RECOVERY OF AN ALPINE *BOMBUS* COMMUNITY FOLLOWING DROUGHT-MEDIATED POPULATION CONTRACTIONS

A Thesis
by
LEIGH RIMMER

Submitted to the Graduate School
at Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

August 2015
Department of Biology

RECOVERY OF AN ALPINE *BOMBUS* COMMUNITY FOLLOWING DROGHT-MEDIATED POPULATION CONTRACTIONS

A Thesis
by
LEIGH RIMMER
August 2015

APPROVED BY:

Jennifer Geib
Chairperson, Thesis Committee

Matthew Estep
Member, Thesis Committee

Ray Williams
Member, Thesis Committee

Sue Edwards
Chairperson, Department of Biology

Max C. Poole, Ph.D.
Dean, Cratis Williams Graduate School

Abstract

RECOVERY OF AN ALPINE *BOMBUS* COMMUNITY FOLLOWING DROUGHT-MEDIATED POPULATION CONTRACTIONS (AUGUST 2015)

Leigh Rimmer
B.S., Appalachian State University
M.S., Appalachian State University

Chairperson: Jennifer C. Geib

Worldwide pollinator decline has increased the urgency of assessing native pollinator abundance and genetic fitness of existing populations (Darvill et al. 2006, Ellis et al. 2006, Goulson et al. 2008, 2015, Geib et al. 2015). In 2012, an extreme and persistent drought in the intermountain western US caused severe population contractions within a *Bombus* (Order: Hymenoptera, Family: Apidae) community at Pennsylvania Mountain Natural Area, Park County, Colorado. There was a 99% decrease in overall forager abundance compared to previous years, and no reproductives were seen during the sampling season. The severe population contractions resulting from the drought served as a natural opportunity to investigate how bumble bees can recover from catastrophic events. This study aimed to 1) measure the post-drought recovery of the alpine *Bombus* community and 2) measure the "effective population size" and search for evidence of a corresponding genetic bottleneck in a historically dominant alpine bumble bee, *Bombus balteatus*.

I conducted field samples to collect all *Bombus* foragers present at the peak of the late flowering/foraging season in 2013. Samples were used conducted comparisons of 1) the overall size of the *Bombus* community, 2) relative species abundances, 3) relative abundances of ecologically relevant groupings of species (alpine vs. recently invading subalpine *Bombus* and long vs. medium, and short-tongued bees), 4) mean tongue length of the *Bombus* community, and 5) measures of species diversity or richness. Statistical analysis of these measures revealed that the community is relatively unchanged from pre-drought estimates. The 2013-year yielded the highest rate of forager capture, no change in overall species or ecologically grouped species abundance, and no change in frequency of tongue length or mean tongue length. Furthermore, 2013 had higher levels of species diversity and richness than any other pre-drought year. These observations suggest that alpine bees may have mechanisms to recovery quickly from severe population contractions and that there may be nearby source populations responsible for replenishing the community.

The second goal of this study was to obtain information from genotypes from *Bombus balteatus* individuals to 1) document size of breeding population ("effective population size," or N_e) and 2) identify any evidence of a genetic bottleneck after an observed population bottleneck. PCR amplification of 9 microsatellite loci provided genotypic data for the 2013 population, and pre-drought genetic information from 2008 served as a comparison. Estimates of N_e were made using COLONY 2.0 software by using genotypes to assign sibships. Genotypes were also required to conduct three different bottleneck tests: the *M*-ratio, heterozygosity excess, and mode shift. N_e estimates did not differ significantly from pre-drought estimates and were well above predicted levels. None of the bottleneck tests revealed any evidence that a genetic bottleneck persisted in the 2013 *Bombus balteatus* population. This outcome suggests that immigration and dispersal to high alpine mountaintops rates are high, and that these mountaintops may be less isolated than previously thought.

Acknowledgments

I give infinite thanks to my advisor, Jennifer Geib, for all of her insight, patience, generosity, and wisdom. I thank Dr. Estep for teaching me new techniques, the use of his molecular lab at ASU, and for spending countless hours to help me troubleshoot my experiments. I'd also like to thank Dr. Williams who first taught me entomology and has provided invaluable insight in the creation of my thesis.

Without the help Jack Stoehr and Ashley Allison, this project may not have been possible. Thank you for all your assistance and optimism in the field and the lab; you will forever be some of my dearest friends. Many thanks to Eric Rayfield, Kimberly, and Mary for help in the lab and field.

To my boyfriend James Montgomery, who shared this journey with me, thank you for always believing in me and being my biggest fan. Brittany Wolff, thank you for being my muse and friend through it all.

My wonderful and saintly parents, Donna and Malcolm Rimmer, are the source of all my accomplishments and strength. Thank you for always having my back and never letting me doubt myself.

Appalachian State University Office of Student Research and Cratis D. Williams Graduate School provided funding for this research.

Table of Contents

Chapter 1

GENERAL INTRODUCTION

Plant-pollinator mutualisms are important ecosystem relationships in angiosperm communities (Allen-Wardell et al. 1998, Kearns et al. 1998, Ghazoul 2005), where both the pollinator and the plant receive benefits to varying degrees (Stanton 2003). Pollinators benefit by receiving nutrient rewards in the form of pollen or nectar, and plants benefit by receiving aid in sexual reproduction via mechanical movement of their pollen to another individual, or the receipt of pollen themselves (Lautenbach et al. 2012). Negative impacts on these relationships are of particular concern because of evidence of worldwide declines in pollinator abundance (Goulson et al. 2015) which may endanger pollinator-dependent plants (Klein et al. 2003, Inouye 2008, Gong and Huang 2011).

While declines of managed honeybees have gained the most attention (Goulson et al. 2015), wild bees, including bumble bees, have been sparsely studied worldwide, although monitoring efforts have intensified (e.g., pollinatorwatch.org and bumblebeewatch.org). European bumble bees have experienced significant range declines, localized extirpation, and four documented extinctions across the continent (Kosior et al. 2007, Goulson et al. 2008). South American bees have faced invasions from North American bumble bees, which has caused native pollinator species to decline in turn (Schmid-Hempel et al. 2014). In North America, two wide-spread and historically abundant bumble bees, *Bombus terricola* and *B. occidentalis* have decreased severely since the 1990s (Williams et al. 2009). The Northern

California bumble bee *B. franklini* has not been reported seen since 2006, and is thought to be extinct. A recent study addressing native bumble bee declines in Illinois has found four species extirpated from the region and substantial declines in overall bumble bee species diversity (Grixti et al. 2009). Despite the limited amount of data currently available for just a few species of North American bees, the predictions drawn from such research forecast a troubling future for the many wild bumble bee populations that have not yet been surveyed.

Economically, pollinator decline can have huge implications for the agricultural industry, as pollinators are estimated to contribute an estimated $14 billion dollars in services to crops each year and are responsible for pollinating 75% of all food crops worldwide (Goulson et al. 2015). Evidence linking pollinator decline and lowered species diversity to diminished crop yields has already emerged (Grixti et al. 2009), as has relationships to loss of pollinator species diversity (Klein et al. 2003). Not only will this breakdown of mutualisms cause economic problems, but a lack of pollination services will reduce the amount of available outcross pollen for plants, and diversity may suffer (Memmott et al. 2004). Smaller populations of bees will also have smaller pools of available genetic diversity, which causes lowered genetic fitness for colonies and inhibit the ability for colonies to fight off parasites and disease (Liersch and Schmid-hempel 1998, Gillespie 2010).

There are several identified causes of bumble bee decline. Habitat loss is responsible for creating a cascade of problems for native pollinators including decreasing availability of nest sites, restricting floral resources, and creating fragmented subpopulations that may become isolated over time and restrict gene flow (Ellis et al. 2006). Competition from non-native bees, such as the introduction of *Apis mellifera* from Europe to North America, can displace and outcompete native pollinators, especially when there are no parasites or diseases in the new region that can limit population sizes or rates of expansion (Goulson 2003, Thomson 2004, Goulson et al. 2005). Parasites, whether native or transported from foreign regions, are becoming a pressing concern for bumble bees. As commercial colony production expands, prevalence of parasites such as *Nosema bombi, N. ceranae,* and *Apicystis bombi* can infect individuals more readily and individuals mingling with wild bees can introduce the parasites to non-managed colonies (Graystock et al. 2013). Pesticides, especially neonicotinoids, are suspected in the decline of managed and wild bee populations within industrial agriculture landscapes (Goulson et al. 2005).

Unfortunately, global climate change (especially higher temperatures and changes in precipitation) may serve to exacerbate many of the problems pollinators are facing today (Goulson et al. 2015). Changing environmental conditions can cause rapid changes in community structure and composition and is expected to drive species range shifts upwards in latitude and elevation. Some preliminary evidence already exists for species range shifts caused by changing temperatures (Williams et al. 2009) and non-native competitive species and invasive parasites may expand their range (Walther et al. 2002, Le Conte and Navajas 2008). Flower phenology may begin to mismatch with pollinator emergence, leading to breakdowns in mutualisms (Parmesan 2006, Memmott et al. 2007, Inouye 2008, Goulson et al. 2015).

Predictions for global climate change include a rise in average global temperature of at least 2°F in the next decade (up to 11.5°F by 2100) (USGCRP 2009). Northern hemisphere snow cover is expected to decrease by 15% by 2100, and snow accumulation will begin later and melt earlier (National Research Council [NRC] 2011). Extreme weather events may become more common, with higher winds, most intense precipitation events, and more frequent and prolonged droughts, especially in the American West (Harrison 2000, Rasmont and Iserbyt 2012).

In the US, the Intermountain West has already experienced significant impacts from climate change. The region has seen above average yearly temperature increases of 2.5°F and in every season, daily minimum temperatures have warmed more than daily maximums (Childress et al. 2015). Since 2000, average snowpack measured by April 1st has declined substantially, and is especially pronounced in the Southern Rocky Mountains below 45 degrees latitude. Furthermore, snowmelt and peak runoff has occurred an average of one to four weeks ahead of usual, which may be contributing to the tendency of more frequent soil moisture droughts also seen in the area (Childress et al. 2015).

Intermountain West predictions for mid-century forecast worsening conditions. Temperatures are projected to increase by an average of 5.5°F, and the early melting of winter snowpack is expected to advance the spring runoff date (when melting usually begins) by one to three weeks. Winter precipitation is more likely to fall in the form of rain rather than snow (McCabe and Wolock 2010), and snowpack is expected to dissipate as early as April 1st. In concordance with worldwide predictions for climate change, this region will experience an increase in number and duration of severe heat waves, drought, and wildfire (Childress et al. 2015). It is currently unknown how pollinator populations and communities will fare under extreme drought conditions.

My thesis research takes advantage of a natural opportunity to investigate implications for pollinators from a particularly intense drought in the Western US in 2012. The context of the study is a native alpine *Bombus* community situated in the Colorado Rocky Mountains, at the Pennsylvania Mountain Natural Area (PMNA), Mosquito Range, Park County. This community was the subject of considerable study in the late 1970s and has been monitored semiannually since 2008.

During 2012, a severe drought struck midway though the water year (measured October through September) and was classified as "extreme" drought by June of that year (droughtmonitor.unl.edu). The early spring months of March – May constituted the second warmest spring in Colorado in the past 118 years and this early warming accelerated the early melting of the already-low snowpack by 4-8 weeks ahead of average. This caused excessive transpiration from soil and vegetation, and more importantly, the loss of the snow responsible for insulating overwintering bumble bee queens (Lukas and Darby 2012). Coinciding with extremely early flowering and senescence of the plant community in 2012 (average bloom time was 45% shorter than normal; Franklin 2014), significant reductions in foraging bumble bee densities were observed compared to previous years (Fig. 1), and no *Bombus* reproductives were observed at the end of the growing season. The mountainous terrain and potential geographic isolation of these populations, along with the *Bombus* life history strategy of re-establishing populations each season from foundress queens of the previous year predicted that carry-over effects would be observed in 2013.

Chapter two outlines my first study documenting the recovery of the *Bombus* community by quantifying species composition and relative abundances of *Bombus* foragers one generation following the drought-mediated population contractions. Foragers are important to the floral community in that they provide direct pollination services and increase plant fecundity for obligate outcrossing plants. Forager samples were also used to estimate the average pollinator tongue lengths within the bumble bee community because pollinators partition themselves among the community based on tongue length and corresponding flower corolla tube lengths (Stang et al. 2006). I compared the diversity and richness of species in the years proceeding, during, and following the drought, and compared post-drought measures to recent data to determine any summary changes resulting from the severe population reductions during the drought year.

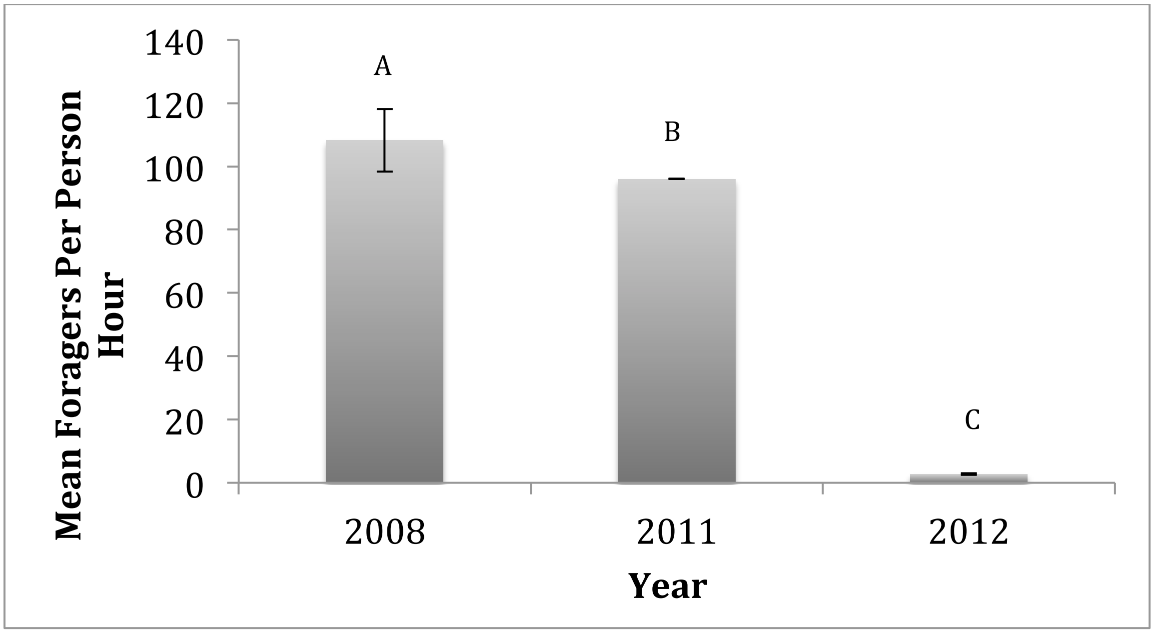
Chapter three focuses on effects of demographic contraction on population genetics of one of two historically dominant alpine *Bombus* species, *Bombus balteatus. Bombus balteatus* is the only long tongue bumble bee in the alpine tundra and is important for the pollination of many alpine plant species; especially those that have long corolla length tubes. Unfortunately, worldwide declines of pollinators are impacting long-tongue species especially hard due to the loss of varied floral resources (Goulson et al. 2008). I sampled DNA from foraging workers and used molecular genetics techniques to examine effective population sizes pre- and post-drought and test for any genetic consequences that would be predicted following severe population reductions, such as loss of heterozygosity (bottleneck effects), that could impact the health and future persistence of these populations.

This study aims to take advantage of this natural opportunity to observe how alpine pollinators will be able recover from an extreme weather event that is expected to become more common and prolonged in the future. It can provide insights into whether instantaneous climactic events may work to facilitate shifts in relative abundances and diversity in pollinator communities. Molecular analyses should set the stage for future research into landscapes factors that facilitate or resist gene flow among pollinator populations.

Figure Legends

Figure 1: Capture rate of foraging worker bumble bees, as a proxy for absolute abundance, during semi-annual surveys at Pennsylvania Mountain, Park County, CO. Columns represent means of sites sampled each year (n = 4, 2, and 4 for 2008, 2011, and 2012, respectively). Error bars are standard error. (Geib and Galen, *unpublished data*).

Figure 1.


140
120
100
80
60
40
20
0
Mean Foragers Per Person Hour
A
B
C
2008
2011
2012
Year

Chapter 2

ABSTRACT

RECOVERY OF ALPINE BUMBLE BEE COMMUNITY FOLLOWING DROUGHT-MEDIATED POPULATION CONTRACTIONS

As pollinator populations continue to decline worldwide, it is important to be able to estimate how populations are able to recover from stochastic declines in population size due to severe weather events, which are expected to increase in frequency. The availability of pollinators is critical for host plants dependent on these mutualistic relationships for sexual reproduction. In 2012, an extreme drought swept the intermountain western US and caused a 99% reduction in overall *Bombus* forager abundance on Pennsylvania Mountain, Park County, Colorado. This study conducts surveys of the post-drought *Bombus* community to make measurements of the ability for pollinators to recover from significant population declines and compares it to *Bombus* community data available from pre-drought years at this site. I conducted comparisons of 1) the overall size of the *Bombus* community, 2) relative species abundances, 3) relative abundances of ecologically relevant groupings of species (alpine vs. recently invading subalpine *Bombus* and long vs. medium, and short-tongued bees), 4) mean tongue length of the *Bombus* community, 5) measures of species diversity and richness. I hypothesized that the severity of the population decline would cause slow recovery of overall abundance, and that relative abundances of alpine species compared to invading non-alpine species would be low. I also expected lower levels of diversity and richness under the

assumption that rarer species would not be able to recover as quickly as well established species.

Analyses of sampled individuals revealed surprising results: the 2013 season had higher rates of capture than any other pre-drought year. There were no significant differences between years of relative species abundance, alpine vs. non-alpine relative abundances, frequency of tongue length, or change in mean tongue length size. Most astonishing, however, was that the 2013-year had the highest measurements of both species diversity and richness when compared to all pre-drought years. These results suggest that alpine bumblebees may have some mechanism to recover from stochastic and severe population reductions, and that there may be unforeseen factors, such as source-sink dynamics and dispersal, maintaining consistent community measurements after extreme weather events. Further research should be conduction to assess long-term recovery of the *Bombus* community and stability of pre-drought communities.

Introduction

The mounting evidence for worldwide declines of pollinator populations has prompted rapid action to increase monitoring efforts across the globe. Native bees, in particular bumble bees, have received significant attention as keystone pollinators ubiquitous to nearly all terrestrial habitats. The most recent efforts to re-survey *Bombus* species' historical ranges have revealed both changes in abundance and range shifts, with some species experiencing significant declines and/or range contractions (Cameron et al. 2011, Goulson et al. 2015). It is unclear how declines will affect bumble bee populations and communities long-term (Goulson et al. 2008, Grixti et al. 2009, Potts et al. 2010, Rasmont and Iserbyt 2012).

