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BIOGEOGRAPHY, POPULATION GENETICS, AND COMMUNITY STRUCTURE OF NORTH AMERICAN BUMBLE BEES

by

Jonathan Berenguer Koch

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Ecology

Approved:

James P. Pitts Major Professor	James P. Strange Project Advisor
Terry Griswold Committee Member	Karen Mock Committee Member
Joseph S. Wilson Committee Member	Mark McLellan Vice President for Research and Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2015

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ABSTRACT

Biogeography, Population Genetics, and Community Structure of

North American Bumble Bees

by

Jonathan Berenguer Koch, Doctor of Philosophy

Utah State University, 2015

Major Professor: James P. Pitts
Project Adviser: James P. Strange
Department: Biology

This dissertation examines patterns of population genetic diversity and structure across multiple bumble bee species in North America. In Chapter 2 I examine the biogeography and population genetic diversity of the Hunt bumble bee, *Bombus huntii* (Hymenoptera: Apidae), a species currently in development as a pollinator for commercial agriculture. In Chapter 3 I test the single species hypothesis for *Bombus californicus* and *B. fervidus* with ecological, morphological, and molecular data. At present there is disagreement as to whether they constitute one or two species, leading to confusion in taxonomic keys, with conservation planning, and even in popular field guides. In Chapter 4, I characterize patterns of phenotypic color variation in bumble bee communities distributed across an elevation gradient in Yosemite National Park, California. In Chapters 5 and 6, I examine patterns of population genetic structure and diversity of four bumble bee species distributed in the Pacific Northwest, and document the range extension of *B. sylvicola* into Olympic National Park. The results of my

dissertation research suggests that (1) Pleistocene climate variability predicts contemporary population genetic structure and divergence, (2) there is clear divergence between *B. californicus* and *B. fervidus*, despite convergence of color phenotypes, (3) bumble bee community structure is predicted by Müllerian mimicry at narrow spatial scales, and (4) bumble bees inhabiting specialized bioclimatic niches, specifically alpine environments, exhibit significant isolation by distance at regional spatial scales. I anticipate that the results of my studies will not only yield effective conservation strategies of bumble bee pollinators, but also advance the study of Quaternary invertebrate phylogeography and population genetic diversity.

(232 pages)

PUBLIC ABSTRACT

Biogeography, Population Genetics, and Community Structure of North American Bumble Bees

Jonathan B. Koch

In 2011, several wild North American bumble bee pollinator species were reported to have declined by up to 96% in relative abundance in comparison to historic estimates, and one species was speculated to be extinct. None of these species have yet been documented to have recovered from these declines and additional species are now suggested to be at risk. Imperiled species in particular show increased specificity to narrow climatic envelopes, as opposed to putatively stable species. My dissertation describes patterns of population genetic diversity, structure, and gene flow pathways associated with climate variation and historical biogeography of bumble bees distributed in western North America. The results of my dissertation research suggests that (1) historic climate variability predicts contemporary patterns of population genetic structure and divergence in an economically important species, (2) color variability in bumble bees is likely associated with lineage diversification and phylogeography, (3) bumble bee community structure across evolutionary time is likely driven by Müllerian mimicry at narrow spatial scales, and (4) bumble bees inhabiting specialized ecological niches are associated with high levels of genetic fixation at regional spatial scales in the Pacific Northwest. The results of my research directly contribute to current efforts to effectively manage, conserve, and advocate for wild bumble bee pollinators in the context of global change.

DEDICATION

For my mother and father, the first two people who taught me the value of an education.

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Finally, I would like to extend my deepest appreciation to my partner, Armen Armaghanyan, for his love, companionship, and encouragement. Armen, you have seen me in my best and my worst, and I am excited to start my next chapter with you.

Aloha, Jonathan Berenguer Uhad Koch

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CHAPTER 1

INTRODUCTION[1]

Examining the relationship between geography and the distribution of genetic diversity is an underlying goal in biodiversity and conservation studies. Genetic diversity is the raw material of evolution, and a reflection of neutral and adaptive processes facilitating the fitness and ultimate survival of a population or species. While contemporary climate change has exacerbated biodiversity loss across a variety of plant and animal taxa (Walther et al. 2002; Rubidge et al. 2012; Miller-Struttmann et al. 2015), the role of past climate continues to illuminate patterns of genetic diversity observed at present (Hewitt 1996; Hewitt 2004). It is only recently that steps have been taken to examine population genetic diversity in North American insect fauna in the context of historic climate variability (Wilson & Pitts 2010; Lozier et al. 2013). Identifying patterns of population structure and gene flow in contemporary populations of a species may elucidate the influence of historic climate processes on current genetic diversity and structure (Hewitt 2000; Galbreath et al. 2010). The results of these studies have the potential to make science-based predictions on how future climate change may affect biodiversity.

Bumble bees (Hymenoptera: Apidae, *Bombus*) are an excellent model to study the role of contemporary and historic climate on the evolution of population genetic diversity and demographic change. These bees belong to a monophyletic lineage of primitively eusocial insects that depend on pollen and nectar from angiosperms for development and survival. Their dependence on a diverse bouquet of flowering plants, along with their

[1] References in this chapter are formatted as in *Conservation Genetics*.

robust body size and setaceous integument, make them some of the most important commercial and wild pollinators on the planet (Velthuis & Van Doorn 2006). It is estimated that there are 250 bumble bee species worldwide, 30 of which are found in the western United States (Williams et al. 2014). With the exception of a few arid- and tropic-adapted species, bumble bees are primarily cold adapted, and are the most diverse in alpine and temperate environments with highest species diversity found in the mountains of central China (Hines 2008). Within the past decade, several bumble bee species have declined at local and regional spatial scales across the planet (Goulson et al. 2008; Cameron et al. 2011; Koch 2011). In the western USA *Bombus franklini* may already be extinct, while a closely related species, *B. occidentalis,* has rarely been detected in most of its former range in the last decade (Cameron et al. 2011).

Threats to bumble bee survival and proliferation include entomopathogens, climate change, agricultural intensification, pesticide misuse, and land-use change (Goulson et al. 2008; Williams & Osborne 2009). It is likely that high-elevation distributed bumble bee species will be the most sensitive to climate change in this century due to their narrow climatic niche (Williams et al. 2009; Casey et al. 2015). Populations of a temperate-adapted species distributed at the geographic edge of its climatic tolerance also may suffer population extinctions and genetic erosion (Williams et al. 2009; Rubidge et al. 2012). Sensitivity and adaptation to a warming climate has been observed in populations of *B. impatiens* and *B. bimaculatus* in the northeastern USA (Bartomeus et al. 2011). In the past several decades, both species are estimated to be emerging from winter dormancy earlier each year in comparison to emergence dates recorded during the early 20th century. Furthermore, a recent study by Miller-Struttmann et al. (2015) found that

bumble bee proboscis lengths have shrunk in response to changes to the decline of floral resources due to rapid climate change. Considering the accumulating evidence on the decline of bumble bees, and their importance as wild pollinators (Kearns et al. 1998), it is increasingly important to identify and assess the distribution of rare and imperiled bumble bee fauna (Cameron et al. 2010).

RESEARCH OBJECTIVES

The primary objective of this study is to uncover species boundaries and population genetic patterns of bumble bee species distributed across western North America. Identifying population genetic patterns of biodiversity has the potential to guide conservation and management of wild lands and agricultural ecosystems (Schwartz et al. 2007). We live in a time of rapid global change where human-induced climate change increasingly threatens biodiversity in the 21st century (Walther et al. 2002). Bumble bees have received a great deal of political, agricultural, and economic interest over the past 20 years due to the importance in crop production and sustainability (Kearns et al. 1998; Cameron et al. 2010; Velthuis & Van Doorn 2006). Recent documentation of pollinator decline (Cameron et al. 2011), coupled with federal proclamations by national governments and non-profit organizations make it ever more timely and pressing to examine the geographic patterns of population genetic diversity in western North American bumble bees (Cameron et al. 2010). Finally, I anticipate that the results of this dissertation will advance the study of invertebrate phylogeography and population genetic diversity of the Quaternary.

List of Dissertation Chapters

I. **Range-wide population genetic diversity of a viable pollinator for commercial agriculture.** Bumble bees contribute significantly to crop pollination worldwide, second only to the European honey bee (Delaplane & Mayer 2000; Kleijn et al. 2015). However, since the collapse of commercial *B. occidentalis* populations due to pathogen outbreaks in commercial rearing facilities in the 1990s, western North America has been without a commercially available native bumble bee pollinator (Cameron et al. 2010). In Chapter 2, I explore wild population genetic diversity and niche space of the commercially viable bumble bee, *B. huntii* (Strange 2015). My aim is to determine whether *B. huntii* across its range is panmicitic, or displays a degree of lineage diversification with respect to climate variation across the North American continent. Based on the results, I discuss how the commercial movement of *B. huntii* populations for agriculture has the potential to impact the population genetic structure and disease ecology of locally adapted *B. huntii* populations.

II. **Illuminating cryptic species.** Although a comprehensive bumble bee phylogeny supports species level-status for *B. fervidus* and *B. californicus* (Cameron et al. 2007), the convergence of color banding patterns in their sympatric distributions continues to make it difficult to correctly identify specimens to species (Milliron 1973). Despite strong molecular and morphological support for two distinct species (Thorp et al. 1983; Cameron & Williams 2003; Cameron et al. 2007) some authors elect to synonymize the two based on the barcoding gene, Cytochrome c oxidase I (Williams et al. 2014). Uncertainties in species

identification pose a significant barrier to conservation biology and agricultural sustainability and security (Carolan et al. 2012; Williams et al. 2012). However, the same impediment also provides an opportunity to study diversification and convergence of color phenotypes across conspecific populations and related species (Wilson & Pitts 2010; Duennes et al. 2012; Wilson et al. 2012). My objective in Chapter 3 is to use population genetics, morphology, and estimates of bioclimatic niche space to illuminate the species boundaries and evolutionary histories of *B. californicus* and *B. fervidus*, two recently diverged sister-taxa that converge on color phenotypes (Hines 2008)**.**

III. **The role of Müllerian mimicry in structuring bumble bee communities.** The coexistence of closely related taxa in an ecological community has long intrigued evolutionary biologists and ecologists. Resource and habitat partitioning have been implicated to be important mechanisms driving the diversity and abundance of species that coexist within a community (i.e., community structuring) (Ranta & Lundberg 1980; Schoener 1974; Inouye 1980). However, phenotypic variation, particularly when expressed as Müllerian and automimicry, has also been found to an important trait associated with the composition of closely related organisms in a community (Brown & Benson 1974; Alexandrou et al. 2011; Beatty et al. 2004; Pfennig et al. 2001). In this study I examine the distribution of bumble bees and their setal phenotypes in Yosemite National Park in California. Bumble bee setal coloration patterns are significant as they are hypothesized to be important features of bumble bee mimetic evolution. My objective in chapter 4 is to test the hypothesis that bumble bee setal color phenotypes are spatially distributed, which

may suggest that community structure and abundance in bumble bees are largely driven by mimetic evolution.

