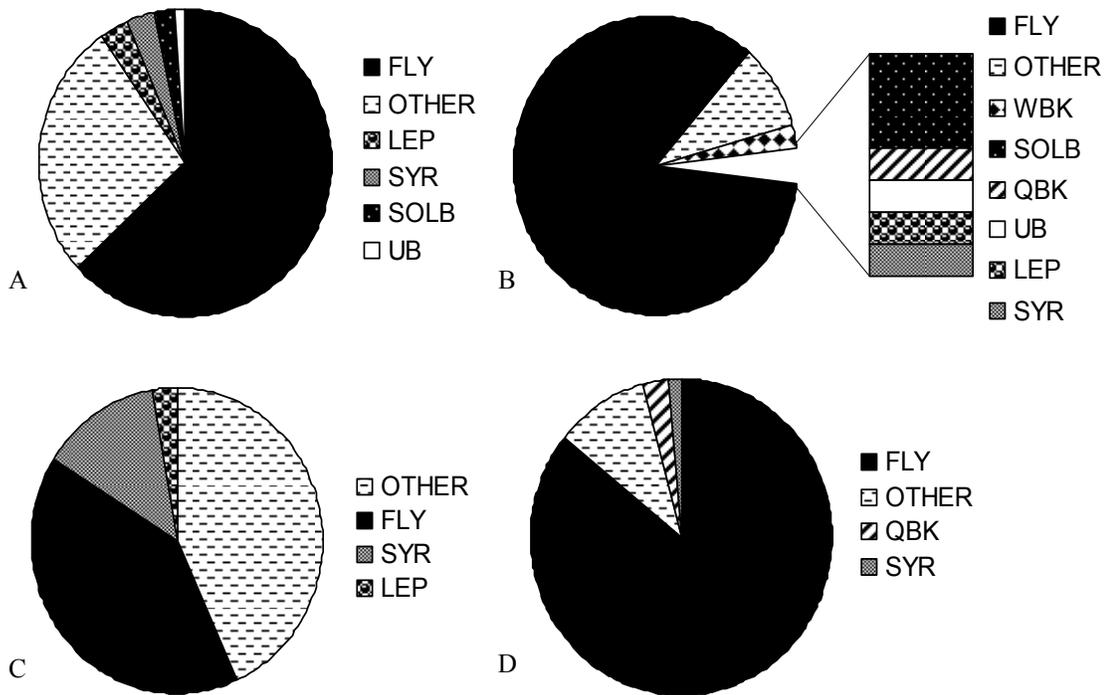
APPENDIX 2: ALPINE BUMBLEBEES SUPPLEMENTARY DATA

RELATIVE POLLINATION EFFICIENCY OF BUMBLEBEES FOR ALPINE CLOVERS

In June-July 2005 I assessed the relative per-floret pollination efficiency of the alpine bumblebees *Bombus balteatus* and *B. sylvicola* while foraging on two alpine clovers, *Trifolium dasyphyllum* and *T. parryi*. Inflorescences of each clover species were collected in the bud stage within the krummholz or lower alpine areas of Pennsylvania Mountain and were immediately placed in florist waterpiks. Inflorescences were kept in cold frames (part shade, part sun throughout the day) until they opened fully to prevent non-experimental visitors from gaining access to the florets. Once fully opened, inflorescences were randomly selected for inclusion in single-species experimental arrays (five inflorescences per array) for pollination efficiency trials.

Efficiency trials were conducted in a 12 x 12 m neutrally colored mesh tent (Coleman, USA) in the krummholz on Pennsylvania Mountain. *B. balteatus* (N=3) and *B. sylvicola* (N=3) queens used in the trials were caught primarily in low alpine areas at the study site, and brought to the tent on iced vials to induce torpor and prevent trauma. During trials, each bee foraged on one array of each clover species, presented in a random order. New arrays were used for each new bee. Within arrays inflorescences were arranged in a 10 cm by 10 cm square, with a fifth "starter" inflorescence in the center. The bee was first allowed to awaken and forage on the "starter" inflorescence, which was removed once the bee transitioned to the array. Each experimental inflorescence was also removed once it had been visited. As I removed the inflorescences from the array, I marked a floret that had received only one visit. Twenty four hours later, I removed the pistil from each marked floret just above the ovary and placed it in a vial with preservative (3:1 glacial acetic acid to ethanol).