Severe population reductions have the potential to trigger a cascade of adverse effects. In bumble bees, which are eusocial pollinators, reduction of colony numbers can reduce the overall fitness of the species or population (Allen-Wardell et al. 1998, Bouzat et al. 1998, Darvill et al. 2006, Osborne et al. 2007, Whitehorn et al. 2009), decrease ability to fight off parasites and disease (Gillespie 2010), and lower genetic variability in future colonies (Ellis et al. 2006), further increasing the risk of local extinctions.

The implications of pollinator loss span beyond the pollinators themselves; loss of pollinators correlates with loss of functionally linked native plants, and decreases in native plant diversity (Biesmeijer et al. 2006) and agricultural productivity (Harrison 2000, Dukas 2005). This isn’t surprising given that insect pollinators alone provide an estimated 14 billion dollars’ worth of services to the agricultural industry (Calderone 2012).

There are several causes of decline in insect pollinators including the intensification of farming practices, monocropping, fragmentation of suitable habitats, loss of nesting sites, increased competition from invasive and non-native species, and potentially pesticide use (Goulson et al. 2005, 2008, 2015, Kosior et al. 2007, Potts et al. 2010, Pauw and Hawkins 2011). Findings from rural landscapes implicate climate change in driving recent losses (Potts et al. 2010, Burkle et al. 2013). Extreme and prolonged droughts are of particular concern because they are expected to increase in severity and persistence as climate change continues (Dai 2012). However, in human-dominated landscapes, climate change impacts are generally confounded with historical changes in land use. Studies in more pristine ecosystems can better offer insights into climate impacts on local extinctions and other responses of pollinator populations and communities.

This study takes advantage of a natural opportunity to observe climate impacts to pollinators and their ability to recover from extreme weather events in a relatively untouched alpine ecosystem in the Central Rocky Mountains. Pennsylvania Mountain Natural Area ("Pennsylvania Mountain"), Park Co., CO, has provided the backdrop for studies of pollinator populations since the 1970s and semi-annually since the late 2000s. These surveys of bumble bee demography and diversity provide a baseline against which impacts of extreme climate events can be gauged. Here, we propose to evaluate such impacts, focusing on the aftermath of an extreme drought in 2012 for the Pennsylvania Mountain alpine bumble bee community that has also experienced recent introductions of new species from lower elevation subalpine habitats since 2008 (Geib et al. 2015).

In 2012 a severe and pervasive drought hit the intermountain western US, including Colorado, an event that is predicted to become more common in this area as shifts in rainfall frequency and abundance become more erratic with increased warming due to climate change (Gutzler and Robbins 2011, Pederson et al. 2013). Extreme drought extended from March-November with impacts exacerbated by "persistent and anomalous" heat (National Oceanic and Atmospheric Administration 2013). During the 2012 season, snowpack in the central Rockies melted over 4 weeks ahead of average, and vegetation was drying out by June (Lukas and Darby 2012). It was the worst drought in this area since 2002, which was considered a benchmark year. Drought severity was on par with conditions generating the dust bowl of the 1930s (National Oceanic and Atmospheric Administration 2013). On

Pennsylvania Mountain, such dry conditions were further magnified by the rain shadow effect of the Continental Divide (Peterson and Billings 1982).

Coinciding with extremely early flowering and senescence of the plant community (average bloom time was 45% shorter than normal; Franklin 2014), dramatic reductions in foraging bumble bee density were observed in the summer of 2012 for all species normally present compared to the previous five years (99% reduction overall, Geib and Galen, *unpublished data*). This event provided an interesting opportunity to observe re-establishment of the *Bombus* community following the drought and compare relative species composition in climatically more normal years before and after the 2012 population contractions.

Pollinator populations naturally exhibit annual, seasonal, and spatial variation in abundance driven by changes in weather patterns (suitable temperatures, adequate rainfall) (Rasmont and Iserbyt 2012), resource availability (including temporal and spatial floral abundances) (Itioka et al. 2001), and competition (Zimmerman and Pleasants 1982, Thomson 2004). However, it is unclear whether alpine bumble bees, potentially isolated by mountainous habitat, can recover from such extreme population reductions as observed in 2012 or whether local extinction is a more likely possibility.

The primary objectives of this study were to assess recovery of the alpine *Bombus* community following the drought-mediated population reductions in 2012 and compare pre- and post-drought community composition. We specifically conducted comparisons of 1) the overall size of the *Bombus* community, 2) relative species abundances, 3) relative abundances of ecologically relevant groupings of species (alpine vs. recently invading subalpine *Bombus* and long vs. medium, and short-tongued bees), 4) mean tongue length of the *Bombus* community, 5) measures of species diversity and richness.

We expected to see slow re-establishment of alpine bumble bee populations, leading to generally fewer foragers of alpine species during the 2013 blooming season. We also hypothesized that the drought might facilitate further upward movements of subalpine species above tree line, altering community composition and relative species abundances.

Methods

Site and Study System

All samples for this study were collected at Pennsylvania Mountain Natural Area ("Pennsylvania Mountain"), Park County, Colorado, USA, in late July-August 2013 and 2014. Pennsylvania Mountain is divided by the headwaters of an east-flowing stream, Pennsylvania Creek. The southern half of the mountain is characterized by a number of east-facing slopes, each topped by ridges or plateaus, extending from tree line (~3500 m) upward to the summit 3963 meters. Above tree line, slope faces comprise a matrix of interspersed alpine meadows and fell fields, and flora consists mainly of flowering perennials characteristic of alpine habitats, grasses, and sedges.

Historical surveys of bumble bees (*Bombus*) on Pennsylvania Mountain in the late 1970s revealed a community that typically consisted of *Bombus balteatus, B. sylvicola,* and *B. frigidus* (Byron 1980). *Bombus balteatus* and *B. sylvicola* dominated the alpine tundra regions above 3600 meters (99% of foraging individuals, Byron 1980), with rare appearances of *Bombus frigidus*. Low alpine bumble bees, *Bombus flavifrons* and *B. bifarious,* were most abundant at elevations below 3100 meters. More recent surveys at Pennsylvania Mountain in2008, 2011, and 2012 sampled the bumble bee community at elevations between 3600 and 3800 meters. Surveys in 2008 and 2011 revealed surprising proportions of low-elevation bumble bees sharing elevation and niche space with the historically dominant alpine species (Geib et al. 2015). Though it is unclear when the shift in the bumble bee community composition first occurred, it is possible that the warmer, dryer climate and more frequent droughts that have characterized Colorado since the 1990s (Saunders et al. 2008) facilitated establishment of non-native species in this habitat. Such upward shifts in species ranges in response to climatic warming have been widely forecast.

Bumble Bee Surveys

In 2013, foraging bumble bee workers were lethally sampled at four sites above timberline on Pennsylvania Mountain, following the methods of Geib et al. (2015). Foraging bumble bees of the worker caste were the targets of these surveys because they are the primary agents of critical pollination services to plants; benefits to plants from pollination services are density-dependent, correlating with abundance of their mutualist pollinators (Dewenter 2003, Geib and Galen 2012). Surveys were conducted between July 25th and August 6th, for 26 person-hours, with equal effort at all sites. In 2014, a reduced survey was conducted at the lowest alpine site only on August 4th for six person hours. The collection site locations coincided with those in 2008 and 2011, and are described in Geib et al. (2015). All sites contained extensive patches of bee-pollinated plant species (Byron 1980), and were in full bloom during the sampling period, which coincides with the peak abundance of foraging worker bees. These semi-annual surveys are conducted at this time to minimize detrimental impacts to brood rearing and production of reproductive castes (which occurs at the end of the summer). In all surveys, bees were collected using nets and stored in iced vials to minimize stress. After all daily collections, worker bees were placed in a freezer until torpor and transferred to centrifuge tubes filled with 95% ethanol. They were stored at -20°C until return to Appalachian State University, Boone, NC, where they were dried, pinned, and identified to species.

Metrics and Statistical Analyses

To assess changes to the bumble bee community following drought-mediated population contractions of 2012, I compared all dependent variables among survey years, with2013 and 2014 surveys considered “post-drought” and 2008 and 2011 considered “pre-drought”. Drought classification data was gathered from the US Drought Monitor based on June 17-July 1 reports of respective years. All precipitation accumulation data was gathered from the Natural Resources Conservation Service SNOTEL website (http://www.wcc.nrcs.usda.gov/snow/). 2008 was considered “normal” in temperature and precipitation for Colorado by July of the water year. Around the same time in 2011, the region was teetering between “abnormally dry” to moderate drought levels. After the historic 2012 drought, precipitation had improved conditions to “abnormally dry” and moderate drought levels by July of 2013, and following an exceptionally wet winter, normal non-drought conditions had been reestablished for the region by late June of 2014. The years 2008, 2011, 2013, and 2014 all had winter (December – May) precipitation averages ranging from 67.3 mm - 75.8 mm. In contrast, the in the severe drought year, 2012, the region received only 38.5 mm of snow water equivalent for the winter, which amounts to 57% of precipitation received compared to the next driest year. Winter precipitation rates are particularly important for bumble bees as snow accumulation insulates overwintering queens and provides early water resources in the form of runoff when queens and plants begin to emerge in the spring. Averaging winter and summer precipitation combined for non-drought years ranged from 63 mm - 69.7mm, while the 2012 drought year received 51.3 mm.

I assessed pre-post drought changes in the forager community from a number of perspectives. Unless otherwise noted, all statistical analyses were conducted using JMP (Version 9.0.01, SAS Institute, Inc.).

Total Forager Abundance

I compared capture rates during forager surveys as a proxy for comparing the overall size of the *Bombus* community among survey years. Given that the total effort invested in bumble bee surveys was not equal every year, I standardized capture rates by dividing the number of bees sampled at each site by the number of person hours of surveying time. Despite potential for differences in capture rate among individual researchers, this measure appears to be the most appropriate index of "absolute" abundance (Pyke et al. 2012). I conducted comparisons using analysis of variance (ANOVA), with site as the unit of analysis (n = 4, 2, and 4 sites, respectively for 2008, 2011, and 2013), and year as the main fixed effect. Because the 2014 bumble bee survey lacked replicate sites (n = 1), it could not be statistically compared to other years, although the data is included in figures for comparison.

Species Composition of the Bumble Bee Community

To assess whether the makeup of the *Bombus* community varied significantly pre- and post-drought I used ANOVA to test for differences in relative abundances (proportions) comprised by each species among survey years. Proportions were arcsine-square-root transformed prior to analysis. Site was the unit of analysis, and year, species, and year x species were included as fixed model effects. Pairwise comparisons of Least Squares Means (LS Means) were conducted with Student's *t*-test.

I then asked whether drought was facilitating upward shifts of subalpine bumble bees into this alpine habitat by comparing relative abundance of species in two groups: proportions of historically dominant high alpine species (*B. balteatus* and *B. sylvicola)* and lower alpine species that have begun to populate the high alpine within the last ten years (*B. flavifrons, B. frigidus, B. bifarius, B. mixtus)*. Statistical comparisons were conducted for years with multiple sample sites (2008, 2011, 2013) via ANOVA, with year, ecological grouping, and year x ecological grouping as fixed model effects. Proportions were arcsine-square-root transformed prior to analysis. Pairwise comparisons of LS Means were conducted with Student's *t*-test.

Distribution of Traits Relevant to Pollination

Pollinators partition themselves among the plant community based on matching between tongue and corolla tube length (Rodríguez-Gironés and Santamaría 2006, Pyke et al. 2012). Changes in relative bumble bee species abundances may alter the relative proportion of long vs. short-tongued bees in the *Bombus* community with implications for benefits to host plants. *Bombus balteatus* is an especially important pollinator in alpine habitats of this region as it is the only long tongued pollinator. To ask whether the relative frequency of tongue lengths varied following drought-mediated population contractions, I grouped bumble bee species by three tongue length categories: long (*B. balteatus*), medium (*B. flavifrons,* and

B. centralis), and short (*B. bifarius, B. frigidus, B. sylvicola, B. mixtus,* and *B. melanopygus*) (Pyke et al. 2012). ANOVA was used to test for differences in proportions of tongue length groups among years with site as the unit of analysis. Proportions were arcsine-square-root transformed prior to analysis. I also calculated mean tongue length of the bumble bee community by multiplying the relative species proportions at each site by the species-specific mean tongue length for workers as determined by Macior (1974). Again, differences in mean tongue length among years were assessed using ANOVA with site as the unit of analysis.

Species Diversity and Richness

Species diversity and richness were calculated in R using the Vegan package, which implements the Shannon-Weiner Index (SWI, value increases when number of species and the evenness of species increases). Diversity was calculated as follows:

$$H = \sum_{i=1}^{s} - (P_i * \ln P_i)$$

Here, H is the Shannon diversity Index (SWI), P_i is the fraction of entire population made of species i, S is the number of species encountered, and $\sum$ is the sum from species 1 to species S.

Species richness is derived from the number of species present, but does not take into account abundance or distribution. It is calculated by averaging the number of species found per site by year. Differences in species diversity among years were assessed using ANOVA with site as the unit of analysis.

Results

Total Forager Abundance

Pre-post drought comparisons of foraging worker bumble bee capture rates as a proxy for total forager abundance revealed that the overall availability of foragers varied drastically among years (One-way ANOVA F (3,10) = 42.19, p = <0.0001; Figure 1, Table 2). However, there were just as many *Bombus* foragers present in 2013 compared to pre-drought years; in fact, there were more bees caught per person-hour per site on average that year. The 788 workers caught in 30 person hours represented the highest rate of bee capture of 26.3 bees/ hour compared to 18 bees/hour in 2008 and just 12 bees/hour in 2011. 2014 capture rates were in line with those of pre-drought years, although no statistical comparisons could be conducted due to lack of site replication.

Species Composition of the Bumble Bee Community

Comparing relative species proportions among years revealed significant temporal oscillation in community structure. (ANOVA whole model F (23,56) = 19.78, $p < 0.0001$; ANOVA year x species F (14) = 2.86, $p < 0.0027$) due to the differences in species proportions within years, F (7) = 3.45, $p < 0.0001$. *Bombus balteatus* comprised overall the greatest proportion of the community, on average (Pairwise LS Means comparisons $p < 0.05$).

Comparing alpine vs. non-alpine bees in the three sampled years revealed temporal stability, with no significant differences among years (Figure 3, Table 2, ANOVA whole model F (5,14) = 0.7390, p = 0.6067).

Distribution of Traits Relevant to Pollination

The average pollinator tongue length on Pennsylvania Mountain did not vary in any of the three years sampled (Figure 4, Table 2, *F* (2,7) = 1.119, *p* = 0.3785). The frequency of tongue length groups within the community showed tendency to vary among years but presented no evidence of significantly changing after the drought (Figure 5, Table 2, ANOVA whole model *F* (8,21) = 2.0531, *p* = 0.0892; ANOVA year x tongue group *F* (4) = 2.15, *p* = 0.1096).

Species Diversity and Richness

Worker bee samples from 2008, 2011, 2013, and 2014 revealed 8 different species inhabiting Pennsylvania Mountain. The year 2014 had the lowest amount of species richness (4) and diversity measurements from the Shannon-Weiner D index (1.015), but this year was only sampled at one site and could not be compared. The 2013 season had the highest level of species richness and diversity (richness = 6.25, diversity Shannon's D = 1.459), mostly due to the appearance of two low-alpine species, *Bombus mixtus* and *B. melanopygus*, with the latter species not found in any other year. However, there were no significant differences in diversity among years (Figure 6, Table 2, ANOVA *F* (2,7) = 0.3078, *p* = 0.7445).

Discussion

By comparing characteristics of an alpine bumble bee community over a number of years prior to and following the severe 2012 drought in the Colorado Rocky Mountains, I could find no evidence of carry-over effects from the resulting drought-mediated population contractions. Relative abundances of both native alpine and invading subalpine bees fluctuated in a pattern that did not support drought as a causal agent. When looking at bees grouped by functional traits important to partnering host plants, neither average tongue length in the community nor frequency of observed tongue length changed, and there was no loss in species diversity or richness.

Total Forager Abundance

I hypothesized that there would be slow recovery of total forager abundance in the Pennsylvania Mountain bumble bee community after the dramatic population declines of both native alpine and invasive subalpine bumble bees during 2012, but the 2013-year samples revealed that this year had the highest total forager abundance and rate of capture of all surveyed years. It is possible that a small number of queens were able to produce a new reproductive caste, which contributed to the large number of foragers seen the following year (the old queens will typically die at the end of the season after laying the reproductive caste) (Goulson 2010). It seems more realistic, however, that heavy immigration from other source populations were responsible for the rapid repopulation of the alpine portion of Pennsylvania Mountain. It is thought that high alpine mountaintops may act as “sky islands” and might limit the amount of immigration and dispersal from one mountaintop to another. However, because of the rapid replacement of all *Bombus* species it is likely that the mountains are not limiting bumble bee dispersal. Although mechanisms for *Bombus* dispersal on mountaintops are still unclear, it is possible that queens from nearby mountaintops immigrated early in the season and were able to quickly find suitable habitat to establish nests. Literature on flight distance of bumble bees supports that most individuals, especially queens, should be able to traverse the linear distance between mountaintops (Knight et al. 2005, Geib et al. 2015). However, it is unclear how the broader landscape characterized by tall mountains and deep, windy valleys or gulches may promote or limit queen dispersal.

Species Composition of the Bumble Bee Community

Looking at differences in relative species abundances in semi-annual surveys of Pennsylvania Mountain bumble bees revealed no clear pattern between pre- and post-drought alpine *Bombus* communities. Surveys on Pennsylvania Mountain since the late 2000s showed evidence of upward shifts in the ranges of bumble bee species that typically occupy subalpine zones when compared to historical surveys conducted in the 1970s (Geib et al. 2015), resulting in homogenization of the subalpine and alpine bumble bee communities. Similar findings have been reported in other parts of Colorado (e.g., Front Range; Miller-Struttman et al. *in review*) as well as globally (e.g., Spain, Ploquin et al. 2013). In Spain, these events have occurred coincident with a regional warming of 0.9 °C, conditions which parallel those experienced by Colorado over the last decade (warming of 1.11°C over the last 30 years; Gordon and Ojima 2015), suggesting that the prolonged warmer, dryer conditions experienced by Colorado over the late 1990s and 2000s facilitated increases in relative proportions of subalpine species observed above tree line. Such predictions of upward shifts in species ranges associated with warming have long been forecast (Walther et al. 2002, Parmesan 2006), and similar upward shifts in Colorado plants have been attributed to this warming (Sproull et al. 2015). However, there was no evidence that the short-term 2012 drought, despite its severity further promoted significant upward shifts of lower elevation

Bombus species. The 2008 and 2011 (pre-drought) Pennsylvania Mountain bumble bee communities shared fewer similarities than did the communities in 2008 and 2013 (the year following the severe drought).

Most interestingly, the 2014 survey of the Pennsylvania Mountain bumble bee community appeared to suggest an unexpected resurgence in relative abundance of alpine bumble bee foragers. Although this survey data could not be statistically compared to others due to lack of site replicates, the data is likely trustworthy in that only the lowest elevation site was surveyed, which should bias data in favor of subalpine species. This finding is surprising, since when invasive species are established and on the rise, as they appeared to be on Pennsylvania Mountain in recent years, they rarely undergo spontaneous decline (Simberloff and Gibbons 2004).