IV. **Range extension of two bumble bees in Olympic National Park.** The geographic distribution of different bumble bees species are thought to be well documented in North America, given recent efforts to catalog their distribution and diversity (Williams et al. 2014; Koch et al. 2012; Cameron et al. 2011; Thorp et al. 1983; Stephen 1957). However, I recently extended the known geographic range of two species, *B. vandykei* and *B. sylvicola* into the Olympic Mountains of western Washington. Prior to my surveys in 2013, both species were not recorded to be in the Olympic Mountains. In addition to describing the distribution of *B. sylvicola* and *B. vandykei* in chapter 5, I discuss the value of surveying bumble bees in national parks and the importance of conducting research away from major transportation corridors.

V. **Community structure and population genetic diversity of bumble bees in the Pacific Northwest.** Identifying populations and species that have reduced genetic diversity has the potential to guide effective management and conservation (Schwartz et al. 2007). Mechanisms of population genetic variability include mutations, gene flow, and reproduction. Theory predicts that low-elevation species that have broad geographic distributions have more opportunities for gene flow (Lozier et al. 2013), whereas high-elevation species, such as alpine specialists, would have less opportunities for gene flow (Galbreath et al. 2009), facilitating unique evolutionary histories across populations. In chapter 6 I investigate the effect of geographic isolation on population genetic structure and

diversity of four bumble bees in the Pacific Northwest. I predict that bumble bee species that are distributed in high-elevation habitats are associated with high levels of genetic fixation, whereas species that are broadly distributed across lower elevations are associated with low levels of genetic fixation. Tis research will provide novel information on the dynamics of gene flow in geographically sympatric invertebrate taxa in the Pacific Northwest.

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CHAPTER 2

RANGE-WIDE ASSESSMENT OF *BOMBUS HUNTII* GENETIC DIVERSITY AND NICHE SUGGESTS POPULATION DIVERGENCE DURING THE PLEISTOCENE[2]

Aim. The goals of my study are to estimate contemporary and Pleistocene habitat suitability, population genetic diversity, and population genetic admixture of an economically important pollinator species, *Bombus huntii* (Hymenoptera: Apidae).

Location. Western North America

Methods. Habitat suitability was estimated using the program MaxEnt by associating 1,035 unique georeferenced locality records spanning the known range of *B. huntii* in western North America with 10 bioclimatic variables. To estimate genetic diversity, I genotyped 380 diploid individuals from 29 localities across the distribution of *B. huntii* in western North America at 10 nuclear microsatellite loci. I conducted a Bayesian analysis with STRUCTURE to examine genetic structure and compared isolation by distance (IBD) and isolation by resistance (IBR) models.

Results. My analyses recovered distinct *B. huntii* population lineages inhabiting three unique regions in western North America: (1) the Intermountain West/Front Range, (2) the Sierra Madre Occidental/Oriental and (3) the Trans-Mexican Volcanic Belt. Average population genetic diversity was highest in areas with historically high climate instability during the Pleistocene. I found genetic fixation (F_{st}) to be correlated with geographic distance (IBD) and environmental resistance (IBR) across the Sierra Madre Occidental/Oriental and Trans-Mexican Volcanic Belt clusters. In the Intermountain

[2] This chapter is co-authored with Dr. Rémy Vandame, Jorge Mérida-Rivas, Philippe Sagot, and James Strange. Permission has been granted by the required coauthors for this research to be included in my dissertation (Appendix A). I intend to submit this chapter to the *Journal of Biogeography*.

West/Colorado Front Range cluster, genetic fixation was significant under the IBR model but not with the IBD model.

Main conclusions. Oscillations between low and high habitat suitability across geographic space since the Pleistocene have likely influenced the mosaic of genetic diversity observed in *B. huntii*. Specifically, I found that areas of high climate instability are associated with high genetic diversity, and areas with low climate instability (*i.e.*, stable) are associated with low genetic diversity. As *B. huntii* is a species of agricultural importance, my study provides information on the distribution of population genetic diversity in wild populations that will likely be useful in future endeavors in their conservation and management.

INTRODUCTION

Since the Pleistocene, geographic instability of ecosystems due to a changing climate has left a lasting imprint on the diversity and composition of populations and species across the planet (Hewitt 2000; Hewitt 1996). Specifically, climate oscillations during the Pleistocene have been found to promote both population divergence and speciation through isolation and recolonization (Knowles 2000; Galbreath et al. 2010; Callahan et al. 2013). The availability of microsatellite loci and habitat suitability modeling techniques provides the opportunity to examine the effects of late Pleistocene climate variability on population genetic structure and diversity (Callahan et al. 2013; López-Uribe et al. 2014). There is converging evidence that species have responded to climate oscillations in a diversity of ways, depending on their associated life history traits and ecological demands (López-Uribe et al. 2014; Callahan et al. 2013; Galbreath et al. 2010). Small rodents with limited dispersal capacity due to narrow bioclimatic niches

have been found to exhibit strong population divergence as suggested by highly conserved gene regions during the Pleistocene (Galbreath et al. 2009). However, animals with great dispersal ability and broad bioclimatic niches have received less attention until recently (López-Uribe et al. 2014). Furthermore, many studies have examined species with strong bioclimatic specialization (*e.g.*, montane/alpine specialist), with few studies examining species that may exhibit a degree of regional adaptation to climate variability. Investigating the effects of Pleistocene climate variability on population genetic diversity has the potential to elucidate the evolutionary history of biodiversity and examine genetic diversity in the context of global environmental change.

Bumble bees (Hymenoptera: Apidae, *Bombus*) are fantastic models for studying the effects of climate oscillations on contemporary bioclimatic niches, range dynamics, and population-level divergence during the Quaternary. They are large-bodied insects and have the capacity to disperse over several kilometers in search of food, nesting sites, and hibernacula (Woodard et al. 2015; Jha 2015; Lozier et al. 2013). Furthermore, they are densely covered in setae and can fly at low temperatures by warming their flight muscles prior to flight (Heinrich & Esch 1994). As bumble bees are dependent on pollen and nectar to feed developing brood, dispersal and colonization during the Pleistocene was likely affected by changes in the distribution of flowering plants. In western North America, the majority of the bumble bee fauna are primarily associated with temperate and alpine environments that can act as sky islands (Heald 1951), particularly in the Intermountain West and the major mountain provinces of Mexico: Sierra Madre Oriental, Sierra Madre Occidental, and Trans-Mexican Volcanic Belt. These sky islands and adjacent valleys may have served as refugia for bumble bees during climate oscillations

where they may have tracked their preferred habitat across a diversity of mountain provinces (Galbreath et al. 2009).

In this study I examine patterns of contemporary population genetic diversity of the Hunt bumble bee, *Bombus huntii* Greene, 1860 across its large geographic range. The latitudinal distribution of *B. huntii* extends from the southern edge of Canada, south throughout the Intermountain West and Front Range of the Colorado Rocky Mountains to southern Mexico, specifically on the Trans-Mexican Volcanic Belt province (Thorp et al. 1983; Koch et al. 2012; Labougle 1990). The longitudinal range of the species is primarily bound by the crest of the Sierra Nevada and Cascade Mountains on the west and the Black Hills of South Dakota on the east. Although populations of *B. huntii* have been found east of the montane environment of South Dakota, they at lower absolute abundances relative to bumble bee communities surveyed throughout Utah, Colorado, and Nevada (Koch et al. submitted). *B. huntii* and its sibling species *B. vosnesenskii* diverged from their most recent common ancestor by the early-Pliocene (~5 mya) (Hines 2008; Cameron et al. 2007). Thus, contemporary population genetic diversity of *B. huntii* can be investigated using climate scenarios estimated for the Pleistocene.

B. huntii has many properties that would make it a viable managed pollinator for agriculture. A recent survey of bumble bees in the USA found *B. huntii* to be the 6th most abundant of the 39 bumble bee species detected throughout the conterminous USA and Alaska (Koch et al. submitted). *B. huntii* has been identified as an effective pollinator for greenhouse crops and is under consideration for large-scale commercialization (Strange 2015). The species produces relatively large nests and an abundance of queens (*i.e.*, gyne) (Hobbs 1967), important biological traits that are comparable to other

economically significant bumble bee species like *B. impatiens* and *B. terrestris* (Velthuis & Van Doorn 2006). Unlike its commercially defunct predecessor, *B. occidentalis*, wild *B. huntii* populations have been associated with low pathogen prevalence in the US (*i.e.*, *Nosema bombi* and *Crithidia bombi*) (Cordes et al. 2012; Blaker et al. 2014), and do not appear to exhibit the debilitating symptoms of pathogens in lab-controlled colonies (Strange et al., unpublished data). The broad geographic distribution of *B. huntii*, coupled with favorable life history traits for domestication, affirms the timeliness of my study as it characterizes wild population genetic diversity and structure prior to the impending distribution of commercial populations for pollination service delivery (Goulson & Hughes 2015; Cabrera et al. 2015). Most studies on the genetic variability of wild pollinator species come after they have been domesticated and distributed (*e.g.*, *Apis mellifera*, *B. occidentalis*, *B. impatiens*, and *B. terrestris*) (Velthuis & Van Doorn 2006, Woodard et al. 2015). However, my study has the potential to reconstruct ancestral states, natural population genetic variability, and biogeography before *B. huntii* is commercialized and transported to areas with novel habitats, and potentially exposed to new diseases.

The primary objective of this paper is study patterns of population genetic structure and diversity across *B. huntii* populations in western North America. My secondary objective is to examine the role of climate variability since the late Pleistocene on the observed genetic diversity and gene flow patterns. To purse these objectives, I first constructed habitat suitability models based on contemporary occurrence records and climatic associations with species distribution modeling techniques. Then, operating under the principle of niche conservatism (Peterson et al. 1999), I estimated the

distribution of *B. huntii* during the Last Glacial Maximum (~ca 22,000 years before present, ybp) of the Pleistocene. Next I PCR amplify a suite of microsatellite loci to estimate genetic diversity and population assignment. Since *Bombus huntii* diverged from its sister taxon, *B. vosnesenskii*, during the mid-Pliocene (Hines 2008), I predict that contemporary population genetic diversity and structure has likely been influenced by climate variability that has been observed since the Pleistocene (Galbreath et al. 2009; Lopez et al. 2014; Peterson et al. 1999; Tzedakis et al. 2002; Rubidge et al. 2014; Callahan et al. 2013).