Upon return to the lab (Columbia, MO) in September, pistils were rinsed twice with distilled water and cleared in 8M (N) NaOH for 24-26 hours. Upon removal from the NaOH, I again rinsed the pistils twice in distilled water, gently placed each on a microscope slide in one drop of 50% decolorized aniline blue/50% glycerol, and carefully squashed it with a cover slip. I used a compound microscope with epifluorescence capabilities (Carl Zeiss, Inc.) to visualize and count pollen grains on each stigma surface. Because clovers allow self pollen to germinate on the stigma, I assumed that constant amounts of self pollen were deposited by each bee for each species.

I analyzed differences in pollen deposition on clover stigmas using ANOVA (Proc GLM: SAS 9.2) with bee species, plant species, and their interactions as fixed effects in the model. Results from efficiency trials (Fig. 1; Table 1) showed that there were no differences among clover species in the amount of pollen deposited per bumblebee visit. The two bee species were comparably efficient as pollinators to both *T. dasyphyllum* and *T. parryi*, suggesting that their service is substitutable, in quality.

RELATIVE PREFERENCE OF BUMBLEBEES FOR ALPINE CLOVERS AMONG OTHER CO-FLOWERING SPECIES

In June-July 2005 I assessed the relative preference of the alpine bumblebees *B. balteatus* and *B. sylvicola* for alpine clovers among five co-flowering plant species (or species group if species could not be easily delineated) known to be visited by bumblebees (Byron 1980): *Erysimum* sp., *Mertensia* sp, *Oxytropis sericea*, *Phacelia sericea*, *Polemonium viscosum*. I conducted these preference trials in a 12 x 12 m neutrally colored mesh tent (Coleman, USA) during at least partly sunny weather conditions. Inflorescences of the seven species were arranged in an interspersed star-shaped array (Fig. 2). Each bee was placed on the ground in the center of the array so that it was equidistant from an inflorescence of each of the seven species. Each bee was allowed 30 minutes of foraging time in the array, not counting time spent on the tent walls grooming. Results are shown in Fig. 3.

BUMBLEBEE TRAIT SURVEYS

To test whether body characteristics of alpine bumblebees differ, I conducted surveys of newly emerged queen *Bombus balteatus* and *B. sylvicola* during June-July, 2008, on Pennsylvania Mountain and at a second site for comparison, Kite Lake, near Alma (Park County), Colorado, elevation 3666 m.

Bumblebees were caught haphazardly with nets and placed in iced vials for transport to the lab for measurements. I measured bee wet body mass (g) with a digital scale; combined length of the abdomen and thorax, as well as length of the head, front, middle and back legs (mm) were measured with a digital micrometer. Bumblebee measures were then analyzed for statistical differences between species using ANOVA (SAS 9.2), with bee species, site of collection, and their interactions as fixed effects. Results showed that *B. balteatus* was significantly larger than *B. sylvicola* in all body traits measured (P<.0001 for all traits; Fig. 4, Table 2, 3).

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Table 1. ANOVA testing for differences in queen *B. balteatus* and *B. sylvicola* per-visit pollen deposition to *T. dasyphyllum* and *T. parryi* florets.