There are, however, exceptions. The invasive Mountain Pine Bark Beetle began to spiral out of control in 1996 likely due to warmer temperatures and increasing drought conditions stressing their host tree species and making them more susceptible to a beetle invasion. The peak of the Pine Bark Beetle population explosion occurred in 2008 (beetles were active on 1.2 million acres of pine trees), but has dropped dramatically since then. A 2014 report on Colorado’s forests estimates that the current range of active infestation stands at only 10,000 acres, mostly due to the death of suitable host trees (Colorado State Forest Service 2014). A recent study in Europe investigated the near total replacement of the Eurasian red squirrel population with the invasive North American grey squirrel, mainly due to effects of competition and disease. Researchers found that the recovery of a native predator, the pine martin, was negatively correlated with grey squirrel abundance and positively correlated with abundance of the Eurasian red squirrel (Sheehy and Lawton 2014).

The recovery of the native predator and the native red squirrel has consequently pushed the invasive grey squirrel populations to an unusually low density. Whether these drivers of invasive species declines have any impact on the non-native bees on Pennsylvania Mountain requires more research into community dynamics, resource availability, and habitat quality.

Distribution of Traits Relevant to Pollination

Under optimal foraging conditions, pollinators will partition themselves among the floral community based on tongue length (Rodríguez-Gironés and Santamaría 2006), which was why measurements regarding tongue length traits were made following the 2012 drought. Currently there is evidence to suggest that there have been worldwide declines in the abundance of long tongued pollinators in particular, partly due to declines in deep corolla length plants (Goulson et al. 2005). Measuring the mean tongue size would reveal any trends favoring a particular tongue size, especially if there was a decline in the only long tongued pollinator, *Bombus balteatus*. Comparing pre- and post-drought tongue length frequencies reveals no pattern or shift in the frequency of tongue length sizes, which is consistent with corresponding relative species abundances, so this was unsurprising. That tongue length group distributions remain constant despite varying species composition of the bumble bee community suggests that pollination services to respective guilds of host plants for each tongue length group should remain consistent.

Species Diversity and Richness

Interestingly, the post drought 2013 year experienced the highest level of species diversity and richness. After the 2012 population crash, I hypothesized that the community would recover slowly and possibly lose rare species. Losing rare species would be likely in a degraded drought-stricken environment as they often exist at the edges of their latitudinal range and can be poorly adapted to local conditions (Goulson et al. 2005). On the contrary, there were appearances of three species found in 2013 that have only been observed sporadically since 2008. The increasing number of rare species observed may be caused by the unusually warm temperatures this region has been experiencing within the last decade.

In a similar study conducted in sub-tropical reef-fish communities, warming sea temperatures increased levels of species richness. The researchers hypothesized that there would be a reduction in the abundance of individual temperate (native) species due to predicted range contractions and an increase of abundance of tropical (non native) fish species as a predicted consequence of warming. Instead, they found no support for a general reduction in abundance of temperate species, and richness and diversity increased by 33% and 15% respectively (Lloyd et al. 2012). The measurements of species richness and diversity within this study revealed similar trends.

An important aspect of studying pollinator decline and recovery is assessing implications for plant populations. In alpine ecosystems, many plants are exceptionally long-lived perennials (Billings and Mooney 1968). The trade offs for such lengthy lifespans are low recruitment and low offspring survival rates, a strategy that protects the plant population from occasions where sexual reproduction is resourced limited (Silvertown et al. 1993). The apparent absence of pollinators during the 2012 drought may not have had major impacts the overall plant population, but may have been a source of pollen limitation for new seed production and recruitment. In years of such extreme drought, plant reproduction may be more restricted by water resources, and plants may have low fecundity regardless of pollen availability (Herrera 1991).

Further Research

Further research is necessary to answer the questions that arise from the findings following the 2012 drought. If these mountaintops are not working as isolated island habitats, source-sink dynamics may be working to maintain populations of alpine bumble bees on Pennsylvania Mountain, but so far no research has investigated such metapopulation dynamics and dispersal in bumble bees in mountain habitats or any other save islands (see Goulson et al. 2011). Other research should also be conducted to search for possible survival strategies that bumble bees may utilize when severe weather events and plant failures occur. Although this study measured the bumble bee community in the year directly following the collapse of a community, there may be a lag effect that could be observed in two or three years after the event. Events such as prolonged drought lasting multiple years may also cause more serious and significant changes to a *Bombus* community than can be observed in just one season. This may be the case for the decade long drought of the late 1990s and early 2000s that characterized this region of Colorado, possibly triggering the invasion of alpine habitats by subalpine species.

Table 1: Average precipitation in millimeters on Pennsylvania Mountain.

	Year Average	Winter Average	Summer Average	Summer and Winter Average
2007	61.64	54.67	76.00	61.78
2008	61.45	71.83	60.33	68.00
2011	65.82	72.00	56.00	66.67
2012	50.09	38.50	77.00	51.33
2013	55.00	67.33	54.33	63.00
2014	64.00	75.83	57.67	

Table 2: ANOVA Tables for A) Year effects on rate of capture in 2008, 2011, 2012, 2013, and 2014, B) Year and species effects on species relative abundance proportions in 2008, 2011, and 2013, C) Year and ecological grouping effects for alpine vs. other proportions in 2008, 2011, and 2013, D) Year and tongue length effects on frequency of tongue length in 2008, 2011, and 2013, E) Year effects on mean tongue length in 2008, 2011, and 2013.

	Source	**D.F.**	**SS**	**F Ratio**	**Prob > F**
A - Rate of Capture	Year	4	188.66	1.66E+16	<0.0001*
	Error	5	1.42E-14		
B- Species Proportion	Year	2	0.01	0.63	0.5452
	Species	5	0.27	6.50	0.0013*
	Species * Year	10	0.12	1.41	0.2527
	Error	18	0.15		
C - Alpine VS Other	Year	2	0.03	1.03	0.3687
	Alpine VS Other	1	0.12	9.88	0.0037*
	Year * Alpine VS Other	2	0.04	1.74	0.1931
	Error	30	0.37		
D - Tongue Length Frequency	Year	2	0.01	0.31	0.7346
	Tongue Length	2	0.21	9.92	0.0006*
	Year * Tongue Length	4	0.04	1.03	0.4113
	Error	27	0.28		
E - Mean Tongue Length	Year	2	0.01	0.12	0.8905
	Error	3	0.11		

Table 3: Table of *Bombus* species diversity, richness, and evenness.

	2008	**2011**	**2013**	**2014**
n	238	192	348	112
Richness	5.5	5	6	4
Shannon Index (ln)	1.425922	1.4185015	1.4331715	1.014869467
Evenness	0.83644147	0.881364537	0.799868244	0.732073574

Figure Legends

Fig. 1 Figure 1: Capture rate of foraging worker bumble bees during semi-annual surveys at Pennsylvania Mountain Natural Area, Park County, CO. Bars are means of all sites surveyed that year (n = 4 sites for 2008, 2012, and 2013; n = 2 and 1 sites for 2011 and 2014, respectively. 2014 is included only for visual comparison and was not included in statistical analyses. Error bars represent standard error.

Figure 2: Relative abundance of *Bombus* species in semi-annual surveys conducted at Pennsylvania Mountain Natural Area, Park County, CO. Proportions are means of all sites surveyed that year (n = 4 sites for 2008, 2012, and 2013; n = 2 and 1 sites for 2011 and 2014, respectively. 2014 is included only for visual comparison and was not included in statistical analyses. Error bars represent standard error.

Figure 3: Relative abundance of alpine and sub-alpine worker bumble bees in semi-annual surveys at Pennsylvania Mountain Natural Area, Park County, CO. Bars are means of all sites surveyed that year (n = 4 sites for 2008, 2012, and 2013; n = 2 and 1 sites for 2011 and 2014, respectively. 2014 is included only for visual comparison and was not included in statistical analyses. Error bars represent standard error.

Figure 4: Mean tongue length of all foraging worker bees caught during semi-annual surveys at Pennsylvania Mountain Natural Area, Park County, CO. Tongue lengths of worker bees were based on means reported by Macior (1974). Bars are means of all bees in sites surveyed that year (n = 4 sites for 2008, 2012, and 2013; n = 2 and 1 sites for 2011 and 2014, respectively. 2014 is included only for visual comparison and was not included in statistical analyses. Error bars represent standard error.

Figure 5: Relative frequency of short, medium, and long-tongued worker bumble bees in semi-annual surveys at Pennsylvania Mountain Natural Area, Park County, CO. Bars are means of all sites surveyed that year (n = 4 sites for 2008, 2012, and 2013; n = 2 and 1 sites for 2011 and 2014, respectively. 2014 is included only for visual comparison and was not included in statistical analyses. Error bars represent standard error.

Figure 6: Shannon Weiner Diversity Index (ln) for bumble bee species observed in semi-annual surveys of foraging workers at Pennsylvania Mountain Natural Area, Park County, CO. Bars are means of all sites surveyed that year (n = 4 sites for 2008, 2012, and 2013; n = 2 and 1 sites for 2011 and 2014, respectively. 2014 is included only for visual comparison and was not included in statistical analyses. Error bars represent standard error.

Figure 7: Species richness observed in semi-annual surveys of foraging workers at Pennsylvania Mountain Natural Area, Park County, CO. Bars are means of all sites surveyed

that year (n = 4 sites for 2008, 2012, and 2013; n = 2 and 1 sites for 2011 and 2014, respectively. 2014 is included only for visual comparison and was not included in statistical analyses. Error bars represent standard error.

Figure 1.

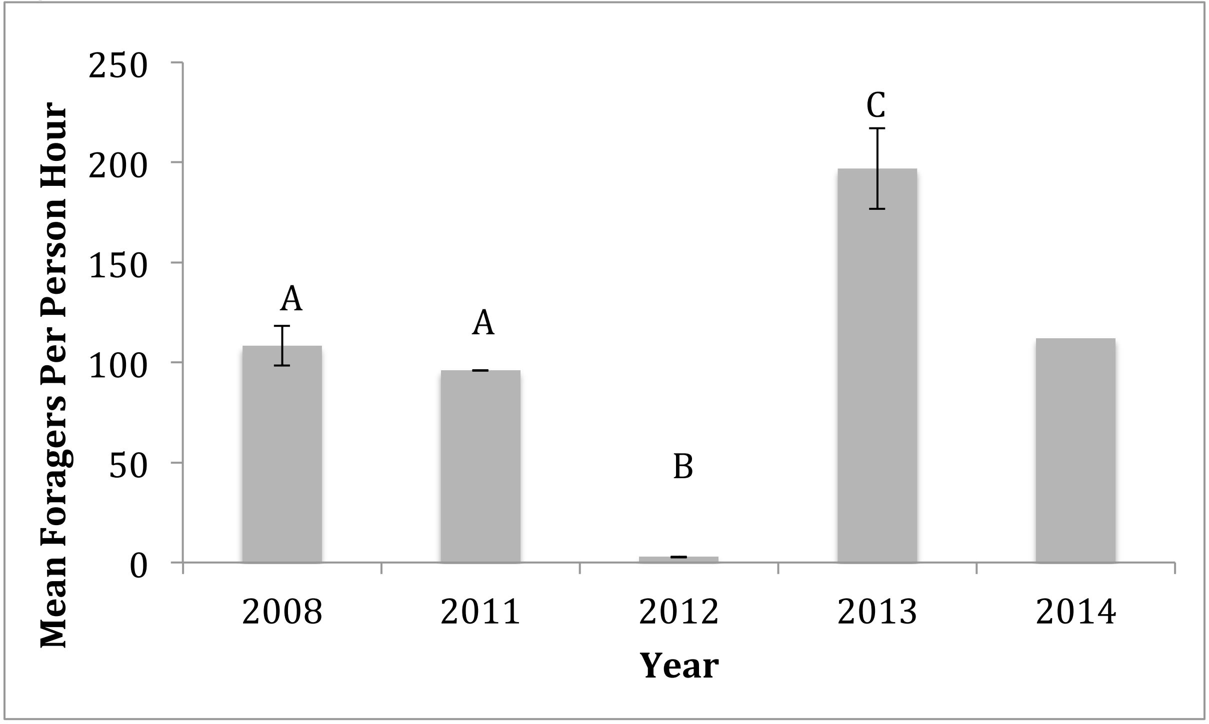
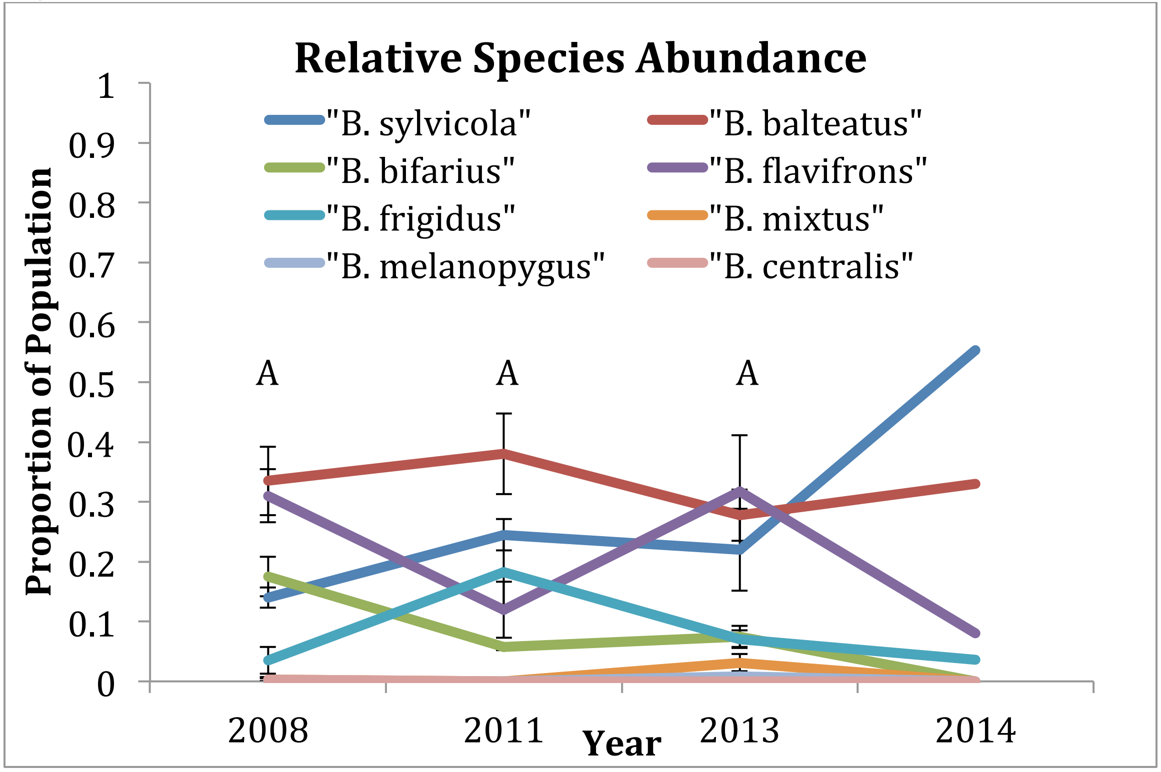

250
200
150
100
50
0
C
A
A
B
2008
2011
2012
2013
2014
Year
Mean Foragers Per Person Hour

Figure 2.



Relative Species Abundance
"B. sylvicola"
"B. balteatus"
"B. bifarius"
"B. flavifrons"
"B. frigidus"
"B. mixtus"
"B. melanopygus"
"B. centralis"
Proportion of Population
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
A
A
A
2008
2011
2013
2014
Year

Figure 3.

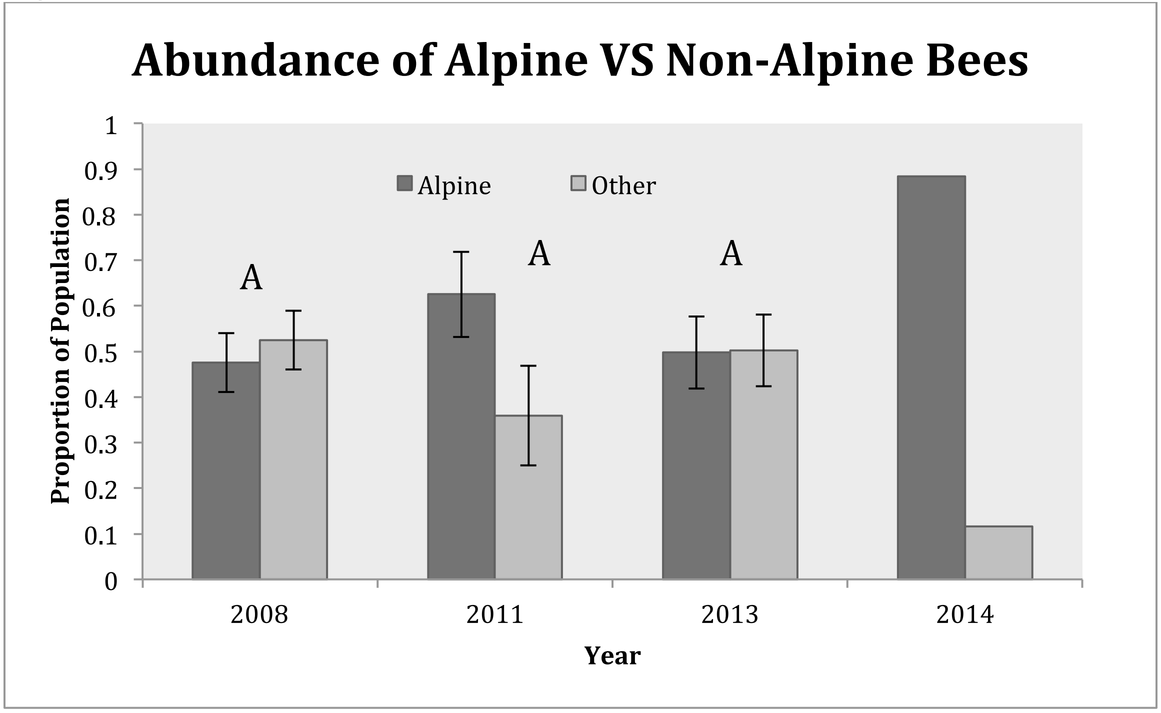

Abundance of Alpine VS Non-Alpine Bees
Proportion of Population
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Alpine
Other
A
A
A
2008
2011
2013
2014
Year

Figure 4.