MATERIALS AND METHODS

B. huntii females were collected across 33 sampling sites throughout North America from 2008 to 2015 (Fig. 2-1). This sampling strategy was able to capture a major portion of the species' range in western North America (Thorp et al. 1983; Labougle 1990). Bumble bees were captured with a diversity of methods including sweep netting, colored bee bowls, and blue vane traps. Given that the aim of my study was to examine population genetic diversity and structure of wild *B. huntii* and had few specimens from certain sites (i.e., small sample size), I elected to pool certain sampling sites together if they were < 9 km from each other. This decision was made based on known biological properties of dispersal and gene flow in bumble bees, in that the sister species to *B. huntii*, *B. vosnesenskii* is estimated to disperse approximately 9 km from her place of origin (Jha & Kremen 2013). At present, there is no data on the population genetic structure or diversity of wild *B. huntii*. Furthermore, barriers to gene flow have been found to be influenced by both land-use change and bioclimatic variability in *B. vosnesenskii* and *B. bifarius* (Jha & Kremen 2013; Lozier et al. 2013), thus limiting the

distance to 9 km for pooling ensures that I did not arbitrarily combine populations together that would artificially alter estimates of genetic diversity and structure (*e.g.*, Wahlund effect).

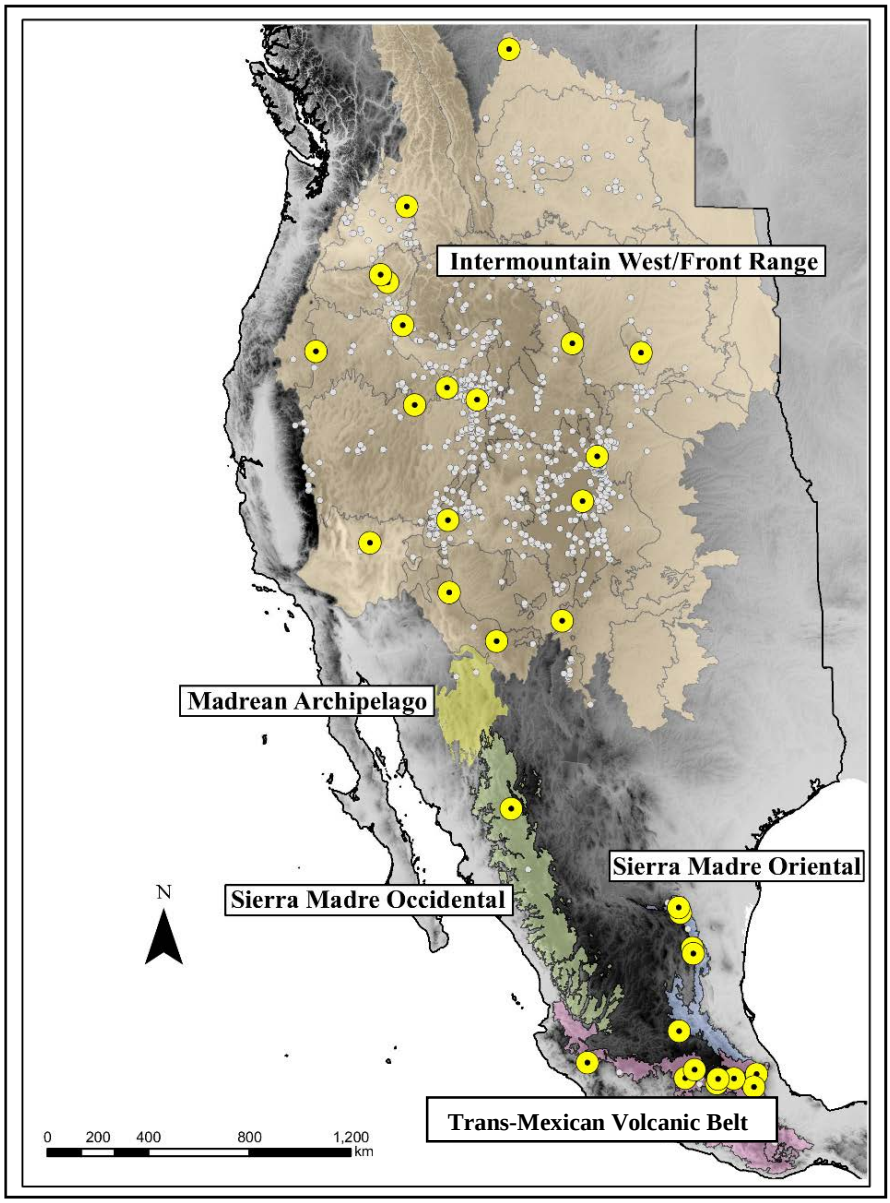

Figure 2-1. Occurrence records (white points) and contemporary surveys of *Bombus huntii* (Hymenoptera: Apidae) (yellow points with black center point) throughout western North America. The shaded polygons represent groups of ecoregions in which *B. huntii* is distributed. Orange = Intermountain West/Colorado Front Range Province, Yellow = Madrean Archipelago, Green = Sierra Madre Occidental, Blue = Sierra Madre Oriental, Purple = Trans-Mexican Volcanic Belt.

Quaternary habitat suitability modelling and bioclimatic niche

To estimate the geographic distribution of habitat suitability (HS) of *B. huntii* throughout its endemic range at present and during the Pleistocene, species distribution models were constructed under the principle of maximum entropy with MAXENT v3.3.1 (Phillips et al. 2004). The software program MAXENT uses presence-only georeferenced locality records and random background points sampled from the study extent to estimate the distribution of the species that is closest to uniform (maximum entropy) under the suite of environmental conditions given to the model (Elith et al. 2011). Georeferenced distribution records were queried in the Global Biodiversity Information Facility database (http://gbif.org) and filtered for unique spatial coordinates (Fig. 2-1) (Appendix B1). Additional records from a contemporary survey of Mexican bumble bees were included in the final set of georeferenced localities of *B. huntii*. Specimen occurrence records that do not agree with historic and contemporary range maps of *B. huntii* were filtered out of the final dataset (Koch et al. 2012; Thorp et al. 1983; Williams et al. 2014).

Occurrence records were aggregated with 18 spatially explicit bioclimatic variables representing contemporary conditions (1950 - 2000) from the WorldClim v1.4 Bioclim dataset (2.5 arc minutes) (Hijmans et al. 2005). To reduce model complexity, I examined the relationship between occurrence records and bioclimatic variables with a pair-wise Pearson Correlation Coefficient (r) test. From each pair-wise correlation coefficient estimate, I retained only one variable for the final model if $r \geq 0.75$. Rather than randomly select a variable for the analyses, I chose to retain the variables that reflect annual and seasonal trends in precipitation and temperature as bumble bees are primarily active during the summer months, but also need to survive winter hibernation (Lozier et

al. 2013). HS models in MAXENT were constructed with default parameters to generate a logistic output (a measure of relative HS), averaged over 100 replicates with a subsampling scheme to evaluate model performance (75% train, 25% test). The *B. huntii* HS models were evaluated in MAXENT using the area under curve (AUC) statistic and a permutation of variable importance. AUC values closer to 0.5 (random) suggest poor predictive performance, whereas values closer to 1 (non-random) suggests high predictive performance. Permutation tests of variable performance executed within the MAXENT software platform used the training points to assess the relative contribution of each variable to the final averaged model in the context of the AUC statistic. If the AUC statistic drops significantly after a bioclimatic variable is removed, it is assumed that that the variable contributes significantly to the estimation of HS. Data processing and geographic visualization of the HSM were implemented in ArcGIS 10.1 (ESRI 2012).

Finally, I visualized the association of bioclimatic variables that contributed significantly to the construction of *B. huntii* HS models with scatterplots. I then tested for differences in bioclimatic niche space between the Intermountain West/Front Range and Mexican *B. huntii* occurrence records with the Wilcoxon Rank-Sum Test. Because there are significantly more USA/Canada records (n = 985) than Mexico records (n = 50), I subsampled 50 occurrence records within the USA and Canada. In preliminary analyses, I compared the subsampled USA/Canada data to the entire dataset (excluding the subsampled data) and found no significant difference between the subsampled versus the entire data set. I elected to retain the complete USA/Canada dataset for comparison with the Mexico dataset.

Operating under the principle of niche conservatism (Peterson et al. 1999), I predicted the distribution of *B. huntii* during the last glacial maximum (LGM, ~22,000 ybp) using the constraints of the contemporary bioclimatic associations of *B. huntii* HS mapped to paleoclimate data available within the WorldClim database (Hijmans et al. 2005). To identify potential climate refugia (HS Stability) over the Quaternary, I added the HS models based on the Contemporary and LGM bioclimatic data. I then standardized the HS Stability raster by dividing the calculated values by the raster's maximum value, producing values ranging from 0 (low HS Stability) to 1 (high HS Stability).

Microsatellite genotyping

Within each of the 33 pooled sampling sites, an average of 12.25 (± 1.29 SE) worker bees were collected (n = 380). DNA was extracted from the mid-leg of each bumble bee using a modified-Chelex100™ protocol described in Strange et al. (2009) and screened at 13 microsatellite loci: B124, BTERN01, BTERN02, BT28, BT10, BT30, B96, BTMS0081, BTMS0066, BTMS0062, BL13, BTMS0044, BTMS0059 (Estoup et al. 1995; Estoup et al. 1996; Reber-Funk et al. 2006; Stolle et al. 2009). Multiplex polymerase chain reactions (PCR) were performed in final volumes of 10 μL, containing approximately 1 μL of extracted DNA, 1x Promega (Madison, WI) reaction buffer, 0.6 mM dNTP mixture, 0.2–0.4 μM primer, 0.001 mg BSA, 0.4 units Taq polymerase (Promega, Madison, WI). The $MgCl_2$ concentration was adjusted to 1.4 mM. The PCR conditions for both multiplex reactions were one 3:30 min cycle at 95°C, 30 cycles of 95°C for 30 s, annealing temperature 55/58°C for 1:15 min, 72°C for 45 s and a final extension period of 15 min at 72°C. The DNA amplifications were performed with

fluorescent 5’ dye-labeled primers (6-FAM, NED, VIC, or PET) and separated on an Applied Biosystems 3730xl automatic sequencer at the Center for Integrated Biology at Utah State University. The allele sizes were scored manually using GENEIOUS version 8 (Kearse et al. 2012), and only samples with ≥ 7 loci scored per individual were included in my analyses.