Source	DF	Type III SS	F ratio	Prob>F
bee species	1	0.168	0.0435	0.8400
plant species	1	1.108	0.2876	0.6063
bee species*plant species	1	0.522	0.1355	0.7223
error	8	30.84		

Table 2. *B. balteatus* and *B. sylvicola* mean body measures at each site with standard errors in parentheses.

| | *B. balteatus* | | *B. sylvicola* | |
	Penn. Mtn. (N=32)	Kite Lake (N=32)	Penn. Mtn. (N=9)	Kite Lake (N=19)
Mass (g)	0.542 (.017)	0.514 (.010)	0.286 (.033)	0.361 (.010)
Proboscis length (mm)	9.48 (.195)	9.47 (.177)	5.65 (.230)	5.87 (.095)
Body length (mm)	20.63 (.477)	19.88 (.150)	15.23 (.923)	16.33 (.179)
Head length (mm)	7.30 (.111)	7.01 (.080)	5.57 (.154)	5.76 (.092)
Front leg (mm)	13.2 (.269)	13.2 (.127)	10.59 (.347)	10.69 (.181)
Middle leg (mm)	16.74 (.288)	16.91 (.142)	13.65 (.452)	13.88 (.179)
Back leg (mm)	19.55 (.388)	19.87 (.181)	16.13 (.313)	16.64 (.251)

Table 3. ANOVA testing for differences in body measures of *B.balteatus* and *B. sylvicola*

Source	DF	Type III SS	F Ratio	Prob>F
Body mass				
site	1	0.0084	1.9954	0.1619
bee species	1	0.6480	153.63	<.0001
site*bee species	1	0.0409	9.7025	0.0026
error	75	0.3163		
Proboscis length				
site	1	0.1474	0.2476	0.6204
bee species	1	195.80	328.76	<.0001
site*bee species	1	0.1952	0.3277	0.5689
error	68	40.498		
Body length				
site	1	0.5194	0.1888	0.6651
bee species	1	337.61	122.69	<.0001
site*bee species	1	14.461	5.2552	0.0245
error	80	220.14		
Head length				
site	1	0.0313	0.1540	0.6959
bee species	1	34.419	169.29	<.0001
site*bee species	1	0.8678	4.2683	0.0423
error	75	15.248		
Front leg length				
site	1	0.0230	0.243	0.8766
bee species	1	109.98	116.01	<.0001
site*bee species	1	0.0614	0.0648	0.7998
error	79	74.891		
Middle leg length				
site	1	0.6766	0.5927	0.4437
bee species	1	157.63	138.08	<.0001
site*bee species	1	0.0119	0.0105	0.9186
error	79	90.185		
Back leg length				
site	1	2.9099	1.6153	0.2074
bee species	1	186.71	103.64	<.0001
site*bee species	1	0.1560	0.0866	0.7693
error	80	144.12		

FIGURE LEGENDS

Figure 1. Pollen grains deposited per visit on alpine clovers by *B. balteatus* and *B. sylvicola*.

Figure 2. Multispecies interspersed preference array. Numbers 1-7 represent inflorescences of seven co-flowering alpine plant species placed in florist waterpiks.

Figure 3. *B. balteatus* and *B. sylvicola* preference (% of total visits) among seven co-flowering alpine plant species.

Figure 4 Comparison of alpine bumblebee anatomical characteristics: A) length and B) mass. Black bars are *B. balteatus queens*, white bars are *B. sylvicola* queens. Within each character, different letters indicate significant differences between the bee species at $p<0.05$.