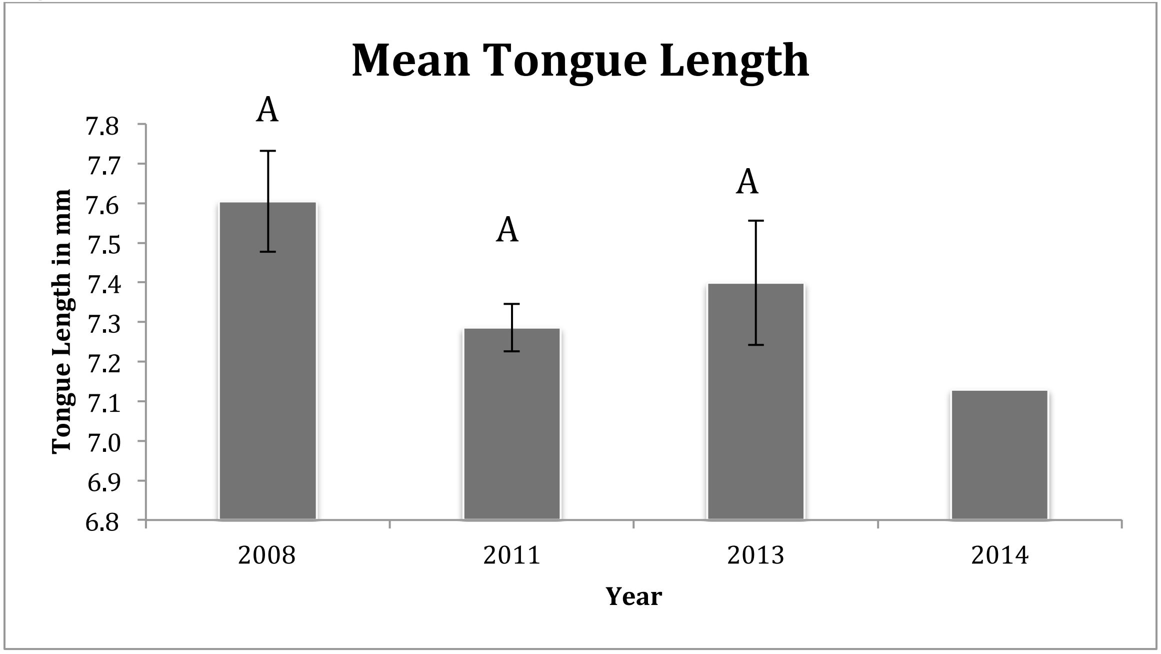
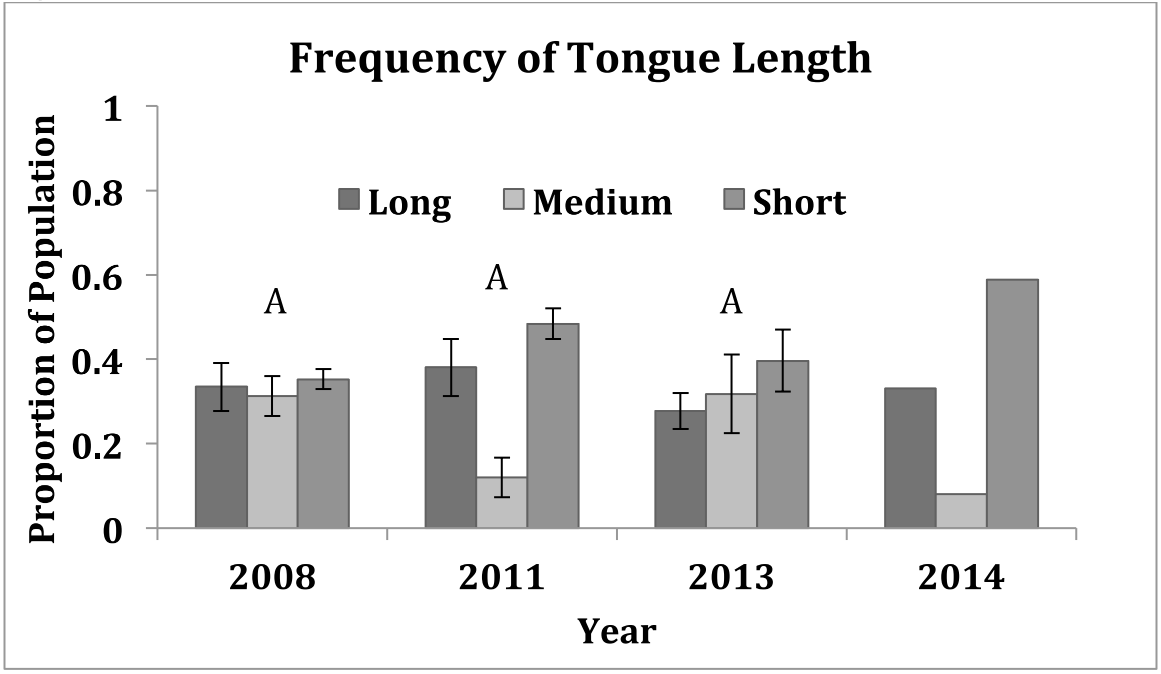

Mean Tongue Length
Tongue Length in mm
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
A
A
A
2008
2011
2013
2014
Year

Figure 5.



Frequency of Tongue Length
Proportion of Population
1
0.8
0.6
0.4
0.2
0
Long
Medium
Short
A
A
A
2008
2011
2013
2014
Year

Figure 6.

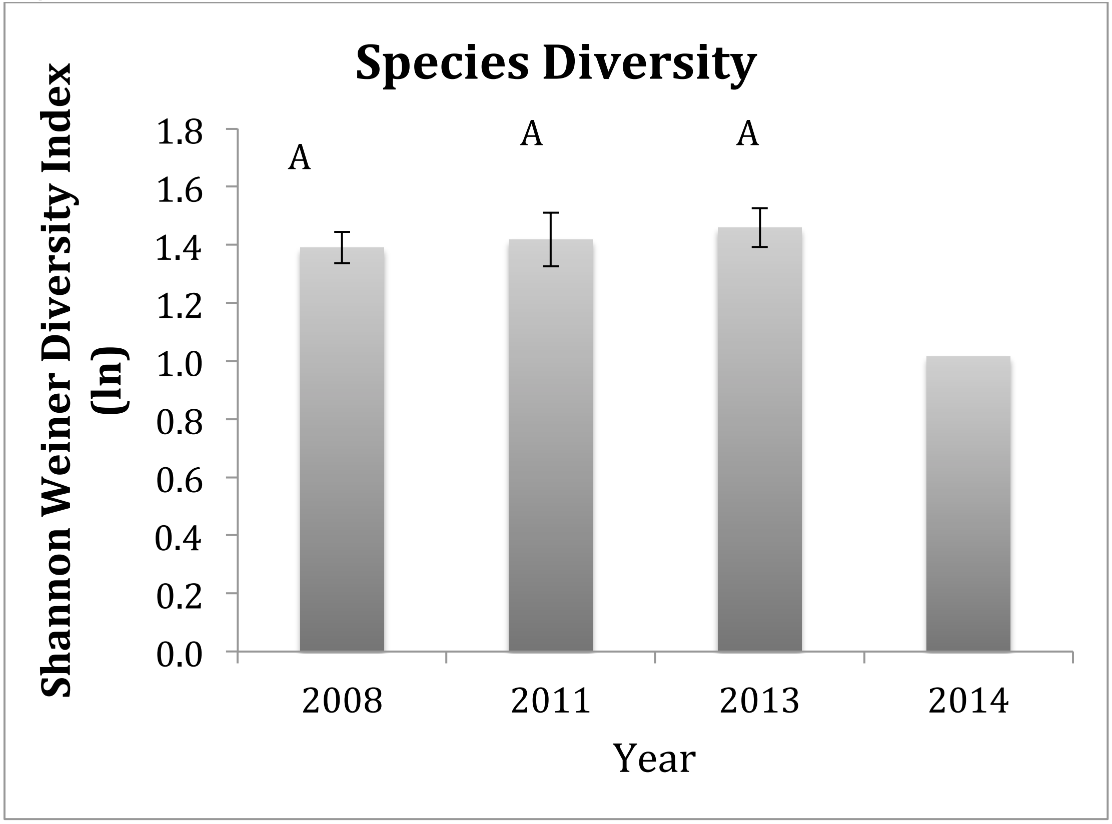
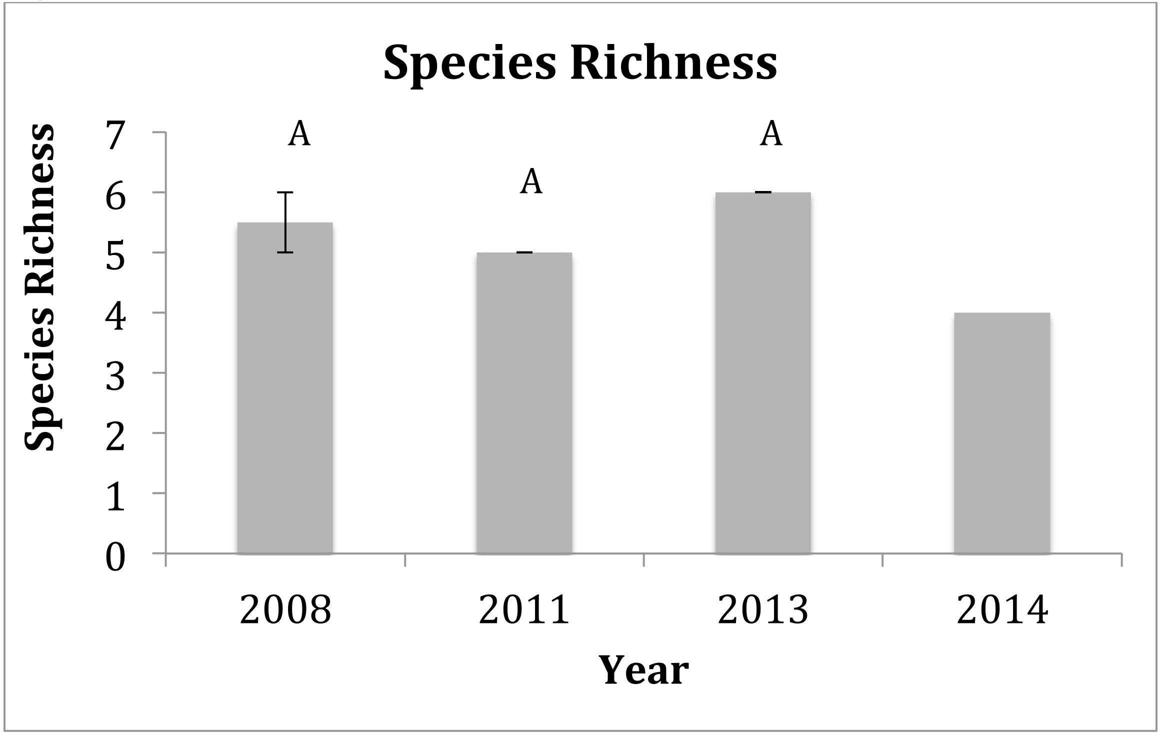

Species Diversity
Shannon Weiner Diversity Index (ln)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
A
A
A
2008
2011
2013
2014
Year

Figure 7.


Species Richness
Species Richness
7
6
5
4
3
2
1
0
A
A
A
2008
2011
2013
2014
Year

Chapter 3

ABSTRACT

NO EVIDENCE OF A GENETIC SIGNATURE FOLLOWING A DROUGHT-MEDIATED DEMOGRAPHIC BOTTLENECK IN AN ALPINE BUMBLE BEE, *BOMBUS BALTEATUS*

When populations experience severe and rapid declines in population size, large amounts of genetic variation can be lost and result in a genetic bottleneck. In 2012, an extreme drought decimated the alpine *Bombus* community on Pennsylvania Mountain, Park County, Colorado, and surveys conducted found no reproductive individuals at the end of the season. Because of this population contraction and lack of reproductives, I hypothesized that samples from 2013 would reflect a genetic bottleneck and that measurements of effective population size would be lower than in previous non-drought years. I collected samples of all available *Bombus balteatus* foragers present at the end of the 2013 season and used genotypes to assess population health. PCR was used to amplify nine microsatellite loci to provide genotype data for analysis. Genotypes were analyzed in COLONY 2.0 to assign sibships of individuals and estimate effective population size. Three commonly used bottleneck testing methods were implemented to look for evidence of a genetic bottleneck signature: the *M*-ratio, the heterozygosity excess, and the mode shift test.

There was no significant decrease in effective population size in 2013 when compared to pre-drought years, even though the 2012 effective population size was effectively zero. Despite the significant observed demographic contraction that occurred in

the 2012 *Bombus balteatus* population, none of the tests used were able to detect any evidence of a genetic bottleneck within the 2013 population. These results suggest that bumble bees may have some method to tolerate or recover from stochastic and sharp declines in population numbers. Results refute the idea of alpine mountaintops as “sky islands”, and hint that immigration and dispersal among peaks may work to maintain genetic diversity. In order to increase power and statistical strength of bottleneck tests, sampling of the *Bombus balteatus* community should continue for several generations to ensure there is not a “false negative” period directly after the bottleneck that may be confounding the results. Findings provide hope for *B. baltaeatus* and its linked specialist host plants, given dire predictions for increasing drought frequency in the US Intermountain West under climate change models.

Introduction

When a population experiences a significant rapid decrease in size (a “demographic bottleneck”) it can lead to a corresponding decrease in genetic variability. The loss in allele diversity leads to a smaller pool of available genetic variation within the following generation and can severely impact the species ability to adapt to change and can increase chances of local extirpation (Freeland 2005). Specific detrimental effects that can negatively impact the future of the population include more frequent inbreeding (Darvill et al. 2006) and inability to fight parasite and disease (Liersch and Schmid-Hempel 1998, Gillespie 2010). Genetic fitness is negatively impacted after a bottleneck because the frequency of rare deleterious recessive alleles can increase (fixation) and cause inbreeding depression (Ellis et al. 2006). These impacts can vary from species to species and are heavily influenced by demographic factors such as metapopulation dynamics, genetic drift, mutation rates, and adaptive pressures (Freeland 2005).

Searching for evidence of genetic bottlenecks is a commonly used technique in conservation, especially as it relates to worldwide species decline (Bouzat et al. 1998, Whitehouse and Harley 2001, Sinclair et al. 2002, Darvill et al. 2006). The molecular methods used in searching for bottlenecks can provide insights to the genetic structure and history of a population. Some methods assume that bottlenecked populations will experience reductions in allelic diversity faster than heterozygosity (in the sense of Nei 1987), so a bottleneck can be detected when observed heterozygosity is larger than expected at mutation-drift equilibrium based on a particular mutation model (Cornuet and Luikart 1996). Other tests operate under the assumption that rare alleles will be lost after a large population decline and create a shift in allele distribution (Luikart et al. 1998a). A third testing method predicts that a bottleneck will cause a shift in the ratio of number of alleles at a locus to the range in allele size (Garza and Williamson 2001).

There are several challenges when searching for evidence of a genetic bottleneck. In many instances, genetic data is only collected after a suspected bottleneck event has occurred, with no past genetic information available (Queney et al. 2000, Whitehouse and Harley 2001, Sinclair et al. 2002). This makes it difficult to decide as to whether or not a past bottleneck can explain low levels of genetic diversity. The ability to detect a bottleneck also depends on the severity, which is measured by how significantly the population size has been reduced and how quickly the population has been able to recover (Freeland 2005). Current methods used to detect the genetic signature of a bottleneck from a single population census often require prior knowledge of mutation rate, initial size of the population, and details of the mutation process (Williamson-Natesan 2005), which may be unknown. Furthermore, the quality of molecular genetic markers, available sample size, and time passed since the bottleneck event will influence the ability to accurately detect a population bottleneck (Luikart and Cornuet 1998, Freeland 2005, Selkoe and Toonen 2006).

This study focuses on genetic impacts to a population of native alpine bumble bees (*Bombus balteatus*) following drastic reductions to their numbers during a severe drought in 2012 in the American intermountain west. The *B. balteatus* population located within Pennsylvania Mountain Natural Area, Park County, Colorado, has been well studied, with demographic information available since the 1970s and genetic data available since 2008 (e.g., see Geib et al 2015). In 2012, a severe and pervasive drought struck the intermountain western US, including Colorado (National Oceanic and Atmospheric Administration 2013). Drastic declines were observed for all resident bumble bee species at Pennsylvania Mountain during the census that year (99% fewer foragers overall and no observed reproductives of any species; Geib and Galen *unpublished data*). This was likely caused by early senescence of the plant community, as average bloom time was 45% shorter than normal (Franklin 2014).

Most social insects such as bumble bees follow a caste system that has few breeding individuals and many sterile workers. Some have expressed increased queen-male relatedness and positive inbreeding coefficients when populations are divided or decline in number (Sundstrom et al. 2003, Ellis et al. 2006). However, some argue that even if there is a loss in genetic diversity in hymenopterans, the sex determination by the haploid sex will purge deleterious recessive alleles (Sorati et al. 1996). An additional risk of low genetic variability in social hymenoptera species is that the single-locus complementary sex determination system can create sterile diploid males, which are a cost to the colony

(Whitehorn et al. 2009, Charman et al. 2010). This creates lowered overall fitness of a population even if deleterious recessive alleles do not persist. Despite the growing evidence that bumble bees around the world have experienced significant range contractions and population declines (Colla and Packer 2008, Goulson et al. 2015), it is unknown how widespread these genetic issues may be in their populations.

The objective of this study was to assess the genetic health of the 2013 *B. balteatus* population one year following a severe drought, by 1) documenting size of breeding population on Pennsylvania Mountain ("effective population size", N_e) and 2) testing for signatures of the population contraction in allele frequencies and distribution (bottleneck effects). Pre-drought genetic information from 2008 serves as a comparison for populations under normal conditions.

I hypothesized that the high elevation mountaintops occupied by *B. balteatus* may act as "sky islands" and limit rapid dispersal and immigration. I expected that the *B. balteatus* breeding population (effective population size, N_e) on Pennsylvania Mountain in the year following the drought would be reduced compared to a relatively normal year. Estimates of N_e serve as important indicators of bumble bee health as they measure the number of individuals that can breed and contribute genetic diversity to future generations. Drought-mediated reductions of *B. balteatus* populations on Pennsylvania Mountain in 2012 may have created a genetic bottleneck that could be observed by analyzing genotypes of subsequent populations, and I expected that the collected genotypes from 2013 would reveal that such an event had recently occurred.

Methods

Study Site and System

All samples used in this study were collected as part of a semi-annual survey of foraging worker bumble bees at Pennsylvania Mountain Natural Area (Pennsylvania Mountain), Park County, Colorado, July 25 - August 5, 2013. Pennsylvania Mountain (4000 m) is situated in the Mosquito Range of Central Colorado. The peaks of the range are quite high (five are > 14,000 ft) and form a north-south running ridge along the boundary between Lake and Park Counties. High elevation ridges connect many peaks. Higher peaks and ridges surround Pennsylvania Mountain on three sides (Fig 1), but are separated from its slopes by steep gulches to the north and south and a wide valley to the west.

Pennsylvania Mountain itself is divided by the headwaters of Pennsylvania Creek (Fig 1). The northern half of the mountain is characterized by significant land use from mining, but the southern half that comprises the Pennsylvania Mountain Natural Area and research station is relatively pristine. From treeline (3530 m) to the false summit (3800 m) it is comprised of a number of east-facing slopes characterized by a matrix of dry and moist alpine meadows and rocky fell fields.