Population genetic diversity and assignment

As bumble bees are generally monandrous (Estoup et al. 1995), primitively eusocial, and live in annual colonies, it is possible to capture sibling female workers in the wild. To avoid pseudo-replication within sampling locations, full-siblings were first assigned to colonies with COLONY 2.0 (Jones & Wang 2010). In the colony-assignment exercise, I set the genotyping error rate to 0.005, based on error rates documented in previous studies (Lozier et al. 2011) and the sex-determination system to “haplodiploid”. If full-siblings were detected in the colony assignment tests (≥ 95% genotype similarity), I randomly retained only one representative from each family using a coin-toss. The removal of full-siblings and individuals with < 50% amplified genotypes resulted in an average population size of 10.30 (± 1.79 SE) diploid females (n = 340). Finally, I retained 28 of the 33 sampled populations as they were represented by at least four individuals.

The probability of null alleles was estimated with the software program MICRO-CHECKER (van Oosterhout et al. 2004). Pairwise linkage disequilibrium (LD) and deviations from Hardy-Weinberg equilibrium (HWE) across populations and loci were tested with the web-based software program GENEPOP v 4.0.10 (Raymond & Rousset 1995). Sequential Bonferroni corrections were applied to the HWE and LD *P*-values estimates to minimize type I errors associated with multiple comparisons (Rice 1989).

Population structure was examined with an individual-assignment Bayesian clustering method with the software program STRUCTURE v 2.3.4 (Pritchard et al. 2000). I elected to use the admixture model in STRUCTURE to assign my genotypes, which assumes that individuals comprise *K* unknown populations, to which an individual can be assigned based on their genotype without a priori delineation of populations. I set the model to run with 20,000 burn-in steps and 100,000 samples, with 10 iterations for each *K*, where *K* ranged from 1 to 10. To determine the optimal *K* (*i.e.*, populations or lineages), the distributions of the probability of the data ($ln\ P(D)$) and ΔK (as described by Earl & vonHoldt 2012; Evanno et al. 2005) were visualized with the web-based software program STRUCTURE HARVESTER 0.6.94 (Earl & vonHoldt 2012). To account for multimodality associated with individual STRUCTURE runs, I averaged each individual's admixture proportions over the 10 replicates for the best *K* using CLUMPP v1.1.2 (Jakobsson & Rosenberg 2007) and spatially visualized population-assignment with pie-charts in ArcGIS v10.1 (ESRI 2012).

I estimated allelic variation across populations with two different indices using rarefaction (standardized to four gene copies per population): (1) effective allelic diversity (*Ae*- count of alleles per locus), and (2) private allelic richness (PAR- the number of unique alleles in a population). An additional measure of genetic diversity, heterozygosity (H_e), was estimated using Nei's genic diversity metric. Based on individual-based population assignments with STRUCTURE, I tested for differences in average *Ae*, PAR, and H_e across three cluster identified: (1) Intermountain West/Colorado Front Range, (2) Sierra Madre Occidental and Oriental (*i.e.*, northern Mexico), and (3) Trans-Mexican Volcanic Belt (*i.e.*, southern Mexico). I first employed the Kruskal-Wallis

Rank- Sum Test, followed by a Wilcoxon Rank-Sum Test to determine if differences in average genetic diversity existed between paired clusters. Additionally, I tested for differences in genetic diversity indices relative to elevation, latitude, longitude, the two bioclimatic based HS models, and the additive HS Stability model across populations with the Spearman Rank Correlation Test.

Statistical analyses and indices of genetic diversity were estimated within the R statistical computing platform (R Development Core Team 2005). I specifically used the *gstudio* and *vegan* packages to calculate genetic diversity. PAR was estimated in the software program HP-RARE (Kalinowski 2005).

Population genetic structure and landscape genetics

Based on the clusters identified by STRUCTURE, I performed an analysis of molecular variance (AMOVA) to test for differences in genetic fixation with ARLEQUIN 3.5 (Excoffier et al. 2005). I then tested for correlation between pairwise estimates of fixation based on allelic frequencies with geographic distance (*i.e.*, Isolation by Distance) in GENALEX 6.5 (Peakall & Smouse 2006). I first examined Isolation by Distance (IBD), incorporating all the sampling sites genotyped in a single test. Next, I partitioned the dataset based on clusters identified in STRUCTURE. However, because the Sierra Madre Oriental/Occidental and Trans-Mexican Volcanic Belt clusters have a limited number of sampling sites to make up the two distinct clusters to test for IBD I elected to combine them into a single cluster I defined as “Mexico” (Fig. 6-2).

In addition to testing for a significant correlation in IBD, I wanted to determine the capacity for contemporary, LGM, and additive geographic stability of HS to predict observed patterns of genetic structure (IBR) (McRae 2006; McRae et al. 2008; Lozier et

al. 2013). I applied electrical circuit theory, implemented in CIRCUITSCAPE 3.5.8 (Shah & McRae 2008) to estimate resistance distance matrices between all pairs of sampling sites. CIRCUITSCAPE estimates resistance distance between two points to calculate the likelihood of potential gene flow, integrating over all possible pathways of dispersal. I used the logistic output raster generated from MAXENT, as well as the additive HS stability raster as inputs to estimate resistance within the landscape inhabited by *B. huntii*. I executed CIRCUITSCAPE in pairwise mode with an eight-neighbor cell connection scheme using average resistances and set the MAXENT raster to conductance so that values closer 1 (high HS) would reduce resistance and values closer to 0 (low HS) would increase resistance.

Both IBD and IBR patterns are hypothesized to capture the relationship between gene flow and genetic drift in the context of geographic and environmental distribution of populations, respectively. However, my final deduction on the best predictor (geographic or environmental) was based on the ability for the coefficient of determination (r^2) to fit the genetic data. Higher r^2 values suggest a better fit of the IBD or IBR variable to the genetic distance estimates. I elected to not combine the variables in alternative, matrix based, regression analyses (*e.g.*, MMRR) given that both geographic distance and environmental HS values (*i.e.*, resistance) are all highly correlated ($r > 0.95$).

RESULTS

Quaternary habitat suitability modelling and bioclimatic niche

The 18 available bioclimatic variables were reduced to 10 bioclimatic variables after the assessment of colinearity and were incorporated into the final HS models in MAXENT. The bioclimatic variables used to estimate habitat suitability included:

Annual Mean Temperature (BIO 1), Annual Precipitation (BIO 12), Precipitation Seasonality (BIO 15), Precipitation of Warmest Quarter (BIO 18), Precipitation of Coldest Quarter (BIO 19), Mean Diurnal Range (BIO 2), Max Temperature of Warmest Month (BIO 5), Temperature Annual Range (BIO 7), Mean Temperature of Wettest Quarter (BIO 8), Mean Temperature of Driest Quarter (BIO 9) (Table 2-1). Average AUC for the subsampled contemporary HS model was 0.90 for the training occurrences and 0.89 (±0.01 SD) for the test occurrences. The contemporary HS models generated are reflective of the range extent maps generated in the past (Thorp et al. 1983) and in recent explorations to define the distribution of *B. huntii* with maximum entropy approaches (Williams et al. 2014). The LGM paleoclimate HS performed similarly to the contemporary HS model (LGM: AUC Test = 0.90, AUC Train = 0.88 ± 0.01 SD). Following permutation test of all 10 variables included in the contemporary HS model it was found that Annual Mean Temperature (BIO 1), Temperature Seasonality (BIO 2), and Average Temperature of Wettest Quarter contributed 29%, 28%, and 11% to HS model construction, respectively (Table 2-1). The contributions of these variables to the LGM HS model were comparable to the Contemporary HS model (Table 2-1).

The contemporary HS predicts *B. huntii* to be distributed across an elevational gradient throughout the Intermountain West (USA/Canada) and primarily at high-elevation habitats of the Sierra Madre Occidental, Sierra Madre Oriental, and the Trans-Mexican Volcanic Belt (Fig. 2-2A). The LGM HS model reveals a dramatic latitudinal shift in the distribution of *B. huntii* HS, where middle latitude mountain ranges relative to the contemporary distribution of the bumble bee may have been a more suitable environment for *B. huntii* in the cooler Pleistocene (Fig. 2-2B). Specifically, the Madrean

Archipelago is suggested to have possessed the bioclimatic conditions that may have provided *B. huntii* a pathway for dispersal to and from the Sierra Madre Occidental (Fig. 2-1, Fig. 2-2).

Pairwise comparisons between the bioclimatic variables used in the HS models found significant differences in bioclimatic niche space between north (*i.e.,* Intermountain West/Colorado Front Range) and south (*i.e.,* Sierra Madre Oriental/Occidental and Trans-Mexican Volcanic Belt) occurrence records (Fig. 2-3). Specifically, I found that 9 of the 10 bioclimatic variables used to construct the HS models exhibited significant differences between the north and south (Sierra Madre + Trans-Mexican Volcanic Belt) populations of *B. huntii* (Fig. 2-3A) (Wilcoxon rank sum tests, all $P < 0.05$). I found no difference between the mean temperatures of the driest quarter (BIO 9) between northern and southern populations (Wilcoxon rank sum tests, $P = 0.13$). The distribution of *B. huntii* in the Intermountain West/Front Range reveals cooler annual temperatures experienced throughout their range, relative to populations found in Mexico. Temperatures associated with Mexican populations are significantly more mesic year round when compared to temperatures associated with populations of *B. huntii* in the USA and Canada (Fig. 2-3C, Fig. 2-3D). Furthermore, precipitation regimes associated with Mexican populations suggest that there is little seasonal variability in precipitation over time, whereas the Intermountain West/Colorado Front Range populations have much more dramatic seasonality in precipitation (Fig. 2-3E, Fig. 2-3F).

Microsatellite genotyping

The locus BTERN02 did not amplify in >50% of the specimens genotyped and was removed from any further data processing. MICRO-CHECKER results indicated that

the locus BTMS0059 was suspected to have null alleles in 48% of the populations, and was ultimately excluded from the study. The remaining 11 loci had either little or no indication of null alleles across my study populations (< 14% of the populations exhibited null alleles). Before Bonferroni corrections, some populations showed deviations from HWE at three of loci B124, BTERN01, and BTMS0062. After Bonferroni corrections BTERN01 did not show significant deviations from HWE (P = 0.46). Furthermore, deviations in HWE across population by locus combinations were not significant as HWE deviations were observed at only 1 - 2 loci across all 28 populations. Significant LD was also observed at three loci across populations: BTMS0066 with B96 and BTM0066 with BTMS0062. Considering that BTMS0066 was not in HWE, and was found to be in LD with two other loci, I removed it from the final analyses. Remaining locus by population combinations were found to exhibit little to no LD patterns (< 0.03% total LD detected in any combination). In total, I retained 10 loci for analyses.