Figure 1. Pollen grains deposited per visit on alpine clovers by *B. balteatus* and *B. sylvicola*.
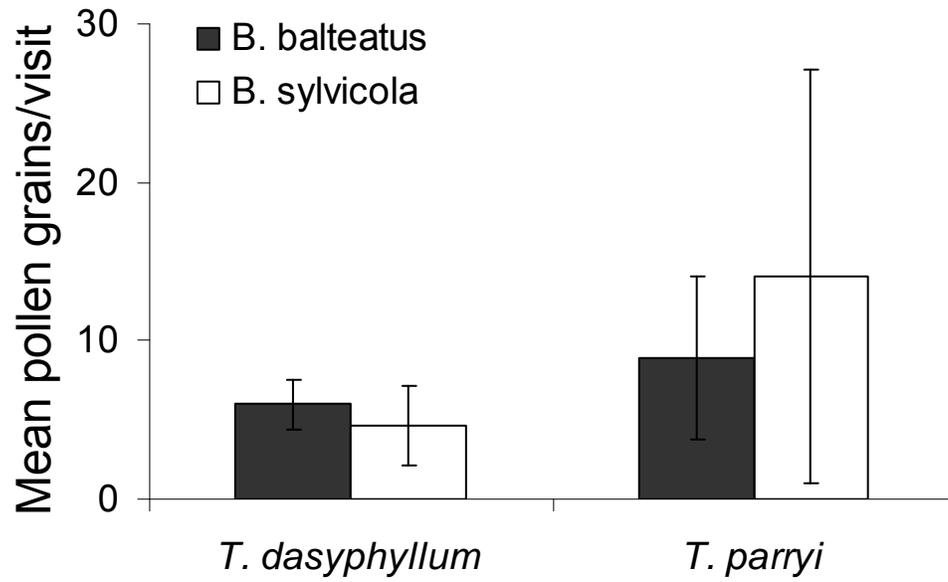
Figure 2. Multispecies interspersed preference array. Numbers 1-7 represent inflorescences of seven co-flowering alpine plant species placed in florist waterpiks.
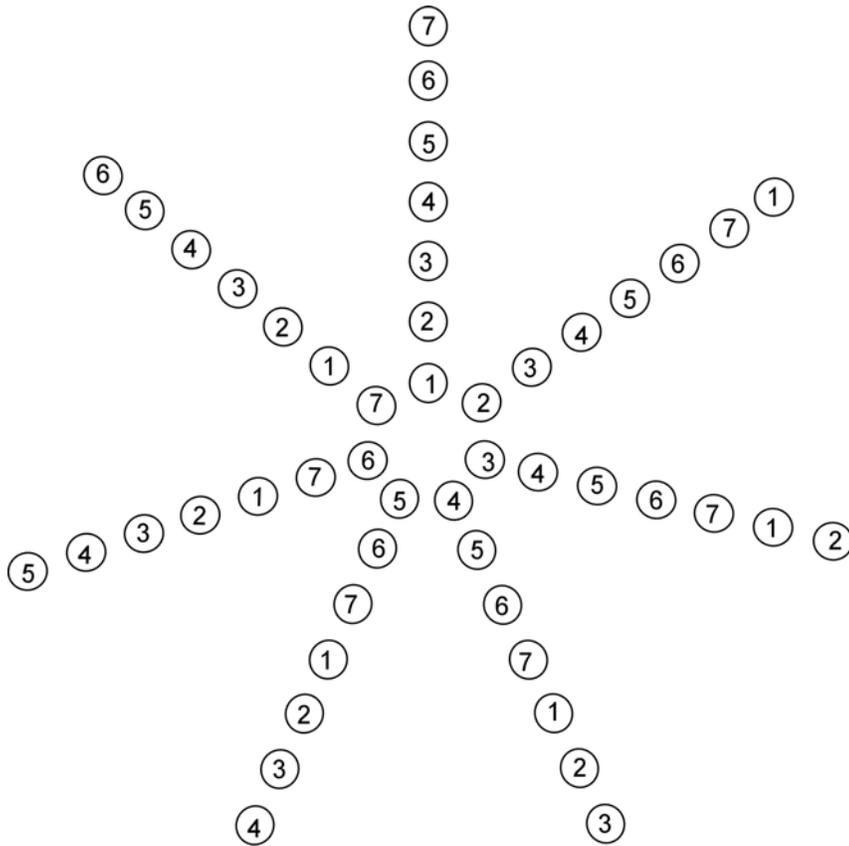