The bumble bee community on Pennsylvania Mountain contains the historically present alpine species *B. balteatus*, *B. sylvicola*, and *B. frigidus,* plus significant numbers of recent subalpine invaders *B. bifarius*, *B. flavifrons*, and rare appearances of *B. centralis*, *B. melanopygus*, *B. mixtus*, *B. nevadensis* and *B. appositus* (Byron 1980, Geib et al. 2015). All species commonly present experienced severe population contractions in 2012, following early senescence of the plant community. Of these species, *B. balteatus* (Hymenoptera:

Apidae) was selected as the focal species because it is a historically dominant alpine species (Macior 1974), is the only long-tongue pollinator on above treeline (Pyke et al. 2012), and is responsible for pollination services to a range of ecologically important plants (Macior 1974, Geib and Galen 2012).

The 2013 survey that provided samples of *B. balteatus* workers for genetic analysis occurred on the relatively pristine southern half of Pennsylvania Mountain. Four alpine sites (50 m radius) were sampled, two high alpine (3730 m and 3735 m) and two low alpine (3659 m and 3698 m). All sites and methods corresponded with those of Geib et al. 2015. Collections occurred during peak abundance of foraging bumble bee workers during the latter half of the flowering season (e.g., see Pyke et al. 2012) when the collection sites were also in full bloom. Bees were collected using nets and stored in iced vials to minimize stress. After daily collections, bees were placed in a freezer until torpor and transferred to centrifuge tubes filled with 95% ethanol. They were stored at -20°C until return to Appalachian State University, Boone, NC, where they were dried, pinned, and identified to species.

Molecular Methods

To conduct the genetic bottleneck tests, I chose to use microsatellites as genetic markers. Microsatellites are often used in population genetics as they can reveal the genetic structure of subpopulations and populations (F-statistics), reveal events in demographic history such as bottlenecks and gene flow, and asses effective population sizes (Luikart et al. 1998b, Piry et al. 1999, Balloux and Lugon-Moulin 2002, Williamson-Natesan 2005). Microsatellites are highly variable between insects and have faster mutation rates compared to other sequences, which makes them useful for determining variation between recent generations (Peery et al. 2013). Microsatellites are typically co-dominant, which makes calculating allele frequencies much easier, as it allows the ability to distinguish between homozygous and heterozygous individuals (Freeland 2005).

DNA was extracted from whole *B. balteatus* workers using a tissue and cell DNA isolation kit (MO BIO, Carlsbad, CA) utilizing the included modified tough-tissue protocol. Modifications included the addition of proteinase-k (20 mg/mL) and placing the extraction tubes in a heat block at 55°C for 30 minutes. Once extractions were complete, PCR was used to amplify nine predetermined microsatellite loci (B124, BL12, BTERN01, BT10, B96, B119, BL11, B10, BT28) (Estoup et al. 1996, Reber, Funk et al. 2006, Lozier et al. 2011) in two multiplex reactions. Each well contained 2 µL 5X buffer (Promega, Madison, WI), 0.56 µL 25 mM $MgCl_2$, 0.6 µL 0.6 mM dNTP, 0.2 µL BSA, 0.08 µL of Taq polymerase (Promega, Madison, WI), and 1 µL of extracted DNA. The wells in plex A contained 3.50 µL H_2O and four primers (10mM) and wells in plex B contained 3.16 H_2O and five primers (10mM). Forward primers were fluorescently dye labeled (Life Technologies, Carlsbad, CA). Both Plex A and B reactions ran for 7 minutes at 95°C, 30 cycles of 95°C for 30 s, 53°C for 30 s, 72°C for 30 s and a final period at 72°C for 10 minutes. Amplified DNA was sent to the Georgia Genomics Facility at University of Georgia (Athens, GA) to be sequenced on ABI 3730xl capillary DNA sequencer (Applied Biosystems, Carlsbad, CA, USA).

Alleles were scored manually using Peak Scanner software (Version 2.0, Applied Biosystems) and samples that lacked data or were ambiguous at more than half of loci were excluded from analysis. Of the nine loci amplified in PCR, only five yielded polymorphic and unambiguous genotypes for 2013 samples (B119 was monomorphic, BTERN01 and BT28 were ambiguous, and B124 did not amplify). After discarding unusable loci, 70 usable genotypes remained, which were used for further analysis. *Bombus balteatus* genetic data from the same Pennsylvania Mountain sites in 2008 (n = 112 individual genotypes, accessed from Geib et al. 2014, 2015) were also included in all analyses for qualitative comparison of post-drought patterns to those prior to the drought. From these samples, six alleles yielded polymorphic and non-ambiguous genotypes (B116, B119, and BT28 were monomorphic and BTERN01 did not amplify). For all analyses, sites within each year were pooled together due to sample size requirements of the software.

Nest Abundance and Effective Population Size

Bumble bees are eusocial species, with colonies originating from one reproductive queen, usually via monoandrous mating, leading to 75% relatedness among offspring. As such, colony abundance rather than that of individual foraging workers is most representative of the size of the breeding population. Because colonies are nearly impossible to physically locate in the field, population size is commonly estimated by assigning genetic sibships to sampled workers (see Knight et al. 2009, Dreier et al. 2014, Geib et al. 2015). To begin analysis, sisters within each survey year were identified in COLONY (Version 2.0, software available online). The program estimates the number of nests in the sample and predicts the number of unsampled nests to find effective population size (N_e). It uses a maximum likelihood algorithm (Wang 2004) which assumes single queen matings, typical for *Bombus balteatus*. Only samples with a minimum of two available loci were included, with a maximum of six. Colony testing parameters were set for a full-likelihood algorithm for haplodiploid species. The mating system was described as having no likelihood of inbreeding or cloning, with monoandrous queens and polyandrous males. Allele frequencies were updated with no prior sibship construction, and each year's dataset was analyzed three times with a different random seed number to ensure convergence of results.

Preliminary Analysis

In order to ensure markers met assumptions for further testing, probability tests for departure from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were conducted in GENALEX software for samples from each survey year (Version 6.5, Peakall and Smouse 2006). Assumptions of single-sample methods require that no genetic substructure exist within a population (Busch et al. 2007) which can be measured by F statistics. For both years, and F_{ST} was calculated in GENALEX to measure the level of genetic substructure between sites (F_{ST}). I used GENALEX to calculate observed heterozygosity (H_O) and mean number of alleles (A) for each year to compare general measures of genetic diversity before and after the 2012 drought.

Genetic Diversity and Bottleneck Signatures in the *B. balteatus* Population

I used a number of different tests to assess evidence for a genetic bottleneck signature in the genotyped samples of *B. balteatus* from Pennsylvania Mountain.

M- Ratio—The M ratio test (Garza and Williamson 2001) detects a bottleneck via the relationship M=(k/r) where k is the number of alleles and r is the range in allele size. If a population experiences a sharp decrease in size, increasing genetic drift would cause k to decrease quickly, but r would only decrease if the smallest or largest allele were lost and at a slower rate than k. Under bottleneck conditions, the M-ratio would be expected to be smaller in reduced populations than in equilibrium populations. The significance of an observed *M* value is determined by comparing it to a range of *M* values that are calculated from theoretical populations in mutation-drift equilibrium. The critical value is the lower 5% of the distribution.

The program M_P_VAL (Garza and Williamson 2001) calculates *M* ratios from genotype information including allele size and number. The ratios generated here are compared to critical *M* (M_C) values which are generated in the program CRITICAL_M (Garza and Williamson 2001). This program requires three input parameters for that estimate the mutation model for the sample population: θ ($\theta = 4N_e\mu$), p_g (percent of mutations that are larger than single steps), and Δ_g (the mean size of the mutations larger than single steps). Values for θ were calculated from known N_e values estimated in COLONY 2.0 and $\mu = 5.0$ X 10^{-4}/locus/generation, a common estimate of microsatellite mutation rate suggested by Garza and Williamson (2001). Generic values determined by Garza and Williamson (2001) were used for the other two parameters under the two-phase model (TPM) so that $p_g = 0.1$ and $\Delta_g = 2.8$ steps. The simulation consisted of 10,000 iterations.

Mode Shift and Heterozygosity Excess—Two tests were conducted in BOTTLENECK software (Version 1.2.02, Piry et al. 1999) to look for evidence of recent population reductions: the mode-shift test and the heterozygosity excess test (Cornuet and Luikart 1996, Luikart and Cornuet 1998).

The mode-shift test is a test for allele frequency distribution. This method determines if the frequency of alleles represented in the population align to a normal L-shaped distribution that would be expected when alleles are in mutation-drift equilibrium. Under this equilibrium, the test finds many rare alleles (i.e. <0.1%) that are usually lost during a genetic bottleneck. When there is a bottleneck, the L-shape distribution will experience a mode-shift where the allele proportions of rare vs. common alleles is lost. Although the mutation model of a marker has a strong effect on the estimation of heterozygosity excess, it does not distort the allele frequencies distribution measured in the mode-shift test because rare alleles are expected to be abundant regardless of the mutation model (Nei et al. 1976).

The second test conducted, the sign test, used allele frequency data to look for heterozygosity excess (Nei et al. 1975). If a bottleneck occurred, the mutation-drift equilibrium that would normally equal the measured HWE heterozygosity (H_e) would be disrupted and the heterozygosity at a locus would exceed the heterozygosity computed from the number of alleles sampled. In short, the test would reveal a deviation from 50:50 heterozygosity deficiency/excess. There are three mutation models available when computing the sign test: the infinite allele model (IAM), stepwise mutation model (SMM), and the two-phase model (TPM), which is a combination of IAM and SMM with configurable percentages and variance of each model. Prior research suggests that the mutation model of most microsatellites follows the intermediate TPM model (Di Rienzo et al. 1994), while others have argued that microsatellites more closely follow the SMM (Luikart and Cornuet 1998). Still, other published methods that use markers within *Bombus* have used the IAM and argue that microsatellites in bumble bees do not follow the SMM (Estoup et al. 1995, Shao et al. 2004). Since the question of most appropriate mutation model for bumble bees is unresolved in the literature, I conducted the test under all three models, with TPM parameters set at variance = 10, $p_g = 0.1$, or 10% (Ellis et al. 2006). The standardized differences test is not reported because it requires a minimum of 20 polymorphic loci and only 6 loci reliably amplified for use.

Results

Nest Abundance

Sample years 2008 and 2013 yielded 112 and 70 complete genotypes, respectively. In the 2008 sample set of 112 individuals, all sisters were removed leaving 98 representatives of singular nests for further analysis. In 2013, only three sisters were found out of 70, resulting in 67 nest representatives. Calculations from COLONY 2.0 software estimated 350 nests in 2008 (95% CI [195, 364]) and 265 nests in 2013 (95% CI [140,305])(see Fig. 2).

Preliminary Analysis

No significant linkage disequilibrium ($P > 0.05$) was found within genotypes of either year. No significant departure from Hardy-Weinberg Equilibrium (HWE) was detected in the 2008 population in either global test by population or by locus. In the 2013 samples, only the BL11 marker deviated significantly (P=<0.001), but because it was in equilibrium in the 2008 year it could still be used for the heterozygosity excess tests (Busch et al. 2007) (Table 1).

The F-statistics tests reveal low fixation indexes across alleles for both years, suggesting there is enough gene flow in the area to prevent the formation of a strong substructure. The mean F_{ST} value in 2013 was 0.043, which indicates very little genetic differentiation among the population. By comparison, 2008 had a mean F_{ST} value of 0.033, slightly less differentiation than in 2013 (Table 2). General measures of H_O and number of different alleles are described in Table 3. The markers in 2013 revealed an average of 13 alleles per locus compared to 10.3 in 2008.

Genetic Diversity and Bottleneck Signatures in the *B. balteatus* Population

M-Ratio—In the 2013 post-drought year, no loci experienced a drop in the *M*-ratio and values ranged between 0.705 and 1.20 (Table 4). The specific critical *M* value calculated by the software ($M_C = 0.695$) was higher than the upper-limit suggested by Garza and Williamson, so calculated *M*-values were only compared with the generic value ($M_C = 0.68$). All loci exceed the critical value and no bottleneck was detected.

In comparison, the 2008 pre-drought year had high *M*-values that ranged between 0.684 and 1.125. All loci exceeded both the generic critical *M* value determined by Garza and Williamson (M_C=0.68 and the specific critical *M*-value determined by the software (M_C= 0.712) and no bottleneck was detected.

Mode Shift and Heterozygosity Excess—The BOTTLENECK software did not detect a mode shift for neither 2013 nor 2008 populations. Both of the allele frequencies for these populations were in a normal L-shaped distribution, despite the expectation that many rare alleles would be lost after the 2012 drought. The heterozygosity excess test which outputs a deficiency:excess ratio value detected no evidence of a bottleneck in either of the years (Table 5). In 2013, samples exhibited no heterozygosity excess under any of the models. The sign test revealed a 2:3 ratio under the IAM ($P = 0.650$), 3:2 under the TPM ($P = 0.317$), and a 5:0 ratio under the SMM ($P = 0.0127$). If a genetic bottleneck existed in 2013, we would expect to see significant heterozygosity excess, but surprisingly we see a large heterozygosity deficiency under the SMM model. In comparison, the 2008 the sign test revealed a 1:5 ratio under the IAM ($P = 0.24$) and TPM ($P = 0.23$), and a 3:3 ratio under SMM ($P = 0.48$) and no bottleneck was detected.

Discussion

The available information and data for the pre-drought *B. balteatus* community served as a useful baseline for post-drought community comparisons. Often times, research into population bottlenecks is conducted only after a suspected bottleneck has already occurred, usually without pre-bottleneck demographic or genetic data to make comparisons (Whitehouse and Harley 2001, Sinclair et al. 2002). Because data is available for the Pennsylvania Mountain *Bombus* community, we had the unique opportunity to compare a "normal" demographic year against a year where there was a known population contraction. I utilized three tests to search for evidence of a corresponding genetic bottleneck immediately after a known population contraction and with specific parameters for the microsatellites used. I hypothesized that I would be able to find genetic evidence that the 2013 *B. balteatus* population had recently suffered severe declines, but tests failed to detect any bottleneck event. Tests showed that the 2008 comparison year did not experience a recent bottleneck event.

Nest Abundance and Effective Population Size

Assessing nest abundance is of particular importance when estimating the health of the *Bombus balteatus* community, as it directly reflects the assumed number of breeding individuals. While estimates for forager abundance may be beneficial when looking at benefits to host plants, nest abundance represents the number of individuals that are able to reproduce and replenish new colonies in the community. These estimates are useful when addressing conservation concerns and population management, such as monitoring managed and wild bumble bee populations.

Although many studies highlight the disproportionate decline of long-tongued bees compared to short-tongue bees (Colla and Packer 2008, Goulson et al. 2015), the *Bombus balteatus* population at Pennsylvania Mountain seems have a mechanism to recover quickly from such a catastrophic population contraction and may contradict predictions for worldwide decline. Nest estimates suggest that although 2013 abundance was slightly less than 2008, number of nests was still comparable and surprisingly high considering estimates in the year prior were effectively zero. Despite the optimistic recovery of nests following the 2013 drought year, further sampling should be conducted on Pennsylvania Mountain to monitor if this influx of colonies can be self-sustaining. If populations are unable to maintain high levels of N_e in the future, the population may become susceptible to inbreeding local extinction (Ellis et al. 2006, Whitehorn et al. 2009).

Genetic Diversity and Bottleneck Signatures in the *B. balteatus* Population

M-Ratio—There may be several reasons as to why the *M*-ratio test did not detect a bottleneck. The *M*-ratio analyzes values of number of alleles per locus and the range in allele size. A neutral allele being lost due to genetic drift is related to its frequency, and rare alleles are lost more than common alleles. The assumption behind this test is that allele frequency distributions are rarely bell-shaped, and it is unlikely for the rarest alleles to always be the largest or smallest in a range of allele sizes. Therefore number of alleles will decrease more rapidly than the range of allele sizes (Garza and Williamson 2001). Because of this assumption, rapid immigration and replacement or addition of new alleles will maintain high ratio values (Williamson-Natesan 2005). Rapid immigration of individuals onto Pennsylvania Mountain and the prompt replacement of alleles would have disguised any evidence of a bottleneck. This test also has limited power directly after a bottleneck and is best conducted more than one generation after the bottleneck event (Peery et al. 2012).

Mode Shift and Heterozygosity Excess—Although a mode shift is a commonly used method for detecting a bottleneck signature (Cornuet and Luikart 1996, Piry et al. 1999), prior research has found this test to have very low type I error and very high type II error (Williamson-Natesan 2005). It is less likely to detect a bottleneck when mutation rates are high (alpha = 20) and when mutations are large (P_g =0.2), and adding more loci does not correct for this, leaving this test a very conservative estimate of a bottleneck. Busch et al (2007) used the mode shift test on populations of kangaroo rats after a known bottleneck occurrence, and the test failed to detect a mode shift in pre- and post- bottleneck years. They determined that while five alleles were lost, four were gained, possibly due to migration, and that this infusion of alleles may have caused the test to maintain a normal L-shaped distribution despite the bottleneck. This may be the reason that no bottleneck signature was detected in 2013, as there were on average 13 alleles per locus in 2013, and compared to an average of 10.3 per locus in 2008.

The heterozygosity-excess test did not detect a bottleneck in 2013. However, it presents a clear explanation as to why 2013 has not retained a bottleneck signature. If there was a bottleneck, the test would reveal heterozygosity excess based on observed and expected measurements of heterozygosity. However, under the SMM model, the test indicates a significant heterozygosity *deficiency*, which is predicted to occur under rapid population expansions (Cornuet and Luikart 1996). If a rapid input of alleles from population replacement did occur in 2013, it would explain why the *M* ratio test maintained high ratio values and why a mode shift did not occur in allele distribution. Of course, this significance is only seen following one of the mutation models, and IAM and TPM maintained non-significant ratio values that suggest there is neither an excess nor deficiency. In comparison, the *Bombus balteatus* population from 2008 had M ratio values above critical M values, no mode shift in allele frequency, and no heterozygosity excess or deficiency.

When a known demographic bottleneck occurs, there may or may not be a resulting genetic bottleneck signature (Holland 2001, Schmid-Hempel et al. 2007). However, problems arise when there is a genetic bottleneck that is simply unable to be detecting using traditional testing methods. If a false negative occurs, populations could be incorrectly considered to be in good health or in HWE. This may present confusion in conservation efforts or in determining how population contractions will affect species in the future. A similar study documents a scenario in which a demographic bottleneck in two banner-tailed kangaroo rat populations was unaccompanied by a genetic bottleneck signature (Busch et al. 2007). The study utilized extensive background demographic information, eight microsatellite markers, and used the three testing methods described above. One population fell to 20% of stable population size, and the other was much more severe: a 10-year, 6-generation decline that spanned two orders of magnitude. Despite having species-specific parameters for tests (mutation rate, N_e and p_g) none of the tests were able to detect a corresponding genetic bottleneck. The authors expect that a combination of high mutation rates, undetected immigration, and a demography marked by stochastic fluctuations interacted together to confound bottleneck analysis. Similarly in this study, there may be several unforeseen attributes of bumble bees and their population dynamics, as well as statistical constraints of the testing methods, that may disguise a bottleneck signature. These alpine species may have some mechanism to recover from the population bottleneck and may be able to tolerate short-term, random, extreme weather events. The mountaintops that were thought to act as isolated "islands" may not, in fact, be so. Dispersal ability across inter-peak valleys and large swaths of pine forest may allow for increased gene flow between subpopulations. These scenarios would help to explain why a bottleneck was undetectable, as bottleneck signatures are rarely retained in mainland populations (Cutrera et al. 2006, Putman and Carbone 2014).