Population genetic diversity and assignment

Based on the admixture ancestry with correlated allele frequencies model, the mean log probability of the data was greatest at K = 7. The ΔK statistic was greatest at K = 3, with significantly less explanatory power gained by additional clusters (K). Assuming K = 3 clusters, demes located in the Intermountain West/Front Range (n = 16) were assigned to cluster 1, demes located in the Sierra Madre Oriental (n = 4) were assigned to cluster 2, and demes located in the Trans-Mexican Volcanic Belt (n = 7) were assigned to cluster 3 (Fig. 2-4). One deme located in Jalisco (CGuz), just west of the

Table 2-1. Habitat suitability model summaries and bioclimatic variable contribution in estimating the distribution of *B. huntii* across North America at present and during the Last Glacial Maximum. Italicized values represent permutation importance of a bioclimatic variable. High permutation values suggest high contribution the model in estimating *B. huntii* habitat suitability.

	Training AUC	**Test AUC**	**AUC STD**	**BIO 1**	**BIO 12**	**BIO 15**	**BIO 18**	**BIO 19**	**BIO 2**	**BIO 5**	**BIO 7**	**BIO 8**	**BIO 9**
LGM	0.90	0.88	0.01	29.96	0.60	8.85	9.72	3.68	28.07	0.65	4.42	10.5	3.55
				37.76	*1.11*	*9.90*	*2.37*	*4.84*	*21.06*	*1.52*	*10.61*	*6.04*	*4.79*
Contemporary	0.90	0.89	0.01	29.69	0.55	8.87	9.92	3.74	27.92	0.59	4.44	10.8	3.49
				37.58	*1.05*	*10.0*	*2.45*	*4.90*	*21.05*	*1.36*	*10.84*	*6.05*	*4.72*

Trans-Mexican Volcanic Belt ecoregion was assigned to deme 2. However, because this sampling site is only represented by two specimens it was not included in additional analyses of population genetic diversity. While the ΔK statistic suggests $K = 3$ clusters, I further investigated the spatial and fractional relationship of *B. huntii* populations with $K = 2$ and $K = 4$ population assignment models (Fig. 2-4, inset). Under a $K = 2$ population assignment scenario, the Sierra Madre Occidental and Sierra Madre Oriental demes are in admixture between the demes located in the Intermountain West/Front Range and the demes in the Trans-Mexican Volcanic Belt (Fig. 2-4). Under a $K = 4$ population assignment model, the CGue site is assigned to a fourth cluster, rather than a deme in admixture between cluster 1 and cluster 2. Given my limited sampling of the Sierra Madre Occidental and its suggested assignment to the Sierra Madre Oriental cluster at $K = 2$, I pooled this sampling site with the Sierra Madre Oriental cluster, hereafter "Sierra Madre" for remaining analyses.

Considering the broad geographic extent of the sampling sites across the Intermountain West/Front Range region, I further investigated the potential for population sub-structure across these ecoregions. The ΔK statistic was greatest at $K = 4$ clusters, with little explanatory power gained by additional clusters. However visual inspection of the geographic distribution of the fractional relationships of the modeled genotypes shows a degree of panmixia (Fig. 2-5).

Average *Ae* across sampling sites is 3.60 (± 0.62 SD), average H_e is 0.66 (± 0.12), and average PAR is 0.12 (± 0.09) (Fig. 2-6). Following the STRUCTURE results, I compared average population genetic diversity patterns across the three genetic clusters. Both average *Ae* and H_e in the Intermountain West/Front Range cluster were significantly

higher than those of the Sierra Madre cluster and the Trans-Mexican Volcanic Belt cluster (Wilcoxon Rank-Sum Tests, both $P < 0.05$) (Fig. 2-6A, Fig. 2-6B). I found no significant difference in average *Ae* between the Sierra Madre cluster and the Trans-Mexican Volcanic Belt cluster (Wilcoxon Rank-Sum Tests, $P = 0.75$), but detected a significant difference in H_e (Wilcoxon Rank-Sum Tests, $P = 0.03$). There was no significant difference in average PAR across all regional clusters (Kruskal Wallis Test, $X^2 = 0.46$, $df = 2$, $P = 0.79$) (Figure 2-6C).

I found a significant and positive correlation across *Ae* (Pearson's Correlation Coefficient, $r = 0.54$, $df = 26$, $P < 0.01$) and H_e (Pearson's Correlation Coefficient, $r = 0.58$, $df = 26$, $P < 0.01$), PAR (Pearson's Correlation Coefficient, $r = 0.34$, $df = 28$ $P = 0.04$) with latitude (Fig. 2-7). Given the outlier survey site with low genetic diversity at low latitudes (CGue in Fig. 2-4), I also examined the significance of genetic diversity patterns with latitude by removing the site from the final dataset. Even when this site was removed I found a similar trend in the data (Pearson's Correlation Coefficient, *Ae*: $r = 0.62$, $df = 25$, $P < 0.001$; H_e: $r = 0.82$, $df = 25$, $P < 0.001$; PAR: $r = 0.38$, $df = 25$, $P = 0.052$). HS stability also increased with decreasing latitudes, suggesting that low latitude environments associated with *B. huntii* habitats were stable during the late Pleistocene (Pearson's Correlation Coefficient, $r = -0.89$, $df = 26$, $P < 0.001$). Given that the latitude is correlated with HS stability (Fig. 2-7A), and that genetic diversity is positively correlated with latitude, I deduce that HS stability is negatively correlated with genetic diversity. Thus, across the sites sampled there is a significant and negative correlation between HS stability and genetic diversity across the distribution of *B. huntii* (Fig. 2-7).

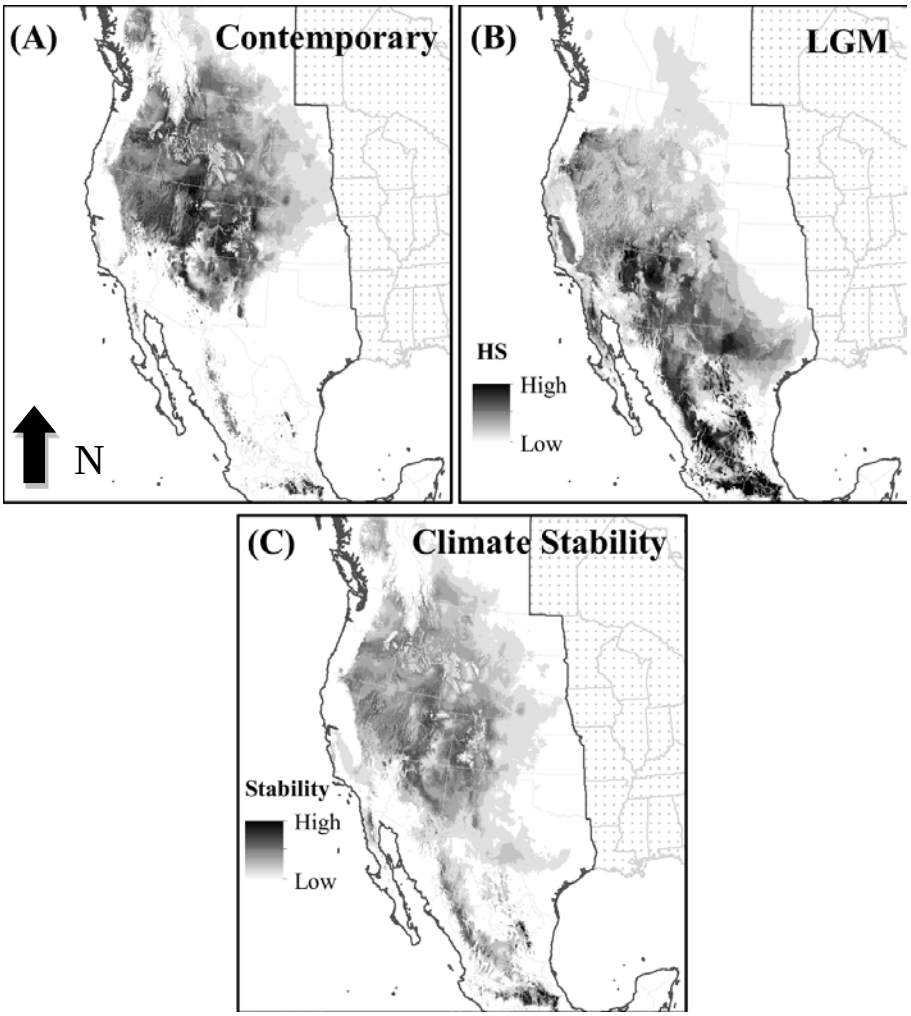

Figure 2-2. Estimate of *B. huntii* habitat suitability (HS). HS values closer to 1 suggest high HS (Black), whereas HS values closer to 0 suggest low HS (White). HS < 0.10 are in white. *B. huntii* HS models were estimated with (A) Contemporary climatic variability (1950 - 2000) and projected onto western North America. Operating under the principle of niche conservatism (Peterson et al. 1999), I projected contemporary *B. huntii* HS to (B) the Last Glacial Maximum (LGM). (C) HS models across time were then added to together and standardized to the maximum HS value calculated to estimate the geographic distribution of HS stability over the past 22,000 years.