Figure 3. *B. balteatus* and *B. sylvicola* preference (% of total visits) among seven co-flowering alpine plant species.
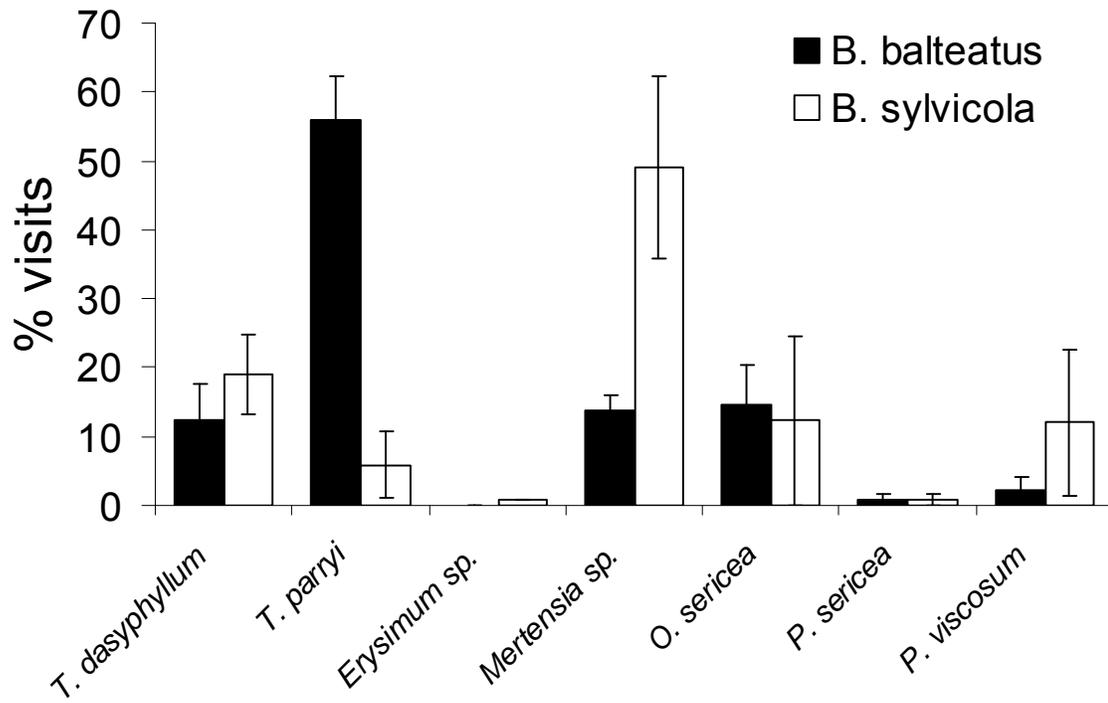
Figure 4. Comparison of alpine bumblebee anatomical characteristics
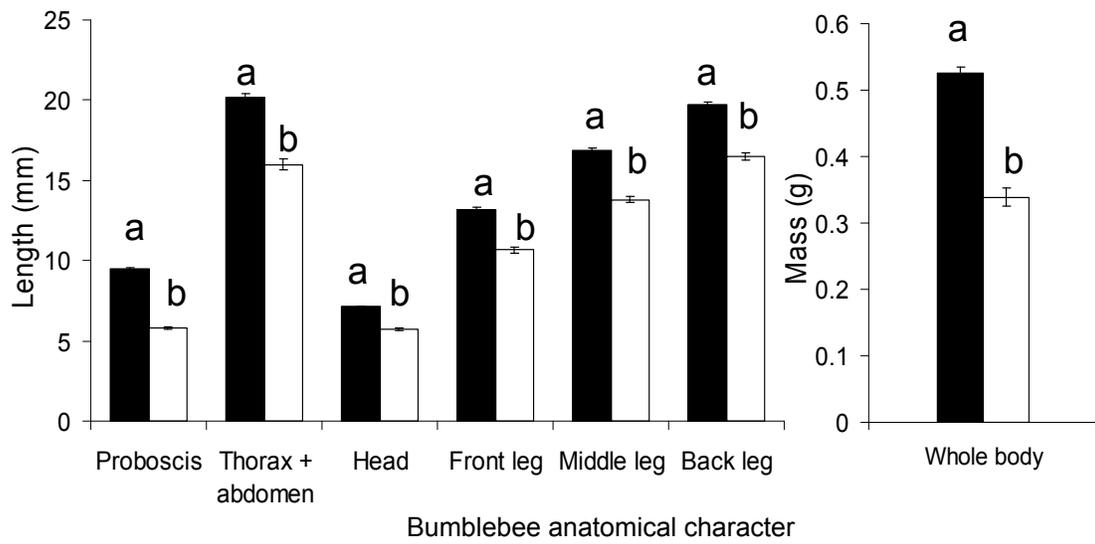
APPENDIX 3: MODEL DEVELOPMENT AND PARAMETERIZATION OF PROJECTION MATRICES FOR *TRIFOLIUM DASYPHYLLUM* AND *T. PARRYI*