Furthermore, the testing methods used are dependent on a variety of conditions to have high statistical power. Power of the tests used in BOTTLENECK depends on the time elapsed since the bottleneck, and are most powerful at 2-4 N_e generations post-population bottleneck (Nei and Li 1976, Luikart and Cornuet 1998). This can leave a period where a false negative, or type II error, is more likely to occur. Power of all testing methods is also affected by the severity of the bottleneck, number of sampled individuals, and number of loci used in analysis. Although minimum requirements were met for number of sampled individuals in BOTTLENECK, many had poor genotyping success and the number of useable loci was low (maximum of six used in all analyses) (Luikart et al. 1998b, Cristescu et al. 2010). Interestingly, reviews of the heterozygosity excess test used in BOTTLENECK reveal that increasing the amount of loci used exacerbates the type I error that may occur (Williamson-Natesan 2005, Peery et al. 2013). The *M*-ratio test loses statistical power when pre-bottleneck θ is small, the bottleneck is short in duration, or when the population is sampled directly after a bottleneck (Williamson-Natesan 2005). Considering the similarities between the constraints of the *M*-ratio test and our post-bottleneck sampling conditions, this may further explain why the test failed to detect a bottleneck.

Not only are the testing methods reliant on situational parameters, they are dependent on the assumptions of microsatellite evolution to generate expected allele distributions for statistical accuracy (Luikart and Cornuet 1998, Garza and Williamson 2001). Three mutation models (IAM, TPM, and SMM) were used in this analysis, although which one is most reliable is relatively unknown. While microsatellites are generally believed to mutate following a TPM with the addition or loss of a single repeat, they may tend toward one extreme more than the other, and there are a small proportion of mutations that result in addition or loss of a larger number of repeats (Di Rienzo et al. 1994). To increase power of these tests, it is important that the estimate of number of multi-step repeats (p_g) as well as the mean size of the multi-repeat (δ_g) is relatively accurate (Williamson-Natesan 2005). Errors in the assumption of these parameters can lead to a variety of false inferences from bottleneck tests (see: Luikart and Cornuet 1998, Piry et al. 1999, Garza and Williamson 2001, Williamson-Natesan 2005).

In order to account for constraints of testing strength, further research should be done to measure how population contractions may impact populations or reveal a genetic bottleneck more than one generation (or season) afterwards. Surveys in 2014-2016 may possess stronger ability to detect a bottleneck. To increase sensitivity of testing ability, it may also be useful to develop microsatellite markers for *Bombus* that are imperfect repeat loci. Their ability to create a more powerful analysis has been noted in Cornuet and Luikart (1996) and suggested in several reviews of these testing methods (Cristescu et al. 2010, Peery et al. 2013). Monitoring efforts should continue at this site to look for long-term impacts of climate change and assess how alpine pollinators may persist through extreme weather events and maintain genetic variability after local populations collapse.

Table 1: Test for departure from Hardy-Weinberg Equilibrium

Key: ns=not significant, * P<0.05, ** P<0.01, *** P<0.001

Year Population	**Locus**	**DF**	**ChiSq**	**Prob**	**Signif**
2008	B124	36	33.808	0.573	ns
2008	BL13	78	94.524	0.098	ns
2008	BT10	36	49.836	0.062	ns
2008	B10	45	44.657	0.486	ns
2008	B96	91	77.255	0.847	ns
2008	BL11	21	24.843	0.254	ns
2013	BL13	66	61.833	0.623	ns
2013	BT10	66	64.166	0.541	ns
2013	B10	91	60.570	0.994	ns
2013	B96	105	91.970	0.814	ns
2013	BL11	66	192.243	0.000	***

Table 2: Analysis of Molecular Variance for 2013 F_{ST}

Summary AMOVA Table

Source	**df**	**SS**	**MS**	**Est. Var.**	**%**
Among Populations	3	16.024	5.341	0.095	5%
Among Individuals	63	154.469	2.452	0.461	22%
Within Individuals	67	102.500	1.530	1.530	73%
Total	133	272.993		2.086	100%

F-Statistics	**Value**	**P (rand >= data)**
Fst	0.046	0.005
Fis	0.232	0.001
Fit	0.267	0.001

Table 3: General measurements of observed heterozygosity (H_O) and number of different alleles per locus (A).

	2013		2008	
Locus	**H_O**	**A**	**H_O**	**A**
B124	NA	NA	0.765	9
BL13	0.718	12	0.816	13
BT10	0.949	12	0.796	9
B10	0.746	14	0.714	10
B96	0.889	15	0.857	14
BL11	0.688	12	0.745	7

Table 4: *M*-Ratio Values and *M* Critical (M_c) values at each locus

Population	**Ne**	**k**	**r**	**Theta**	**Locus**	**M-ratio**	M_c
2008	693.75	9	8	2.775	B124	1.125	.712
		13	19		Bl13	0.684	
		9	10		BT10	0.9	
		10	12		B10	0.833	
		14	16		B96	0.875	
		7	7		BL11	1	
2013	**727.57**	**k**	**r**	**Theta**	**Locus**	**M-Ratio**	M_c
		12	17	2.91	Bl13	0.705	.695
		12	13		BT10	0.923	
		13	13		B10	1	
		15	13		B96	1.153	
		12	10		BL11	1.2	

Table 5: Heterozygosity excess test from BOTTLENECK 2.0 and P values for ratio significance from 50:50

Year	**Mutation Model**	**Ratio (Deficiency: Excess)**	**Probability**
2013	IAM	2:3	0.65
	TPM	3:2	0.32
	SMM	5:0	0.01
2008	IAM	1:5	0.24
	TPM	1:5	0.23
	SMM	3:3	0.48

Figure Legends

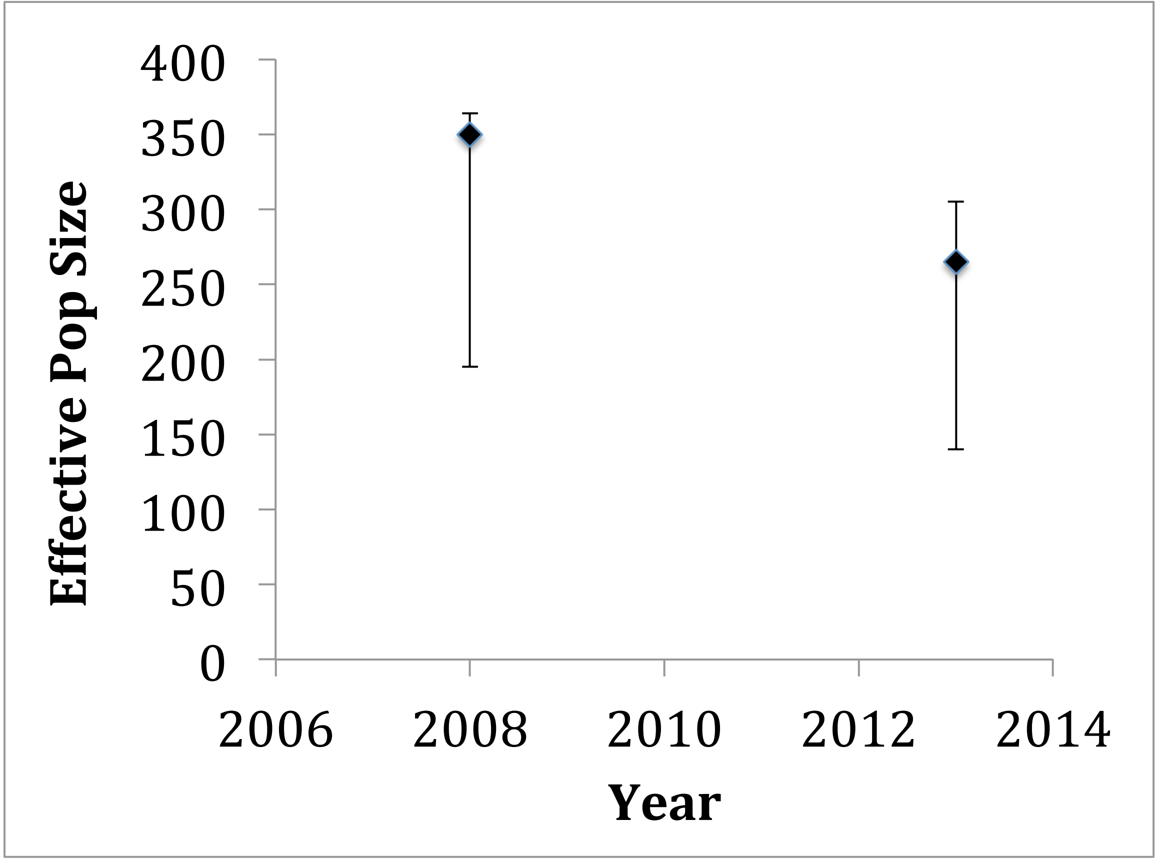
Figure 1. Aerial image of Pennsylvania Mountain, Mosquito Range, Park County, Colorado. Pennsylvania Mountain Natural Area is located on the southern half of the mountain.

Figure 2: *Bombus balteatus* “effective population size”(N_e). Error bars represent the 95% confidence interval.

Figure 3: Capture rate of foraging worker bumble bees, as a proxy for absolute abundance, during semi-annual surveys at Pennsylvania Mountain, Park County, CO. Columns represent means of sites sampled each year (n = 4, 2, and 4 for 2008, 2011, and 2012, respectively). Error bars are standard error. (Geib and Galen, *unpublished data*).

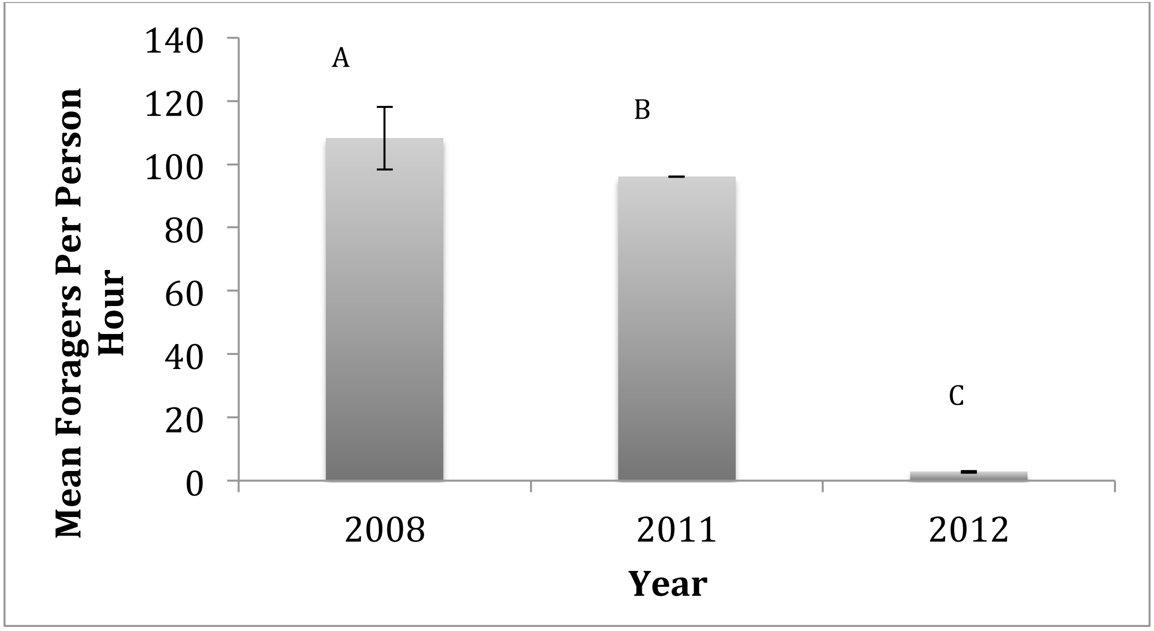
Figure 1.

Figure 2.



Effective Pop Size
400
350
300
250
200
150
100
50
0
2006
2008
2010
2012
2014
Year

1234 **Figure 3**.



1235

Chapter 4

GENERAL CONCLUSIONS

As pollinator abundance and diversity continue to decline worldwide (Grixti et al. 2009, Goulson et al. 2015), it is imperative that research is conducted to understand how pollinator communities will respond to shrinking numbers and increasing threats from climate change, pesticide use, and habitat degradation (Goulson et al. 2005). In the preceding chapters, I contribute to this body of understanding by taking advantage of an opportunity to examine recovery of alpine bumble bees after a severe population contraction on Pennsylvania Mountain. The *Bombus* community at this site was thought to experience geographical isolation from other mountaintop communities due to large inter-peak valleys, high winds, and large conifer stands fragmenting populations, however this research reveals that there may be much higher levels of immigration and dispersal than expected. In a world that is increasingly fragmented by changes in land use, understanding how pollinators can persist is very useful in developing conservation and management policies.

Despite the observed collapse of the *Bombus* pollinator community on Pennsylvania Mountain, it is clear that these bumble bees have some mechanism to recover from short-term population contractions. In Chapter 2, I used a range of measurements to compare the post-drought *Bombus* community to pre-drought measurements and found no evidence to suggest that the population experienced any lasting effects from the 2012 drought. This year had very high rates of capture, and while number of foragers is not particularly indicative of the overall population (sterile workers will not produce any offspring) it is a very important measurement when assessing impacts to host plants.

The 2012 drought provided a unique opportunity to study how the collapse of a pollinator community would affect plant fecundity in the following year. In alpine ecosystems, many plants are exceptionally long-lived perennials (Billings and Mooney 1968). The trade offs for such lengthy lifespans are low recruitment and low offspring survival rates, a strategy that protects the plant population from occasions where sexual reproduction is resourced limited (Silvertown et al. 1993). The apparent absence of pollinators during the 2012 drought may not have had major impacts the overall plant population, but may have been a source of pollen limitation for new seed production and recruitment. In years of such extreme drought, plant reproduction may be more restricted by water resources, and plants may have low fecundity regardless of pollen availability (Herrera 1991).

In Appendix A, I specifically investigate possible carry over effects for two species of linked clovers, *Trifolium dasyphyllum* and *T. parryi*. These are ecologically important plants because they are a few of the only nitrogen fixing plants that exist above tree line, Low nitrogen availability is characteristic of alpine and high-elevation ecosystems, as cold soil and air temperatures restrict transformation rates of organic matter (Makarov et al. 2011). *Trifolium dasyphyllum* is a generalist with most *Bombus* species throughout the season, while *T. parryi* is considered a specialist with *B. balteatus* (studied specifically in Chapter 3) in the early flowering season (Geib and Galen 2012). The results indicate that host plants received adequate pollination services in the 2013 post-drought year, which is not particularly surprising considering the rapid repopulation of Pennsylvania Mountain that same year. If the *Bombus* population contraction was followed by very slow recovery or caused certain species (especially the long-tongued *B. balteatus*) to become extirpated, we may have seen much lower plant fecundity and recruitment. While one season of failed seed production may only be a drop in the bucket for these alpine plants, predictions for increasingly frequent droughts may begin to impact the overall population in future decades.

Although many alpine plants have developed a strategy to persist through stochastic events that may inhibit seed production for entire seasons, most plants have not developed the luxury of such long lifespans. There are large gaps in our knowledge regarding consequences to plants when pollinators are absent, and most recent research has focused primarily on the losses of honey bees (*Apis mellifera*) (Goulson et al. 2015). There is, however, some emerging data specifically connecting the losses of pollinators to the decline of host plants. Beismeijer et al. (2006) found a casual link between local extinctions of functionally linked plants and pollinator species in Great Britain and the Netherlands. The agricultural industry has experienced increases in food production costs due to shortfalls in the availability of pollinators. For example, California almond production has become noticeably more expensive due to shortages of mobile honey bee colonies (Watanabe 1994).

In Chapter 3 I focus on the keystone pollinator *B. balteatus* in part to its historical dominance of this area and because it is the only long-tongued pollinator found on Pennsylvania Mountain (Macior 1974) able to pollinate plants with deep corollas. I used molecular genetic techniques to estimate number of nests and search for evidence of a genetic bottleneck resulting from the population contraction in 2012. While forager abundance is important for plants, estimating genetic diversity within a population is important for species continued fitness (Darvill et al. 2006, Whitehorn et al. 2009). Despite the severity of the 2012 population decline, no evidence of a bottleneck was found, supporting the idea that alpine bees may have developed mechanism for long range dispersal and immigration (Lepais et al. 2010, Geib et al. 2015).

Further research must continue to monitor pollinator abundance and measure how pollinators will adapt, persist, or perish through increased climatic or anthropomorphic pressures. Long-term studies should be conducted to look at possible lag-time affects following seasonal population declines. Linked plants requiring pollination services for reproduction should be investigated in order to form predictions for future populations and create management and conservation strategies to prevent further loss of diversity and richness.

Compiled References

Allen-Wardell, G. 1998. The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. Conservation Biology 12:8–17.

Balloux, F., and N. Lugon-Moulin. 2002. The estimation of population differentiation with microsatellite markers. Molecular Ecology 11:155–165.

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

Billings, W., and H. A. Mooney. 1968. The ecology of artic and alpine plants. Biological Reviews. 43:481-529.

Billings, W. D. and K. M. Peterson. 1974. Arctic and alpine vegetation: plant adaptation to cold summer climates. In Iarctic and Alpine Environmetns, ed. J. D. Ives and R. G. Barry. 403-443. Methuen, London, UK.

Bouzat, J. L., H. H. Cheng, H. A. Lewin, R. L. Westemeier, D. Jeffrey, and K. N. Paige. 1998. Genetic in evaluation of a demographic bottleneck the greater prairie chicken. Conservation Biology 12:836–843.

Bowman, W. D., J. C. Shardt, and S. K. Shmidt. 1996. Symbiotic N_2 fiaxation in alpine tundra: ecosystem input and variation in fixation rates among communities. Oecologia 108:345-350.

Burkle, L. A, J. C. Marlin, and T. M. Knight. 2013. Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. Science 339:1611–5.

Busch, J. D., P. M. Waser, and J. A. DeWoody. 2007. Recent demographic bottlenecks are not accompanied by a genetic signature in banner-tailed kangaroo rats (*Dipodomys spectabilis*). Molecular Ecology 16:2450–2462.

Byron, P. A. 1980. On the ecology and systematics of Coloradan bumblebees. University of Colorado, Boulder, Colorado, USA.

Calderone, N. W. 2012. Insect pollinated crops, insect pollinators and US agriculture: trend analysis of aggregate data for the period 1992-2009. PLoS ONE 7:24–28.