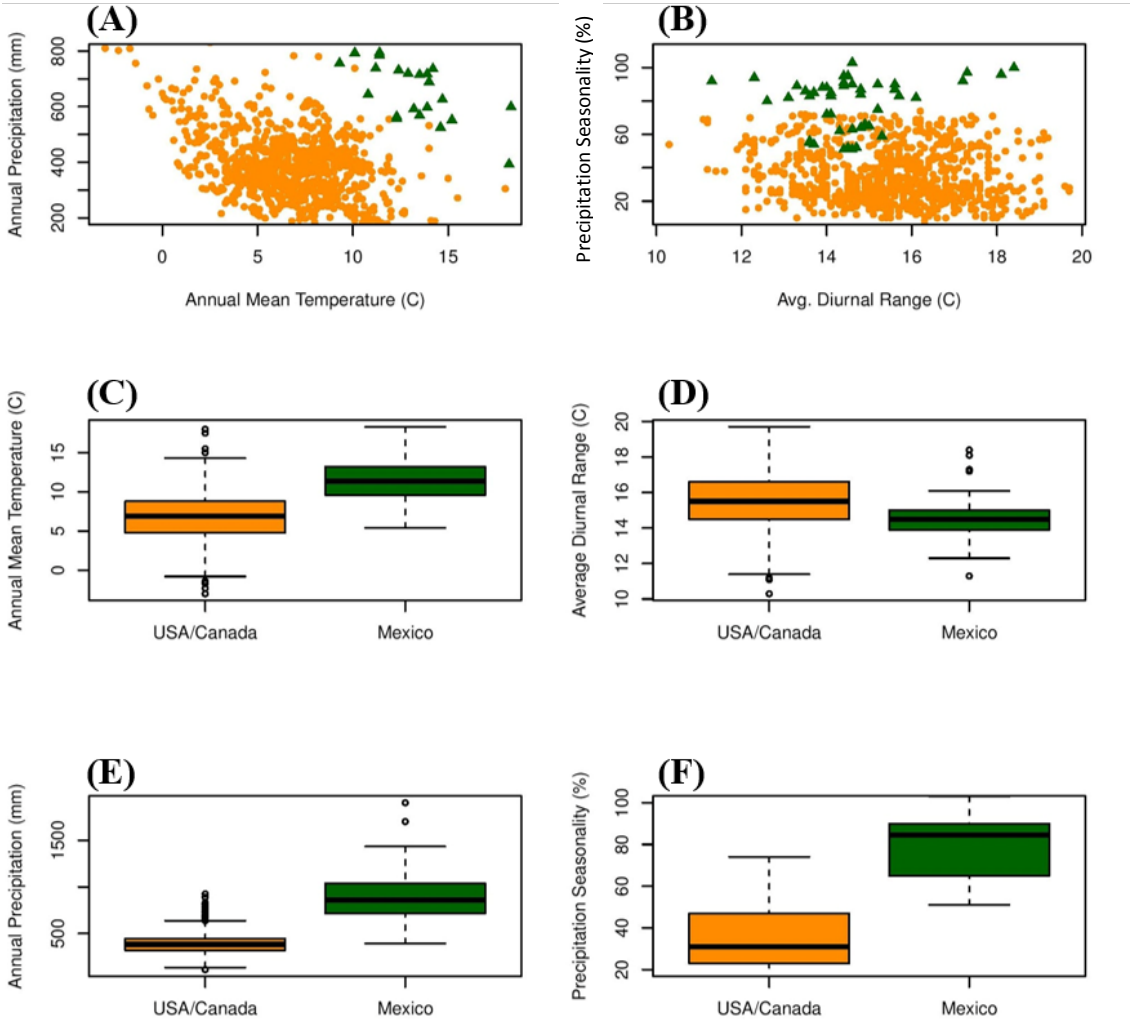

Figure 2-3. The range of select temperature and precipitation values associated with the distribution of USA/Canada (orange circles) and Mexico (green triangles) *B. huntii*. (A) Annual Mean Temperature (°C) with respect to Annual Precipitation (mm); (B) Average Diurnal Range (°C) (*i.e.*, average range of temperatures associated across a day) with respect to and Precipitation of Seasonality (%) (*i.e.*, the temporal distribution of rainfall across seasons), respectively. Comparisons in the distribution of these select variables were investigated with Boxplots and a simple t-test between USA/Canada and Mexico occurrence records: (C) Annual Mean Temperature (°C), (D) Average Diurnal Range (°C), (E) Annual Precipitation (mm), and (F) Precipitation Seasonality (%). High values of Precipitation seasonality suggest relatively high precipitation across all seasons (precipitation distributed across all seasons). Orange boxplots = USA/Canada, Green boxplots = Mexico.

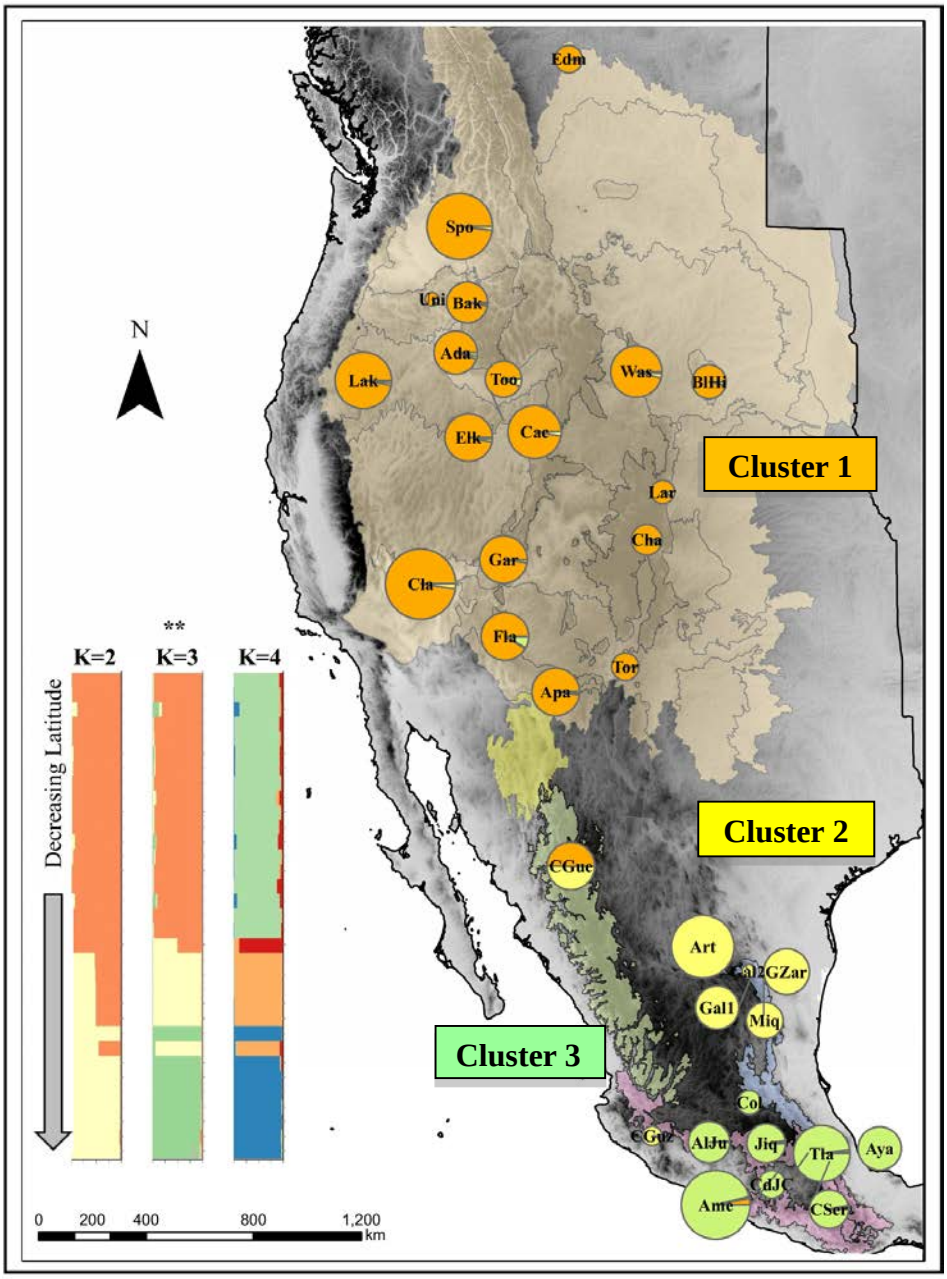

Figure 2-4. Sampling sites of the Hunt bumble bee, *Bombus huntii* (Hymenoptera: Apidae) in North America. The polygons in the background are reflective of Level III North American EPA ecoregions (Olson et al. 2001). Pie charts represent assignment probability of each site belonging to each of the *K* = 3. Pie chart size represents the abundance of individuals genotyped at each site. Intermountain West sites are shown in orange, Sierra Madre Occidental/Oriental are shown in yellow, and Trans-Mexican Volcanic Belt sites are shown in green. Inset bar graphs represent individual STRUCTURE assignment to each of the *K* = 2, 3, and 4, expressed in decreasing latitude (each bar = 1 individual). *Evanno-Method informed # of genetic clusters of *B. huntii* populations (Earl & vonHoldt 2012).

Population genetic structure and landscape genetics.

AMOVA results (Table 2-2) found that 11.67% of the genetic variation was partitioned among the three major genetic clusters (Intermountain West/Front Range, Sierra Madre, and Trans-Mexican Volcanic Belt), 6.55% among the sampling sites within the clusters, and 81.78% among individuals within sites. The overall F_{ST} among sites is 0.12 ($P < 0.001$) and F_{IS} is 0.07 ($P < 0.001$). When sampling site among clusters was pooled to perform the AMOVA, the partitioning patterns were similar (Table 2-2B), except for the estimate of F_{IS}, which was lower and not significant. The estimate of F_{ST} remained significant even when sites were pooled among clusters. An examination of inbreeding coefficients of each population revealed no significant inbreeding patterns, with the exception of one sampling site from Flagstaff, Arizona (USA), which had an F_{IS} that was significantly higher than other sampling sites (Table 2-3).

Examining isolation by distance and resistance patterns based on cluster assignment revealed dramatic differences in allelic fixation across samplings sites. In the combined Mexico cluster, genetic distance was significantly correlated with both geographical (Mantel test: $r = 0.82$, $P = 0.01$) and contemporary environmental resistance (Mantel test: $r = 0.71$, $P = 0.01$). However, in the Intermountain West/Colorado Front Range cluster, I found no significant difference between genetic distance and geographical distance (Mantel test: $r = 0.06$, $P = 0.13$), but a significant correlation with contemporary environmental resistance (Mantel test: $r = 0.91$, $P < 0.01$). However, while IBR was significant, sites within the Intermountain West/Colorado Front range cluster revealed overall low fixation patterns (all $F_{ST} < 0.12$) whereas sites distributed in Mexico