In order to assess whether pollinator density effects on clover individuals translate into effects on population dynamics (Chapter 3), I developed stage-based (Lefkovitch) projection matrices for *T. dasyphyllum* and *T. parryi* based on their life histories (Fig. 1). Both clovers are long-lived alpine plants, with life histories consisting of five stages: dormant seeds, seedlings, juveniles, flowering adults, and vegetative adults. Stage definitions are in Table 1. Models are based on an annual time step with pre-breeding census characteristic of a birth pulse population (Caswell 2001). Vital rates in the projection matrices were based existing literature, data I collected, or unpublished data specific to *T. dasyphyllum* and *T. parryi* when possible (see Table 2). When no data were available, I used vital rates from other alpine plant species with similar life histories.

Because of the pre-breeding (pre-dispersal) census, newborn seeds enter the population as seedlings if they germinate by the next census; otherwise they are dormant seeds in the seed bank. Seedlings that germinate and grow during the first growing season were separated from later pre-adult ('juvenile") stages since overall mortality in alpine plant communities is often concentrated in the first year of life (Forbis 2003). I measured seedling survival ($S_{3,2}$) during my experiments but had to rely on unpublished data from other sources for the remaining juvenile years (Table 2). There were no data available on *T. parryi* juvenile survival rates, thus I used parameters from my study site for another alpine plant with similar life history, *Ranunculus adon*eus (Stanton and Galen 1997).

Alpine plants often remain in pre-adult stages for many years (Forbis 2003), but average time span in this stage is not known for alpine clover. Bonde (1968) showed that *T. nanum*, an alpine congener of *T.*

dasyphyllum and *T. parryi*, gained an average of three leaves in the first growing season and less than one leaf per year after that; however, by end of year three biomass increased three fold over that time span suggesting juveniles spend significant time establishing underground mass. In tracking seedling growth and development, neither *T. dasyphyllum* nor *T. parryi* gained more than a single leaf in their first growing season, suggesting they likely remain juveniles for at least a few years. Lacking exact data on the duration of the pre-adult phase for alpine clovers, I again used data from *R. adoneus*, one of a few alpine species for which this parameter has been estimated. Stanton and Galen (1997) found that *R. adoneus*, on average, begins flowering after its seventh year. Thus I set timing of the pre-adult stage for surviving clover individuals at seven years.

Flowering adult plants are the only phase contributing offspring to the next generation. Because of the pre-breeding annual census, seeds produced by adult plants are seen either as seedlings or dormant seeds in the seed bank the next year. Therefore, fecundity rates ($F_{1,4}$ and $F_{2,4}$) are given by the product of seed production rates per inflorescence $\times$ mean inflorescences per plant $\times$ the proportion either germinating the first year ($F_{1,4}$) or remaining in the seed bank ($F_{2,4}$). These parameters were all measured as a function of pollinator density, as was germination from the seed bank into the seedling class the second year ($S_{2,1}$). Studies of alpine clover seed germination in the lab and *R. adonis* seed germination in the field suggest that germination beyond the second year is negligible (Bonde 1965a, b, Stanton and Galen 1997) . Thus seeds remaining in the seedbank after the second year were assumed to be unviable.

T. dasyphyllum and *T. parryi* often regress to vegetative phases after flowering, as is reflected in their observed transition rates (AF-AV; $S_{5,4}$). However, I was only able to follow plants in the bumblebee addition patches for one year after flowering, so it is unknown how long they remain vegetative before flowering again and whether or not flowering and vegetative forms differ in survival rates. Thus, for vegetative adult plants I divided the mean survival rates evenly between the AV-AV and AV-AF vital rates.