Cameron, S. A, J. D. Lozier, J. P. Strange, J. B. Koch, N. Cordes, L. F. Solter, and T. L. Griswold. 2011. Patterns of widespread decline in North American bumble bees. Proceedings of the National Academy of Sciences of the United States of America 108:662–667.

Charman, T. G., J. Sears, R. E. Green, and A. F. G. Bourke. 2010. Conservation genetics, foraging distance and nest density of the scarce great yellow bumblebee (*Bombus distinguendus*). Molecular ecology 19:2661–74.

Childress, A., E. Gordon, T. Jedd, R. Klein, J. Lukas, and R. McKeown. 2015. Colorado Climate Change Vulnerability Study. University of Colorado, Boulder, Colorado, USA.

Colla, S. R., and L. Packer. 2008. Evidence for decline in eastern North American bumblebees (Hymenoptera: Apidae), with special focus on *Bombus affinis* Cresson. Biodiversity and Conservation 17:1379–1391.

Colling, G., C. Reckinger, and D. Matthies. 2004. Effects of pollen quantity and quality on reproduction and offspring vigor in the rare plant *Scorzonera humilis* (Asteraceae). American Journal of Botany 91:1774–1782.

Cornuet, J. M., and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014.

Cristescu, R., W. B. Sherwin, K. Handasyde, V. Cahill, and D. W. Cooper. 2010. Detecting bottlenecks using BOTTLENECK 1.2.02 in wild populations: The importance of the microsatellite structure. Conservation Genetics 11:1043–1049.

Cutrera, A. P., E. A. Lacey, and C. Busch. 2006. Intraspecific variation in effective population size in talar tuco-tucos (*Ctenomys talarum*): the role of demography. Journal of Mammalogy 87:108–116.

Dai, A. 2012. Increasing drought under global warming in observations and models. Nature Climate Change 3:52–58.

Darvill, B., J. S. Ellis, G. C. Lye, and D. Goulson. 2006. Population structure and inbreeding in a rare and declining bumblebee, *Bombus muscorum* (Hymenoptera: Apidae). Molecular Ecology 15:601–11.

Dewenter, I. S. T. 2003. Seed set of male-sterile and male-fertile oilseed rape (*Brassica napus*) in relation to pollinator density. Apidologie 34:227–235.

Dhar, R., N. Sharma, and B. Sharma. 2006. Ovule abortion in relation to breeding system in four *Trifolium* species. Current Science 91:482-485.

Dreier, S., J. W. Redhead, I. A. Warren, A. F. G. Bourke, M. S. Heard, W. C. Jordan, S. Sumner, J. Wang, and C. Carvell. 2014. Fine-scale spatial genetic structure of common and declining bumble bees across an agricultural landscape. Molecular Ecology 23:3384–3395.

Dukas, R. 2005. Bumble bee predators reduce pollinator density and plant fitness. Ecology 86:1401–1406.

Ellis, J. S., M. E. Knight, B. Darvill, and D. Goulson. 2006. Extremely low effective population sizes, genetic structuring and reduced genetic diversity in a threatened bumblebee species, *Bombus sylvarum* (Hymenoptera: Apidae). Molecular Ecology 15:4375–4386. Molecular Ecology and Evolution 12:1074-1084.

Elliott, S. E. 2008. Reciprocal Benefits in a Plant-Pollinator Mutualism. Dartmouth College, Hanover, New Hampshire, USA.

Ellison, N. W., A. Liston, J. J. Steiner, W. M. Williams, and N. L. Taylor. 2006. Molecuar phylogenetics of the clover genus (*Trifolium*). Molecular Phylogenetics and Evolution 39:688-705.

Estoup, A, M. Solignac, J. M. Cornuet, J. Goudet, and A. Scholl. 1996. Genetic differentiation of continental and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe. Molecular Ecology 5:19–31.

Estoup, A., C. Tailliez, J. M. Cornuet, and M. Solignac. 1995. Size homoplasy and mutational processes of interrupted microsatellites in two bee species, *Apis mellifera* and *Bombus terrestris* (Apidae). Molecular Ecology and Evolution 12:1074-1084.

Franklin, J. 2014. Unpublished Master's thesis. University of Missouri, Columbia, Missouri, USA.

Freeland, J. R. 2005. Molecular Ecology. John Wiley & Sons, Ltd., West Sussex, England.

Galen, C. , T. Gregory, and L. F. Galloway. 1989. Costs of self-pollination in a self-incompatible plant, *Polemonium viscosum*. American Journal of Botany 76:1675-1680.

Garza, J. C., and E. G. Williamson. 2001. Detection of reduction in population size using data from microsatellite loci. Molecular Ecology 10:305–318.

Geib, J. C., and C. Galen. 2012. Tracing impacts of partner abundance in facultative pollination mutualisms: from individuals to populations. Ecology 93:1581–1592.

Geib, J. C., J. P. Strange, and C. Galen. 2014. Data from: Bumble bee nest abundance, foraging distance, and host-plant reproduction: implications for management and conservation. Ecological Applications 25:768–778.

Geib, J. C., J. P. Strange, and C. Galen. 2015. Bumble bee nest abundance, foraging distance, and host-plant reproduction: implications for management and conservation. Ecological Applications 25:768–778.

Ghazoul, J. 2005. Buzziness as usual? Questioning the global pollination crisis. Trends in Ecology and Evolution 20:367-373.

Gillespie, S. 2010. Factors affecting parasite prevalence among wild bumblebees. Ecological Entomology 35:737–747.

Gong, Y., and S. Huang. 2011. Temporal stability of pollinator preference in an alpine plant community and its implications for the evolution of floral traits. Oecologia 166:671-680.

Gordon, E., and D. Ojima. 2015. Colorado Climate Change Vulnerability Study. University of Colorado, Boulder, Colorado, USA.

Goulson, D. 2003. Effects of introduced bees on native ecosystems. Annual Review of Ecology, Evolution, and Systematics 34:1–26.

Goulson, D. 2010. Bumblebees: behaviour, ecology, and conservation. Second edition. Oxford University Press, New York, New York, USA.

Goulson, D., M. E. Hanley, B. Darvill, J. S. Ellis, and M. E. Knight. 2005. Causes of rarity in bumblebees. Biological Conservation 122:1–8.

Goulson, D., J. C. Kaden, O. Lepais, G. C. Lye, and B. Darvill. 2011. Population structure, dispersal and colonization history of the garden bumblebee *Bombus hortorum* in the Western Isles of Scotland. Conservation Genetics 12:867–879.

Goulson, D., G. C. Lye, and B. Darvill. 2008. Decline and conservation of bumble bees. Annual Review of Entomology 53:191–208.

Goulson, D., E. Nicholls, C. Botias, and E. L. Rotheray. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347:1–9.

Graystock, P., K. Yates, B. Darvill, D. Goulson, and W. O. H. Hughes. 2013. Emerging dangers: Deadly effects of an emergent parasite in a new pollinator host. Journal of Invertebrate Pathology 114:114–119.

Grixti, J. C., L. T. Wong, S. A. Cameron, and C. Favret. 2009. Decline of bumble bees (*Bombus*) in the North American Midwest. Biological Conservation 142:75–84.

Gutzler, D. S., and T. O. Robbins. 2011. Climate variability and projected change in the western United States: regional downscaling and drought statistics. Climate Dynamics 37:835–849.

Harrison, R. D. 2000. Repercussions of El Niño: drought causes extinction and the breakdown of mutualism in Borneo. Proceedings of the Royal Society 267:911–915.

Herrera, C. M. 1991. Dissecting factors responsible for individual variation in plant fecundity. Ecology 72:1426-1448.

Holland, B. S. 2001. Invasion without a bottleneck: Microsatellite variation in natural and invasive populations of the brown mussel *Perna perna* (L). Marine Biotechnology 3:407–415.

Holzman, H. P., and K. Haselwandter. 1988. Contribution of nitrogen fixation to nitrogen nutrition in an alpine sedge community (*Caricetum curvulae*). Oecologia 76:298-302.

Inouye, D. 2008. Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. Ecology 89:353–362.

Itioka, T., T. Inoue, H. Kaliang, M. Kato, T. Nagamitsu, S. Sakai, T. Yumoto, S. U. Mohamad, and A. A. Hamid. 2001. Six-year population fluctuation of the giant honey bee *Apis dorsata* (Hymenoptera : Apidae) in a tropical lowland dipterocarp forest in Sarawak. Annals of the Entomological Society of America 94:545–549.

Kearns, C., D. Inouye, and N. Waser. 1998. Endangered Mutualisms: the conservation of plant-pollinator interactions. Annual Review of Ecology and Systematics 29:83-112.

Klein, A.-M., I. Steffan-Dewenter, and T. Tscharntke. 2003. Fruit set of highland coffee increases with the diversity of pollinating bees. Proceedings of the Royal Society 270:955–61.

Knight, M. E., A. P. Martin, S. Bishop, J. L. Osborne, R. J. Hale, R. A. Sanderson, and D. Goulson. 2005. An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species. Molecular Ecology 14:1811–1820.

Knight, M. E., J. L. Osborne, R. A. Sanderson, R. J. Hale, A. P. Martin, and D. Goulson. 2009. Bumblebee nest density and the scale of available forage in arable landscapes. Insect Conservation and Diversity 2:116–124.

Kosior, A., W. Celary, P. Olejniczak, J. Fijal, W. Król, W. Solarz, and P. Plonka. 2007. The decline of the bumble bees and cuckoo bees (Hymenoptera: Apidae: Bombini) of Western and Central Europe. Oryx 41:79.

Lautenbach, S., R. Seppelt, J. Liebscher, and C. Dormann. 2012. Spatial and temporal trends of global pollination benefit. PloS One 7:1-16.

Le Conte, Y., and M. Navajas. 2008. Climate change: impact on honey bee populations and diseases. Revue Scientifique et Technique (International Office of Epizootics) 27:485–497, 499–510.

Lepais, O., B. Darvill, S. O'Connor, J. L. Osborne, R. A. Sanderson, J. Cussans, L. Goffe, and D. Goulson. 2010. Estimation of bumble bee queen dispersal distances using sibship reconstruction method. Molecular Ecology 19:819– 831.

Liersch, S., and P. Schmid-hempel. 1998. Genetic variation within social insect colonies reduces parasite load. Proceedings of the Royal Society 265:221–225.

Lloyd, P., É. E. Plagányi, S. J. Weeks, M. Magno-Canto, and G. Plagányi. 2012. Ocean warming alters species abundance patterns and increases species diversity in an African sub-tropical reef-fish community. Fisheries Oceanography 21:78–94.

Lozier, J. D., J. P. Strange, I. J. Stewart, and S. a Cameron. 2011. Patterns of range-wide genetic variation in six North American bumble bee (Apidae: *Bombus*) species. Molecular Ecology 20:4870–88.

Luikart, G., F. W. Allendorf, J. M. Cornuet, and W. B. Sherwin. 1998a. Distortion of allele frequency distributions provides a test for recent population bottlenecks. The American Genetic Association 89:238–247.

Luikart, G., and J. Cornuet. 1998. Empirical Evaluation of a Test for Identifying Allele Recently Data Bottlenecked Populations from Frequency. Society of Conservation Biology 12:228–237.

Luikart, G., W. B. Sherwin, B. M. Steele, and F. W. Allendorf. 1998b. Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. Molecular Ecology 7:963–974.

Lukas, J., and L. Darby. 2012. The 2012 Drought in Colorado, Utah and Wyoming. University of Boulder, Boulder, Colorado, USA.

Lundberg, H. 1980. Effects of weather on foraging-flights of bumblebees (Hymenoptera, Apidae) in a subalpine/alpine area. Holarctic Ecology 2:104–111.

Macior, L. W. 1974. Pollination ecology of the front range of the Colorado Rocky Mountains. University of Akron, Akron, Ohio, USA.

Macior, L. W. 1996. Pollination ecology of *Pedicularis bracteosa* in the montane-subalpine Ecotone. Plant Species Biology 11:165-171.

Makarov, M. I., T. I. Malysheva, A. A. Ermak, V. G. Onipchenko, A. L. Stepanov, and O. V. Menyailo. 2011. Symbiotic nitrogen fixation in the alpine community of a lichen heath of the Northwestern Caucasus Region (the Teberda Reserve). Eruasian Soil Science. 44:1381-1388.

McCabe, G. J., and D. M. Wolock. 2010. Long-term variability in Northern Hemisphere snow cover and associations with warmer winters. Climatic Change 99:1–2.

Memmott, J., P. G. Craze, N. M. Waser, and M. V Price. 2007. Global warming and the disruption of plant-pollinator interactions. Ecology Letters 10:710–7.

Memmott, J., N. M. Waser, and M. V Price. 2004. Tolerance of pollination networks to species extinctions. Proceedings of the Royal Society 271:2605–2611.

Miller-Struttman, N. E., J. C. Geib, J. D. Franklin, P. G. Kevan, R. M. Holdo, D. Ebert-May, J. A. Kettenbach, E. Hedrick, and C. Galen. (n.d.). The evolution of a functional mismatch in a pollination mutualism under climate change. Submitted to Science.

National Oceanic and Atmospheric Administration. 2013. State of the Climate: Drought Annual 2012. Washington, D.C., USA.

National Resource Council. 2011. Climate Stabilization Targets: Emissions, Concentrations, and Impacts over Decades to Millennia. The National Academies Press, Washington, DC, USA.

Nei, M. 1987. Molecular evolutionary genetics. Columbia Press, New York, New York, USA.

Nei, M., R. Chakraborty, and P. A. Fuerst. 1976. Infinite allele model with varying mutation rate. Proceedings of the National Academy of Sciences of the United States of America 73:4164–4168.

Nei, M., and W. Li. 1976. The transient distribution of allele frequencies under mutation pressure. Genetic Research 28:205–214.

Nei, M., T. Marwarma, and R. Chakraborv. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1–10.

Osborne, J. L., A. P. Martin, C. R. Shortall, A. D. Todd, D. Goulson, M. E. Knight, R. J. Hale, and R. A. Sanderson. 2007. Quantifying and comparing bumblebee nest densities in gardens and countryside habitats. Journal of Applied Ecology 45:784–792.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution, and Systematics 37:637–669.

Pauw, A., and J. A. Hawkins. 2011. Reconstruction of historical pollination rates reveals linked declines of pollinators and plants. Oikos 120:344–349.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295.

Pederson, G. T., J. L. Betancourt, and G. J. McCabe. 2013. Regional patterns and proximal causes of the recent snowpack decline in the Rocky Mountains, U.S. Geophysical Research Letters 40:1811–1816.

Peery, M. Z., B. N. Reid, R. Kirby, R. Stoelting, E. Doucet-Bëer, S. Robinson, C. Vásquez-Carrillo, J. N. Pauli, and P. J. Palsbøll. 2013. More precisely biased: Increasing the number of markers is not a silver bullet in genetic bottleneck testing. Molecular Ecology 22:3451–3457.

Peery, Z. M., R. Kirby, B. N. Reid, R. Stoelting, E. Doucet-Bëer, S. Robinson, C. Vásquez-Carrillo, J. N. Pauli, and P. J. Palsboll. 2012. Reliability of genetic bottleneck tests for detecting recent population declines. Molecular Ecology 21:3403–3418.

Peterson, K. M., and W. D. Billings. 1982. Growth of alpine plants under controlled drought. Arctic and Alpine Research 14:189–194.

Piry, S., G. Luikart, and J. M. Cornuet. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity 90:502–503.

Pleasants, J. 2009. Competition for bumblebee pollinators in Rocky Mountain plant communities. Ecology 61:1446-1459.

Ploquin, E. F., J. M. Herrera, and J. R. Obeso. 2013. Bumblebee community homogenization after uphill shifts in montane areas of northern Spain. Oecologia 173:1649–1660.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010. Global pollinator declines: trends, impacts and drivers. Trends in Ecology and Evolution 25:345–353.

Putman, A. I., and I. Carbone. 2014. Challenges in analysis and interpretation of microsatellite data for population genetic studies. Ecology and Evolution 4:4399–4428.

Pyke, G. H., D. W. Inouye, and J. D. Thomson. 2012. Local geographic distributions of bumble bees near Crested Butte, Colorado: competition and community structure revisited. Community and Ecosystem Ecology 41:1332–1349.

Queney, G., N. Ferrand, S. Marchandeau, M. Azevedo, F. Mougel, M. Branco, and M. Monnerot. 2000. Absence of a genetic bottleneck in a wild rabbit (*Oryctolagus cuniculus*) population exposed to a severe viral epizootic. Molecular Ecology 9:1253–1264.

Rasmont, P., and S. Iserbyt. 2012. The Bumblebees Scarcity Syndrome: Are heat waves leading to local extinctions of bumblebees (Hymenoptera: Apidae: *Bombus*)? Annales de la Société Entomologique de France 48:275–280.

Reber Funk, C., R. Schmid-Hempel, and P. Schmid-Hempel. 2006. Microsatellite loci for *Bombus spp*. Molecular Ecology Notes 6:83–86.

Di Rienzo, A, A. C. Peterson, J. C. Garza, A. M. Valdes, M. Slatkin, and N. B. Freimer. 1994. Mutational processes of simple-sequence repeat loci in human populations. Proceedings of the National Academy of Sciences of the United States of America 91:3166–3170.

Rodríguez-Gironés, M. A., and L. Santamaría. 2006. Models of optimal foraging and resource partitioning: deep corollas for long tongues. Behavioral Ecology 17:905–910.

Saunders, S., C. Montgomery, and T. Easley. 2008. Hotter and drier: The West's changed climate. The Rocky Mountain Climate Organization. Louisville, Colorado, USA.

Schmid-Hempel, P., R. Schmid-Hempel, P. C. Brunner, O. D. Seeman, and G. R. Allen. 2007. Invasion success of the bumblebee, *Bombus terrestris*, despite a drastic genetic bottleneck. Heredity 99:414–422.

Schmid-Hempel, R., M. Eckhardt, D. Goulson, D. Heinzmann, C. Lange, S. Plischuk, L. R. Escudero, R. Salathé, J. J. Scriven, and P. Schmid-Hempel. 2014. The invasion of southern South America by imported bumblebees and associated parasites. Journal of Animal Ecology 83:823–837.

Selkoe, K. A., and R. J. Toonen. 2006. Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. Ecology Letters 9:615–629.

Shao, Z. Y., H. X. Mao, W. J. Fu, M. Ono, D. S. Wang, M. Bonizzoni, and Y. P. Zhang. 2004. Genetic structure of asian populations of *Bombus ignitus* (Hymenoptera: Apidae). Journal of Heredity 95:46–52.

Sheehy, E., and C. Lawton. 2014. Population crash in an invasive species following the recovery of a native predator: the case of the American grey squirrel and the European marten in Ireland. Biodiversity and Conservation 23:753–774.

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

Simberloff, D., and L. Gibbons. 2004. Now you see them, now you don’t- population crashes of established introduced species. Biological Invasions 6:161–172.