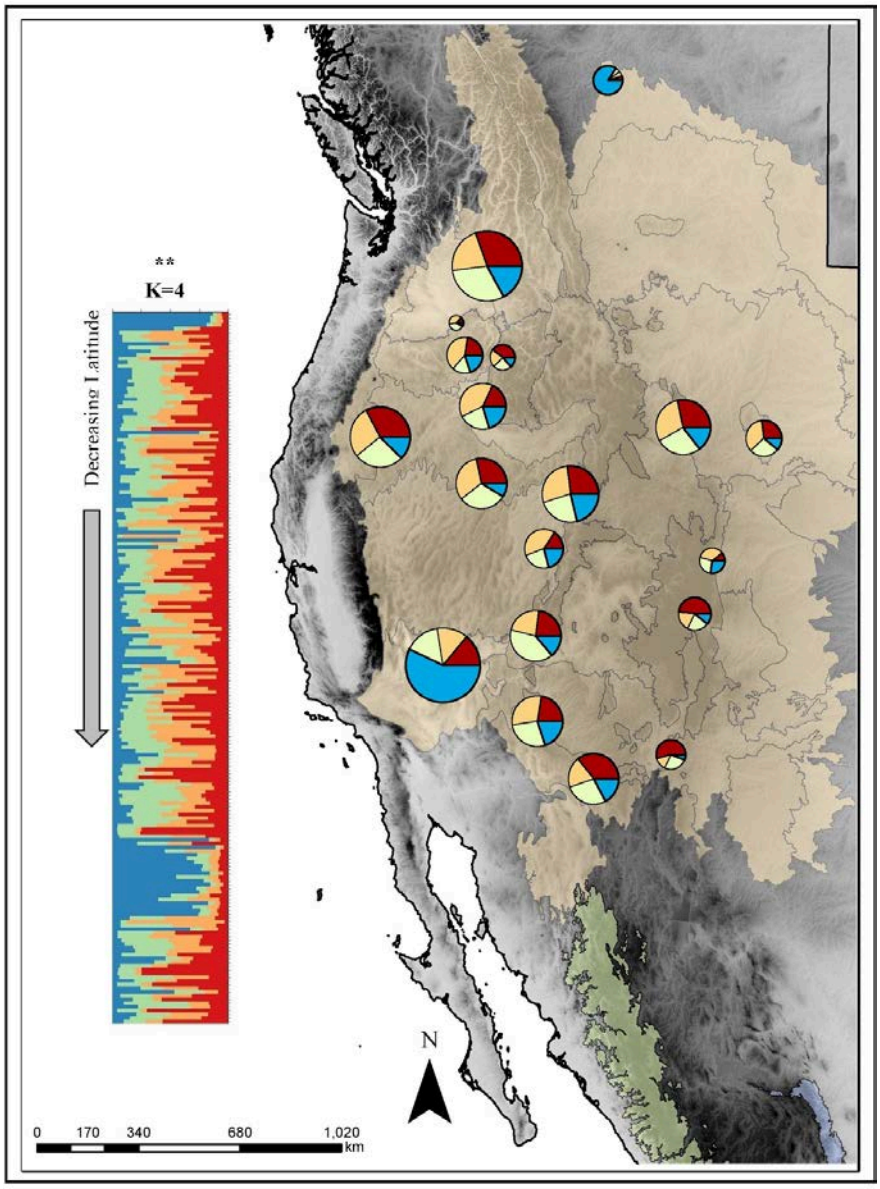

Figure 2-5. Sampling sites of the Hunt bumble bee (*Bombus huntii*) in the USA/Canada. The polygons in the background are reflective of Level III North American EPA ecoregions (Olson et al. 2001). Pie charts represent assignment probability of each site belonging to each of the $K = 4$ clusters identified by STRUCTURE based on microsatellite allele frequencies and values normalized with CLUMPP. Inset bar graphs represent individual STRUCTURE assignment to each of the $K = 4$, expressed in decreasing latitude represents the individuals genotyped at each site. Each bar in the plot represents one individual, with the proportion of color representing fractional assignment to a 1 of four populations. Pie chart size represents the abundance of individuals genotyped at each site.

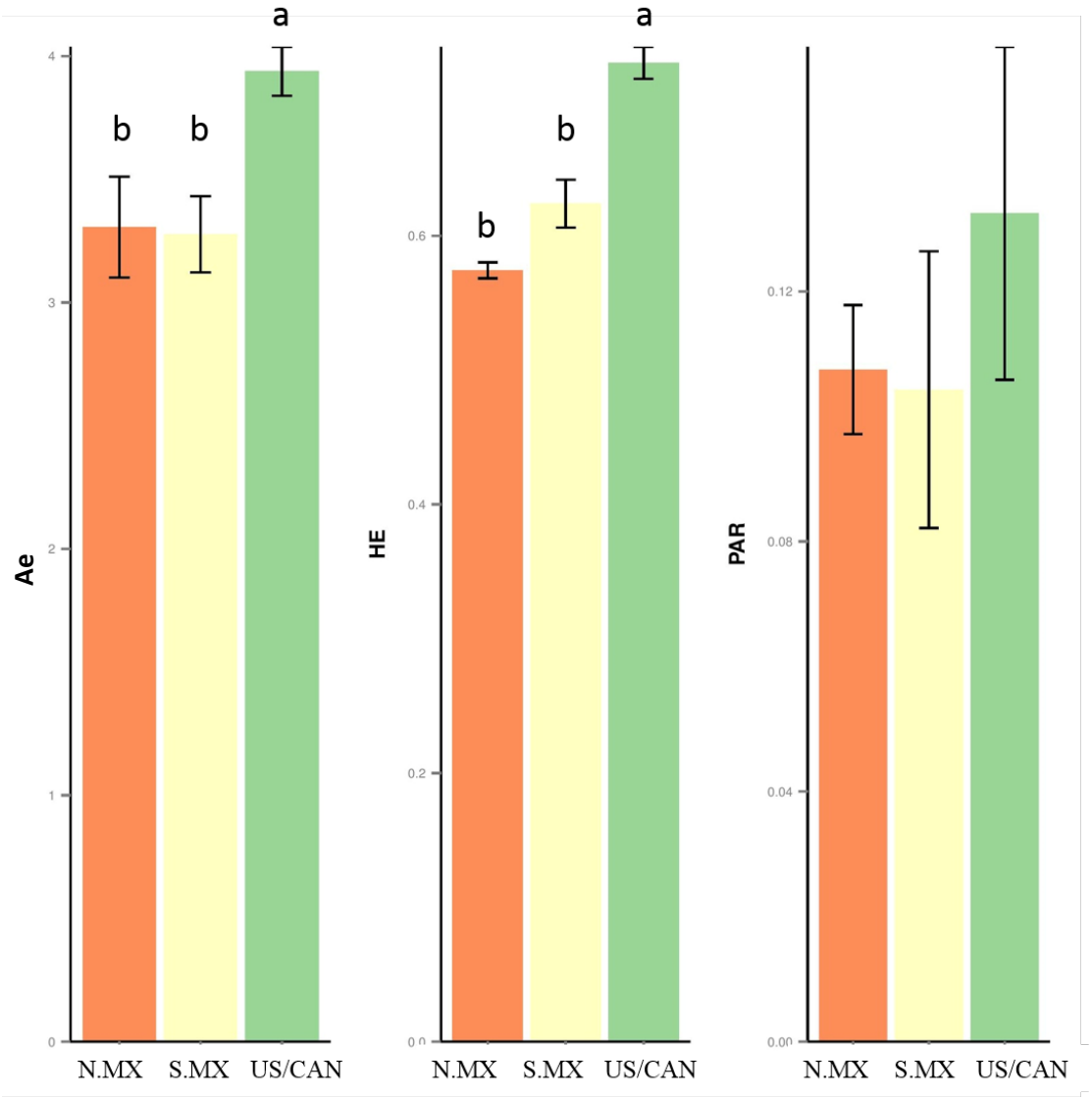

Figure 2-6. Average (± SE) (A) effective allelic diversity (*Ae*), (B) expected heterozygosity (H_e), and (C) Private Allelic Richness across USA/Canada (green), South Mexico (yellow), and North Mexico (orange). Indices with significant differences between groups are symbolized with lower case letters to reflect significant *P* values ($P < 0.05$) estimated with a Wilcoxon rank sum tests. I found no difference in PAR across all three regions. Genetic diversity was estimated with rarefaction techniques to account for variability in sampling across regions.

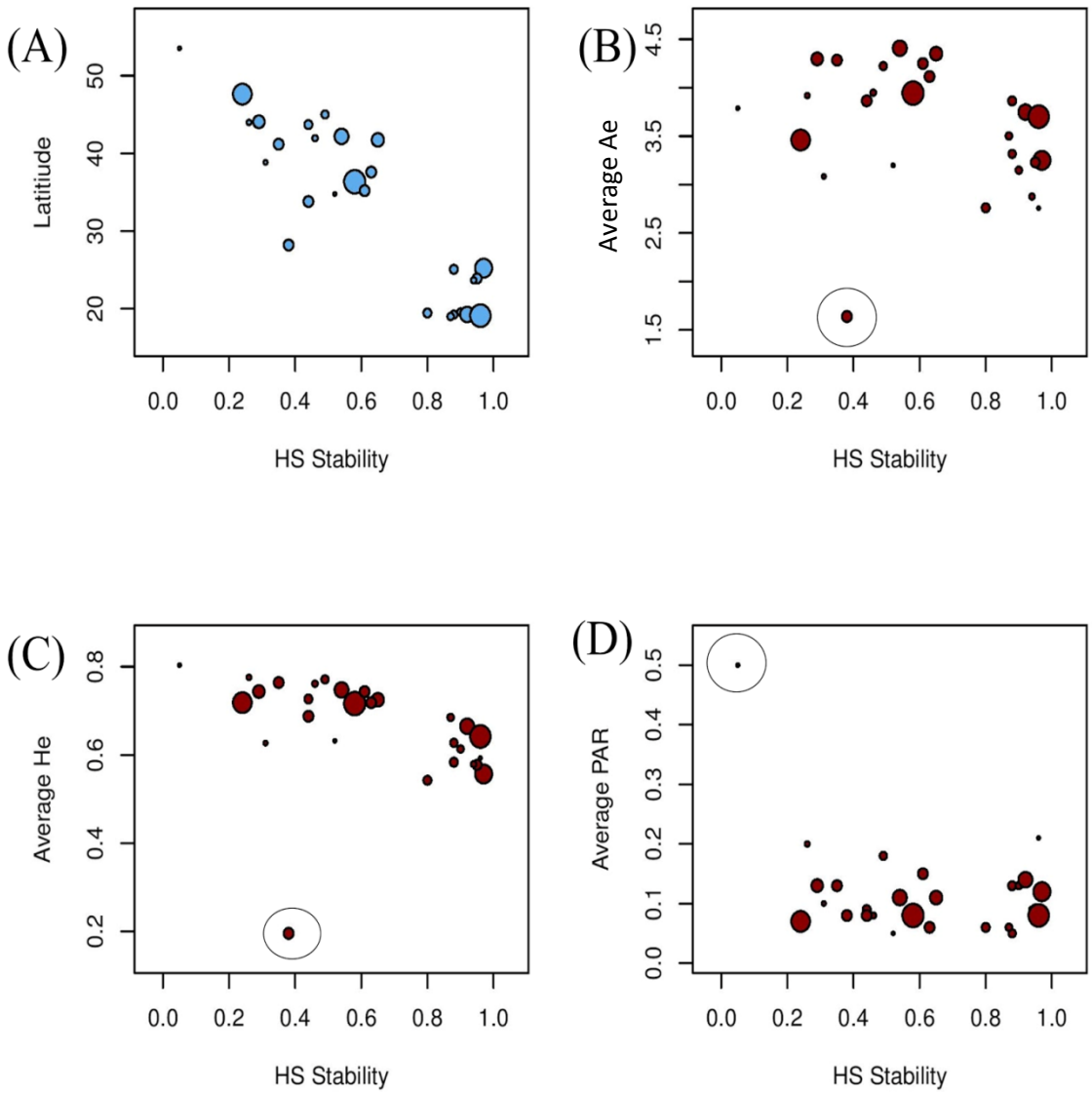

Figure 2-7. Population genetic diversity of *B. huntii* across a latitude gradient in North America relative to the geographic stability of Habitat Suitability (HS) over the past 22,000 years. HS Stability represents the sum of HS associated with each sampling site at Present (1950 -2000), and the Last Glacial Maximum (LGM). Stability values closer to 1 represent high HS stability, whereas values closer to 0 represent low HS stability (highly unstable). (A) Latitude vs. HS Stability, (B) Average Allelic Richness (*Ae*) vs. HS Stability, (C) Average Heterozygosity (H_e) vs. HS Stability, and (D) Private Allelic Richness (PAR) vs. HS Stability. Size of point represents the number of individuals collected at each sampling site (N = 28). Circled sites represent unique data outliers in each plot.

ranged from F_{ST} = 0 - 0.57 at relatively short geographical distances and high resistance (Fig. 2-8).