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Table 1. Stages in alpine clover life history

| Stage	Definition and description
Seed	Ungerminated at the end of the first growing season
Seedling	Cotyledons present +/- true leaves (1st growing season)
Juvenile	Has true leaves, age 2-6 years, not flowering
Adult (flowering)	Has true leaves, $\geq$7 years, produced at least 1 inflorescence
Adult (vegetative)	Has true leaves, $\geq$7 years, but produced no inflorescences

Table 2. Alpine clover vital rates

Life history parameter		Estimated value	Source
A) T. dasyphyllum			
$S_{2,1}$	Seed bank to seedling	*F(pollinator density)*	This manuscript
$S_{3,2}$	Seedling to juvenile	0.40	(Geib 2010)
$S_{3,3}$	Juvenile to juvenile	0.64	T. Forbis unpublished data
$S_{4,3}$	Juvenile to AF	0.06	Based on *R. adonis**
$S_{5,4}$	AF to AV	0.24	Geib 2010
$S_{4,4}$	AF to AF	0.61	Geib 2010
$S_{5,5}$	AV to AV	0.43	Geib 2010
$S_{4,5}$	AV to AF	0.43	Geib 2010
$F_{1,4}$	Fecundity (AF to seed bank)	*F(pollinator density)*	This manuscript
$F_{2,4}$	Fecundity (AF to seedling)	*F(pollinator density)*	This manuscript
B) T. parryi			
$S_{2,1}$	Seed bank to seedling	*F(pollinator density)*	This manuscript
$S_{3,2}$	Seedling to juvenile	0.40	Geib 2010
$S_{3,3}$	Juvenile to juvenile	0.68	Based on *R. adonis**
$S_{4,3}$	Juvenile to AF	0.09	Based on *R. adonis**
$S_{5,4}$	AF to AV	0.64	Geib 2010
$S_{4,4}$	AF to AF	0.14	Geib 2010
$S_{5,5}$	AV to AV	0.39	Geib 2010
$S_{4,5}$	AV to AF	0.39	Geib 2010
$F_{1,4}$	Fecundity (AF to seed bank)	*F(pollinator density)*	This manuscript
$F_{2,4}$	Fecundity (AF to seedling)	*F(pollinator density)*	This manuscript

Notes: S_{ij} = contribution of surviving individuals from class j to i. F_{ij}= contribution of offspring from class j to i. AV=vegetative adult, AF=flowering adult, * source=Stanton and Galen 1997

FIGURE LEGENDS

Figure 1. Loop diagram of alpine clover life history stages and transitions between them (arrows). Bold arrows represent stages through which we traced the effects of increasing pollinator abundance on vital rates. S_{ij} = contribution of surviving individuals from class j to i. F_{ij} = contribution of offspring from class j to i.

Figure 1.
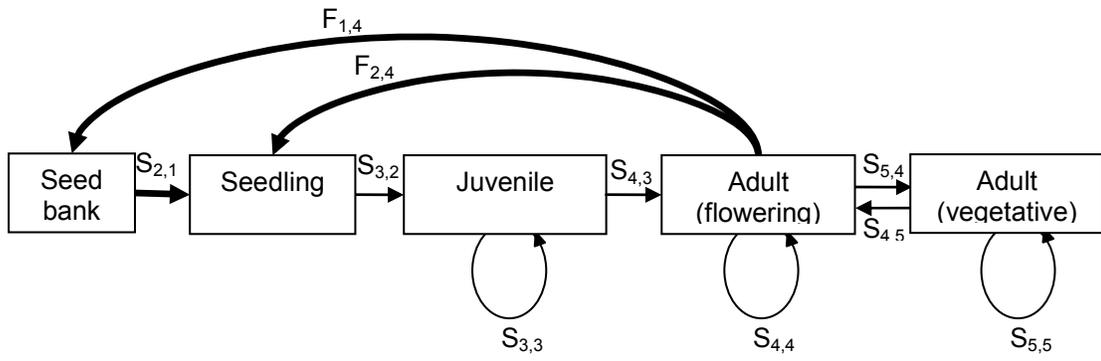
APPENDIX 4: POLLINATOR GARDENS OUTREACH PROGRAM SUPPLEMENTARY MATERIAL