Sinclair, E. A., B. Costello, J. M. Courtenay, and K. A. Crandall. 2002. Detecting a genetic bottleneck in Gilbert’s Potoroo (*Potorus gilbertii*)(Marsupialia: Potoroidae), inferred from microsatellite and mitochondrial DNA sequence data. Conservation Genetics 3:191–196.

Sorati, M., M. Newman, and A. A. Hoffman. 1996. Inbreeding and incompatibility in *Trichogramma nr. brassicae*: evidence and implications for quality control. Entomologia Experimentalis et Applicata 78:283–290.

Sproull, G. J., M. F. Quigley, A. Sher, and E. González. 2015. Long-term changes in composition, diversity and distribution patterns in four herbaceous plant communities along an elevational gradient. Journal of Vegetation Science 26:552-563.

Stang, M., P. G. L. Klinkhamer, and E. Van Der Meijden. 2006. Size constraints and flower abundance determine the number of interactions in a plant-flower visitor web. Oikos 112:111–121.

Stanton, M. 2003. Interacting guilds: moving beyond the pairwise perspective on mutualisms. The American Naturalist 162:10-23

Sundstrom, L., L. Keller, and M. Chapuisat. 2003. Inbreeding and sex-biased gene flow in the ant *Formica exsecta*. Evolution 57:1552–1561.

Thomson, D. 2004. Competitive interactions between the invasive European honey bee and native bumble bees. Ecology 85:458–470.

United States Global Change Research Program. 2009. Global Climate Change Impacts in the United States. (T. R. Karl, J. M. Melillo, and T. C. Peterson, Eds.). Cambridge University Press, New York, NY, USA.

Walther, G., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J. Fromentin, O. H. I, and F. Bairlein. 2002. Ecological responses to recent climate change. Nature 416:389–395.

Wang, J. 2004. Sibship reconstruction from genetic data with typing errors. Genetics 166:1963–1979.

Watanabe, M. 1994. Pollinatoin worries rise as honey bees decline. Science 265:1170.

Whitehorn, P. R., M. C. Tinsley, M. J. F. Brown, B. Darvill, and D. Goulson. 2009. Impacts of inbreeding on bumblebee colony fitness under field conditions. BMC Evolutionary Biology 9:152.

Whitehouse, A. M., and E. H. Harley. 2001. Post-bottleneck genetic diversity of elephant populations in South Africa, revealed using microsatellite analysis. Molecular Ecology 10:2139–2149.

Williams, P. H., J. L. Osborne, P. H. Williams, and J. L. Osborne. 2009. Bumblebee vulnerability and conservation world-wide. Apidologie 40:367-387.

Williamson-Natesan, E. G. 2005. Comparison of methods for detecting bottlenecks from microsatellite loci. Conservation Genetics 6:551–562.

Zimmerman, M., and J. M. Pleasants. 1982. Competition among pollinators : quantification of available resources. OIKOS 38:381–384.

Appendix A: Benefits To Host Plants

Pollination mutualisms are important ecosystem functions that provide plants with cross-pollination benefits while producing food rewards for pollinators in the form of nectar or pollen. Recent estimates in pollination services state that around 80% of wild plant species and 35% of agricultural plants depend on pollinators for fruit and seed set (Food and Agriculture Organization of the United Nations). In fact, the most common proximate cause of reproductive failure in studied wild plants has been pollination limitation, and direct result of lack of pollinators (Colling et al. 2004, Elliott 2008).

In 2012, the intermountain western US experienced a severe drought that resulted in drastic contractions in populations of bumble bees surveyed at Pennsylvania Mountain Natural Area, Park County, CO. Bumble bees are keystone pollinators in alpine tundra ecosystems (Lundberg 1980, Geib and Galen 2012) and can pollinate twice as fast as many co-pollinators, such as solitary bees, flies, and hummingbirds (Pleasants 2009). It is unclear how quickly benefits provided to alpine plants from bumble bee pollination will recover following the drought-mediated reductions of native *Bombus* populations. If reduced population sizes carry over to subsequent years, effects could cascade to *Bombus*-dependent alpine flora. I tested this prediction by examining pollination services and resultant fitness one year following the drought for two related and ecologically important alpine clover species that differ in pollination niche breadth: *Trifolium parryi*, a *Bombus* species specialist and *T. dasyphyllum*, a broad generalist for pollination. Prior studies have documented that fitness and recruitment of *Bombus*-specialized *T. parryi* plants vary with abundance of their primary pollinators at small and large spatial scales (Geib and Galen 2012, Geib et al. 2015), while benefits to *T. dasyphyllum* are independent of variation in abundance of any one species. Quantitative models of the relationships predict dramatic loss of pollination services to *T. parryi* at very low pollinator densities. However, the availability of non-*Bombus* co-pollinators should mediate the outcomes for either clover (Geib and Galen 2012).

Methods

Study site and system

Data for this study were collected at Pennsylvania Mountain Natural Area ("Pennsylvania Mountain"), Park County, CO, in June - July 2013.

Trifolium dasyphyllum and *T. parryi* (Fabaceae, subfamily Faboideae) are closely related native clovers (Ellison et al. 2006), abundant in the central Rocky Mountains (USA) from tree line upward. On Pennsylvania Mountain (Park Co., CO), they flower prolifically from snowmelt in June to late August. *T. dasyphyllum* are cushion-like clover plants that produce an average of eight flower heads per plant, and favor dry, open meadows. In contrast, *T. parryi* does not maintain a cushion-like structure, produces only one to two flower heads, and is more commonly found in moister snow bed environments. They coexist where the boundaries of these two habitats meet.

These clovers are of particular ecological importance because they are responsible for a wide range of annual nitrogen inputs from fixation (Bowman et al. 1996). Nitrogen is a limiting key nutrient in alpine tundra regions, as low average temperatures limit the amount of biological activity that can occur through nitrogen fixing symbiotic bacteria (Billings and Peterson 1974). This increases the biological importance of *Trifolium* species, which have been measured to have a 100 fold nitrogenase activity compared with non-legumes (Holzmann and Haselwandter 1988).

Both clover 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). *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* queens, and has found to be visited more often by *B. balteatus* than *T. dasyphyllum* clovers when in mixed patches. Solitary bees and flies also visit both clovers late in the summer and contribute significantly to seed set (Geib and Galen 2012).

Field Methods

Three sites on the southern half of Pennsylvania Mountain were chosen for the study, two in the krumholtz region below tree line (at 39°15’2”N 106°6’51.3”W and 39°15’177”N 106°6’866”W at approximately 3450 m), and a sprawling site on a high elevation slope above tree line (39°15’14.9”N 106°7’27.5”W at approximately 3740 m). Within each site, I established ten plots (2m by 2m) that contained at least five plants of each clover species. Due to the lack of mixed plots at the high elevation slope, additional single species plots were identified that contained at least 5 plants of the same species for a total of 20 plots (10 *T. dasyphyllum*, 10 *T. parryi*). Plants within each plot were randomly assigned to one of three treatment groups:

- Open pollinated plants (O; n = 3 plant per plot at each site) were left open to natural visitors to assess fecundity attributed to background rates of pollination.
- Hand pollinated plants (HP; n = 1 plant per plot at each site) received hand pollination of a marked flower head daily until all florets had senesced to determine maximum potential fecundity for each patch.
- *B. balteatus* queen exclusion plants (EX; n = 1 plant per plot at each site) were fitted with a cage that excluded the clovers' shared pollinator, queens of *Bombus balteatus*, to assess the contribution of co-pollinators to fecundity. The *B. balteatus* queen exclusion cages were made of wire hardware cloth (64 X 64 mm mesh), too small for *B. balteatus* queens to pass through but passible to smaller bumble bees and a variety of solitary bees, wasps, and ants.

After the flowers had senesced, one pistil from each flower head was collected and stored in a fixative containing a 3:1 mix of ethanol and glacial acetic acid for later assay of pollination. The remaining flower head was wrapped in mesh in order to collect any seeds that would develop throughout the rest of the season. Plastic mesh cages were placed around all flowers to protect them from elk and other grazers. The flower heads were collected in October and returned to Appalachian State University to be analyzed for seed set. However, catastrophic losses from herbivory, grazing and anthropogenic causes significantly reduced sample sizes.

Pistils were cleared with 8 M NaOH for 24 hours, rinsed, and stained with decolorized aniline blue due. Pollen tubes were not successfully visualized, but stigma pollen grains were quantified using a microscope (Olympus XI81) with epifluorescence capabilities. However, stigma pollen grains are not a reliable indication of insect pollination service for clovers, due to the tendency of clovers to allow self-pollen to germinate at least partway down the style.

Flowers were dissected, and seeds and fruits were counted from each sample. Plant fecundity was measured by dividing the number of seeds produced by the number of florets on the flower head. As subsequent seed production is seen as a pollination benefit to these plants, we could compare fecundity of plants between the different treatments, between sites, and between species.

Statistical Analysis

Statistical analysis was conducted in JMP (Version 22; SAS Institute) using analysis of variance (ANOVA) to test for model effects of site location and treatment on pollination benefits to each species using plot as unit of analysis. Raw data from the experiment had non–normal distribution patterns and were square-root transformed. Plant treatment (hand pollinated, HP; open pollinated, O; or excluded from *B. balteatus* queens, EX*)*, site (n = 3), and treatment by site interactions were the fixed effects of the model. Both plant fecundity (seeds/flower) and fruit set (fruits/flower) was tested under this model.

Results

The impact of site and treatment on plant fecundity and fruit production differed slightly between species. The site in which the plots were situated had a significant effect on both seed set and fruit set (*T. parryi* fruit/flower*:* F (2,67) = 3.50, P = 0.0359, seed/flower F (2,67) = 3.60, P = 0.0329, *T. dasyphyllum* fruit/flower: F (2, 65) = 9.77, P = 0.0002, seed/flower *F* (2,65) = 9.97, *P* = 0.0002). In both species, there were neither significant effects from pollination treatment nor interaction effects from site x pollination treatment.

T. dasyphyllum produced essentially no seeds above tree line (slope site) and sites below treeline (ridge and logging road), *T. dasyphyllum* set significantly more seed. There were no differences in seed set per flower between treatments. *T. parryi* experienced lowest seed set at the ridge, significantly different from seed set at the logging road site.

Discussion

Our main objective for this study was to test for evidence of pollination limitation in early-flowering mixed clover patches one year following drought-mediated declines in bumble bee population abundances. We found no evidence for pollination limitation; fecundity for open-pollinator clovers at all sites demonstrated that limitation of seed set due to access to pollinators was not an issue, even just one year following drought-mediated population reductions of all bumble bee species.

For *T. parryi* at all sites (ridge, slope and logging road) seed set was the same for excluded, open, and hand pollinated plants. *Trifolium parryi* experienced lowest seed set at the ridge site, which is consistent with the tendency for this species to favor moister, more sheltered habitats such as on the logging road protected by large willow patches and the slope site that receives snow bed runoff well into summer.

T. dasyphyllum had significantly lower seed set at the high elevation slope site. A possible explanation for decreased seed set may be that plants aborted seed production due to lack of resources or incompatible pollen.

Hand pollination was expected to serve as a control for maximum plant seed set when pollen deposition was not acting as a limitation. Some sites had lower seed set in hand pollinated plants than open pollinated plants, indicating that hand pollinators may not have been successful. Low success from hand pollination may have occurred for several reasons. *Trifolium* plants are unable to self-pollinate, and it may have been possible that donor pollen was selected from a vegetative reproduced clonal plant In this case, self-incompatible pollinations may clog stigmas, inhibit the ability for outcross pollen to create germination tubes, and ultimately reduce plant fecundity (Galen et al 1989). Donor pollen may have failed to properly attach to the stigma for germination, or the pollen may have been deposited at the wrong time. All hand pollinations were done using pollen from a donor plant outside of 5 x 5m patch when flowers were fully open to limit these errors, but it is possible that these scenarios still contributed to low success of hand pollination at some sites.

Previous research suggested that excluding *B. balteatus* from flowering heads would have no consistent impact on *T. dasyphyllum* pollination services, but should negatively impact *T. parryi* in early flowering patches by limiting pollen deposit and subsequent seed set (Geib and Galen 2012). Interestingly in 2013, excluding *B. balteatus* queens did not reduce seed set in *T. parryi*. Either *Bombus* or non-*Bombus* copollinatiors contributed significantly to seed set in the absence of *B. balteatus* queens. In the 2013 season, *B. flavifrons* worker bees were most abundant, and estimates of nest numbers from individual counts predict that this species had the greatest relative abundance in the *Bombus* community. Prior research has found that although *B. balteatus* has the largest tongue size average, thus its position as a primary pollinator for *T. parryi, B. flavifrons* has a range of tongue size that overlaps with *B. balteatus*. Average tongue length for *B. balteatus* is 8.75mm

– 13 mm (mean = 10.0 mm) and *B. flavifrons* has a range of 6.5mm = 13mm (mean = 8.25mm) (Macior 1996). This increasingly abundant low-alpine species may be contributing to pollination services in the long-corolla clover, and may be the reason that no decrease in fecundity was seen in the early 2013 flowering season.

If *B. flavifrons* is not providing these services, then there are several other scenarios that may explain the high fecundity. Other co-pollinators outside of the *Bombus* community may also have established themselves as viable pollinators for *T. parryi*. Solitary bees and flies may be contributing pollination services, even though past studies of the same species found a co-pollinator effect only later in the season (Geib and Galen 2012). It is unknown how the drought may have impacted co-pollinator abundance and/or phenology in 2013.

Table 1: ANOVA for *Trifolium dasyphyllum* and *T. parryi* testing site (ridge, slope, log road) and treatment (hand pollinated, open pollinated, excluded from *B. balteatus* queens) effects on fruits/flower and seeds/flower (plant fecundity).

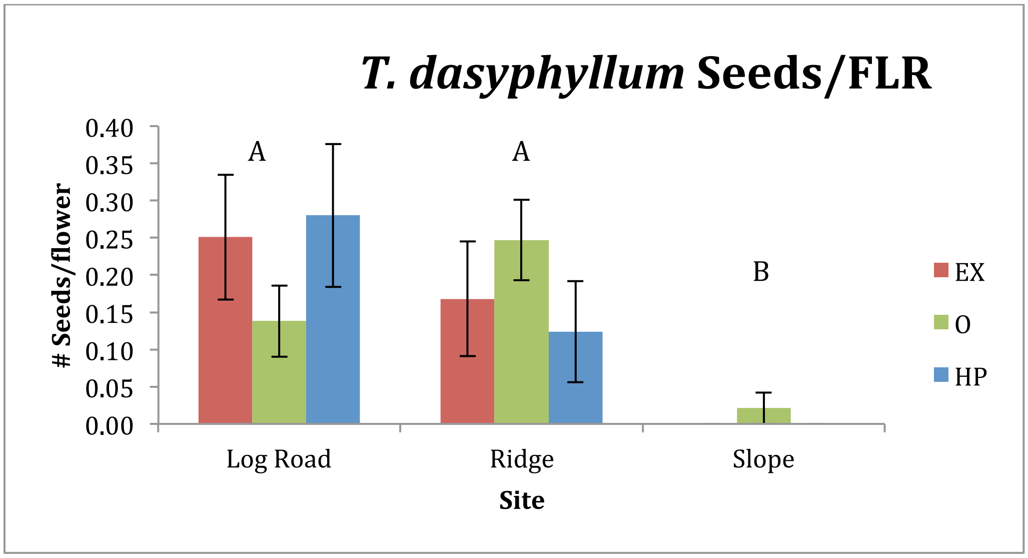
T. dasyphyllum	Source	DF	SS	F ratio	P Value
A - Fruits/Flower	Site	2	1.03	9.77	0.0002*
	Treatment	2	0.01	0.12	0.8889
	Site X Treatment	4	0.33	1.54	0.2016
	Error	65	3.44		
B- Seeds/Flower	Site	2	1.17	9.97	0.0002*
	Treatment	2	0.02	0.16	0.8494
	Site X Treatment	4	0.37	1.56	0.1953
	Error	65	3.80		
T. parryi	Source	DF	SS	F ratio	P Value
A - Fruits/Flower	Site	2	0.33	3.50	0.0359*
	Treatment	2	0.01	0.12	0.8896
	Site X Treatment	4	0.19	1.01	0.4062
	Error	67	3.14		
B- Seeds/Flower	Site	2	0.38	3.60	0.0329*
	Treatment	2	0.01	0.08	0.9253
	Site X Treatment	4	0.22	1.02	0.4031
	Error	67	3.56		

Figure Legends

Figure 1: Number of *T. dasyphyllum* seeds produced per flower under three treatments: excluded from *B. balteatus* queens (EX), open pollinated (O), and hand pollinated (HP). Bars are site means with standard error.

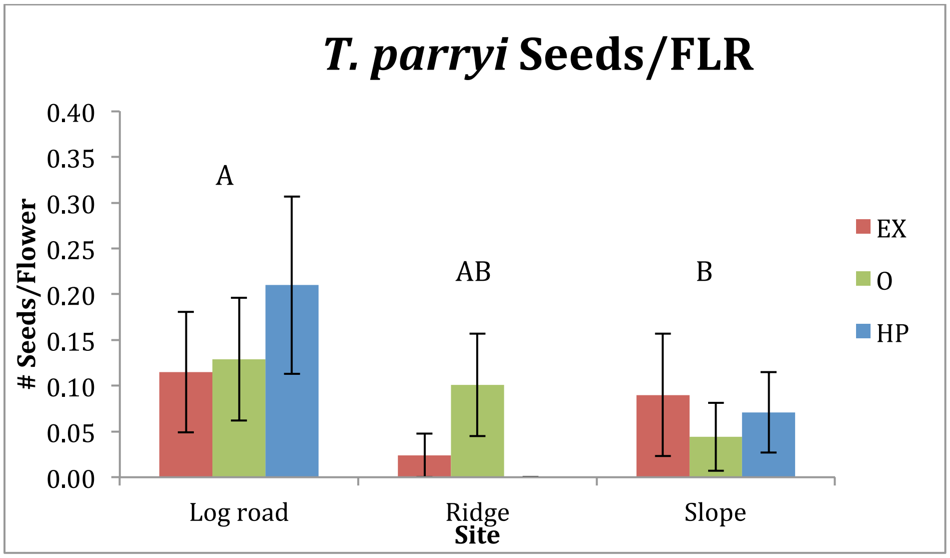
Figure 2: Number of *T. parryi* seeds produced per flower under three treatments: excluded from *B. balteatus* queens (EX), open pollinated (O), and hand pollinated (HP). Bars are site means with standard error.

Figure 1.



T. dasyphyllum Seeds/FLR
Seeds/flower
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
A
A
B
EX
O
HP
Log Road
Ridge
Slope
Site

Figure 2.



T. parryi Seeds/FLR
Seeds/Flower
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
A
AB
B
Log road
Ridge
Slope
Site
EX
O
HP

Vita

Leigh Rimmer went to Appalachian State University where she received her Bachelor of Science degree in Environmental Biology and Ecology. She began a MS program at ASU in 2012 and completed it in 2015. She lives in Washington, D.C. and works for the Environmental Protection Agency.