INTERVIEW PROTOCOL TO ELICIT TEACHER ORIENTATIONS

This interview protocol to elicit and clarify the participant teacher's orientations toward use of pollinator gardens was directly based on methods by Friedrichsen and Dana (2003). As in their paper, the interview consisted of two parts, a card-sort task followed by open-ended interview questions.

Part 1: Card-sort task

I asked the teacher to read a set of scenario cards and sort the cards into three stacks: a) "This scenario best represents how I would use the pollinator gardens," b) "This scenario does not represent how I would use the pollinator gardens," and c) "unsure". I included the following scenarios:

1. You introduce the pollinator gardens by presenting basic background information and terminology before moving into activities.

2. You have each student select a research topic related to pollinators from a list that you provide. Students use the school library and the Internet as resources for writing a report on their topic.

3. You have your students first engage in hands-on activities related to pollination, then follow up with discussion.

4. You introduce the pollinator gardens by presenting basic background information and terminology before moving into activities.

5. You give students access to a wide variety of plants and a wide variety of insect specimens. By letting the students explore on their own they will be able to discover ideas such as reciprocal selection on traits of plant-pollinator partners.

6. Students write to their local or state representatives to voice concerns about the status of pollinators in Missouri.

7. You have students keep a journal about how their ideas about the pollinator gardens have changed over time.

8. To help your students understand pollinator characteristics, you organize a series of stations, with each station containing representatives from a different class of pollinators.

9. You have your students observe pollinators in the gardens and generate research questions. Each small group carries out their own experiment to test a hypothesis related to the group's questions.

10. You have students document daily visits to flowering plants in the gardens and have them measure the resulting seeds set.

11. You have students memorize the names of the plants in the gardens and their most important pollinators.

12. Students play the part of conservation agents. They design reserves for endangered butterflies, "buying acres" with limited "tax dollars". Students use computer simulations to see how their reserve designs impact butterfly populations over the next 100 years.

13. You have students develop a project of their choosing (poster, letter to the editor, skit for elementary students) related to some aspect of pollination biology.

14. In planning your use of the pollinator gardens, you collect a variety of activities for the students to do. You organize each session by doing a different activity each day.

15. In considering how farm practices impact pollinators, you have students debate the positive and negative aspects of the use of pesticides on crop plants.

16. You begin students' learning about pollinator gardens by having students read a chapter in a textbook.

17. You take students on a nature walk to learn about wild plants and insects.

18. Students explore their own ideas about pollinator gardens. One uses library books to research medicinal plants while another investigates whether or not invasive plants reduce pollinator visits to native plants.

19. You have students watch a video about how pollinators are important in their lives.

20. You have students research native plants in the area to decide which plants would be best included in a garden to attract pollinators.

21. Students design the layout of gardens, research plant care, and select tasks for contributing to garden maintenance.

22. You ask students to discuss how a scientist would determine whether invasive plants impact pollination to native plants.

23. Students learn about careers that involve taking care of plants and gardening.

24. To apply what they have learned about pollinator gardens, you have students do a service project – designing a pollinator garden for a local nursing home.

25. You have students share data collected from the gardens, and encourage them discuss what the data means.

Part 2: Open-ended interview questions

As described in the Friedrichsen and Dana (2003) paper, the purpose of this part of the interview was to probe the teacher to articulate reasoning behind the classification of the scenarios. I selected stack A, the scenarios selected by the teacher that best represent how he would use the pollinator gardens. For each card, I asked the teacher to describe as best he could: "How does this scenario support your purposes and goals for teaching science? Which aspects of this scenario are the most appealing to you as a teacher?" At the end I also asked: "If you were going to teach a unit on this subject, what additional strategies would you use?"

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VITA

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