DISCUSSION

The major findings of my study are that (1) there are at least three unique genetic clusters that a deme can be assigned to throughout the range of *B. huntii*, Intermountain West/Colorado Front Range, Sierra Madre, and the Trans-Mexican Volcanic Belt, (2) population genetic diversity is highest in the Intermountain West/Colorado Front Range, relative to demes in the Sierra Madre and Trans-Mexican Volcanic Belt, (3) bioclimatic distinguishes between populations distributed in Mexico and the USA/Canada, and (4) contemporary population genetic diversity suggests that Mountain province in Mexico may have served as a refuge for *B. huntii* populations during the Pleistocene.

Quaternary climate variability and population genetic patterns

Pleistocene climate variability has governed many of the biodiversity patterns observed throughout North America. Specifically, the oscillation between cooling and warming periods have been found to drive the dynamics of species and communities distributed throughout montane environments of western North America in plants (Callahan et al. 2013) and mammals (Galbreath et al. 2009, 2010). One of the most striking examples is the population divergence and local adaptation in the American Pika, *Ochontona princeps* (Lagomorpha: Ochontonidae), an alpine specialist throughout much of the western USA (Galbreath et al. 2009; Galbreath et al. 2010). Studies have revealed extreme population isolation of American Pika to alpine areas during warming events, and recolonization to low-elevation and latitude habitats during cooling (*e.g.*, glaciation) events. Molecular and fossil data suggest that waves of introgressions occurred across

American Pika populations during Pleistocene cooling events, and then events of isolation took place during Pleistocene warming events (Galbreath et al. 2009; Galbreath et al. 2010). The need to remain adapted to a strict thermal niche is suggested to be the mechanism that has driven population divergence and local adaptation in Pika populations throughout North America. In yet another montane example, quaking aspen has been found to colonize southern latitudes during cooling periods, and may have become isolated during extended periods of time in warming periods during the Pleistocene (Callahan et al. 2013).

My population genetic study of *B. huntii* in western North America found populations distributed in the northern extent of its distribution to be associated with high levels of allelic diversity, while populations at low latitudes exhibited reduced levels of allelic diversity (Fig. 2-7). Throughout its Intermountain West/Colorado Front Range distribution, *B. huntii* exhibits a high degree of gene flow, as suggested by the lack of isolation by distance. Populations distributed throughout the Intermountain West and Colorado Front Range experience much colder annual temperatures, a pattern that is certainly correlated to the interplay with latitude and elevation (Fig. 2-3C). Furthermore an examination of the elevation profile of *B. huntii* HS in the northern part of its range suggests that high HS across a broad elevation gradient (Fig. 2-2). However, Mexico populations appear to be limited to high elevation habitats throughout the Sierra Madre and Trans-Mexican Volcanic Belt (Figure 2-2). The broad bioclimatic profile and geographic distribution of *B. huntii* in northern populations likely facilitate range-wide gene flow that I was able to detect with microsatellite loci.

Table 2-2. (A) Results of Analysis of Molecular Variance (AMOVA) for *B. huntii* (n = 330), based on microsatellite allele frequencies across populations in western North America (not Cluster based). (B) Results of Analysis of Molecular Variance (AMOVA) for *B. huntii* (n = 330), based on microsatellite allele frequencies for USA/Canada, North Mexico, and South Mexico clusters identified with STRUCTURE.

A.

Source of Variation	***df***	**Sum of Squares**	**Variance Components**	**% variation**
Among Populations	2	181.015	0.45284	11.67
Among individuals within populations	327	1203.72	0.25419	6.55
Within Individuals	330	1047	3.17273	81.78
Total	659	2431.74	3.87975	

F_{IS} = 0.07414; F_{ST} = 0.11672; F_{IT} = 0.18223; (all $P < 0.001$)

B.

Source of Variation	***df***	**Sum of Squares**	**Variance Components**	**% variation**
Among three clusters	2	181.015	0.41859	10.8
Among sites within clusters	25	217.299	0.23737	6.12
Within sites	302	986.424	0.04679	1.21
Within Individuals	330	1047	3.17273	81.87
Total	659	2431.738	3.87975	

F_{SC} = 0.6867, F_{CT} = 0.10801, F_{IT} = 0.18133; (all $P < 0.001$)

F_{IS} = 0.01453 ($P < 0.098$); **NS**

Table 2-3. Inbreeding coefficient estimates (F_{is}) across clusters and demes (sites) of *B. huntii*. F_{is} values range from -1 (low inbreeding) to 1 (high inbreeding). Bold type indicate site/cluster with significantly high F_{is} values relative to other sites/clusters

Cluster	Site	Fis	P	Cluster Fis	Cluster P
USA/Canada	Ada	0.09	0.06	0.06	0.00
	Apache	0.07	0.11		
	Baker	0.07	0.14		
	Black Hills	0.07	0.14		
	Box Elder	0.09	0.09		
	Cache	-0.03	0.79		
	Chaffee	-0.13	0.84		
	Clark	0.04	0.15		
	Edmonton	0.15	0.09		
	Elko	0.00	0.57		
	Flagstaff	**0.19**	**0.00**		
	Garfield	0.00	0.50		
	Lake	0.03	0.20		
	Spokane	-0.02	0.76		
	Torrance	-0.07	0.72		
	Washakie	0.05	0.13		
South Mexico	Almoloya de Juarez	-0.07	0.79	0.05	0.03
	Amecameca	-0.02	0.74		
	Ayahualulco	-0.15	0.95		
	Ciudad Serdan	-0.07	0.85		
	Contla de Juan Cuamatzi	-0.11	0.93		
	Ixtapaluca	0.05	0.19		
	Jiquipilco	0.09	0.19		
North Mexico	Arteaga	0.02	0.38	**0.16**	**0.00**
	Ciudad Guerrero	0.15	0.15		
	Galeana	-0.10	0.96		
	General Zaragoza	-0.07	0.90		
	Miquihuana	-0.07	0.81		

The lack of fixation and high genetic diversity suggests that *B. huntii* had very little barriers to gene flow across the the Intermountain West/Colorado Front Range since at least since the LGM.

The lack of allelic fixation and genetic structure north of Mexico is not unique to *B. huntii*. Several broadly distributed bumble bee species are found to experience a degree of admixture and low allelic fixation at distances greater than >500 km (Lozier et al. 2011). The ability of bumble bees to disperse and forage on a broad array of pollen sources at great distance is likely a strong factor in facilitating gene flow across populations (Jha 2015; Goulson et al. 2001; Knight et al. 2005; Moreira et al. 2015). Bumble bees are dependent on suitable forage and nesting resources. Bumble bee dispersal as a mechanism in facilitating gene flow is limited to the reproductive caste, namely queens and males. Workers themselves do not disperse and breed, as they have reduced ovaries, and do not have the ability to be a nest foundress. However, while some North American bumble bees exhibit little allelic fixation or low admixture, other, mostly montane species have been found to exhibit isolation by distance and resistance patterns (Hines & Williams 2012; Lozier et al. 2013; Jha & Kremen 2013). For example, *B. bifarius* is primarily associated with montane environments throughout its known distribution. However, populations of *B. bifarius* are known to occur at low-elevation and offshore islands throughout the Pacific Northwest and Central Coast of California (Koch et al. 2012; Williams et al. 2014). These populations have reduced population genetic diversity and exhibit a degree of phenotypic divergence relative to populations distributed across the Colorado Rocky Mountains, the Sierra Nevada, Cascade, Sawtooth, Bighorn, and Uinta Mountains of western North America (Lozier et al. 2013; Lozier et al. 2011).

The variability in HS across these montane provinces has been a major barrier to gene flow, revealing dramatic IBR patterns across *B. bifarius* populations.

The correlation between HS stability and latitude, along with increased population genetic diversity with increasing latitude suggests that *B. huntii* was able to maintain a degree of gene flow across populations during climatic oscillations, at least during the late Pleistocene. The ability of bumble bees to disperse at great distances and tolerate cool temperature supports this hypothesis. However, due to the high mutation rate of microsatellites (Estoup et al. 1996; Estoup et al. 1995), I may not have been able to capture the effect of early Pleistocene climate oscillations on contemporary population genetic diversity and structure. As such, additional, 'more conserved' loci may be able to capture these historic patterns.

Hypothesis of last exchange and dispersal into montane Mexico

HS models constructed from contemporary occurrence records and bioclimatic variables reveal low HS across the Madrean Archipelago, a group of mountains that occur in southern Arizona (Fig. 2-1, Fig. 2-2). This particular group of mountains is a major biogeographic feature that separates the high-elevation San Francisco Peaks near Flagstaff, Arizona from the northern extent of the Sierra Madre Occidental. While little collecting of *B. huntii* has occurred south of Flagstaff (Fig. 2-1), HS models support that the Madrean Archipelago is likely not especially suitable for *B. huntii* (Fig. 2-2A). However, this region may have been suitable during the Pleistocene, and provided a pathway for dispersal and between Mexico and northern populations (Fig. 2-2B, 2-2C). To test this hypothesis I constructed HS models under the principles of niche conservatism (Peterson et al. 1999) and projected contemporary HS limitations to

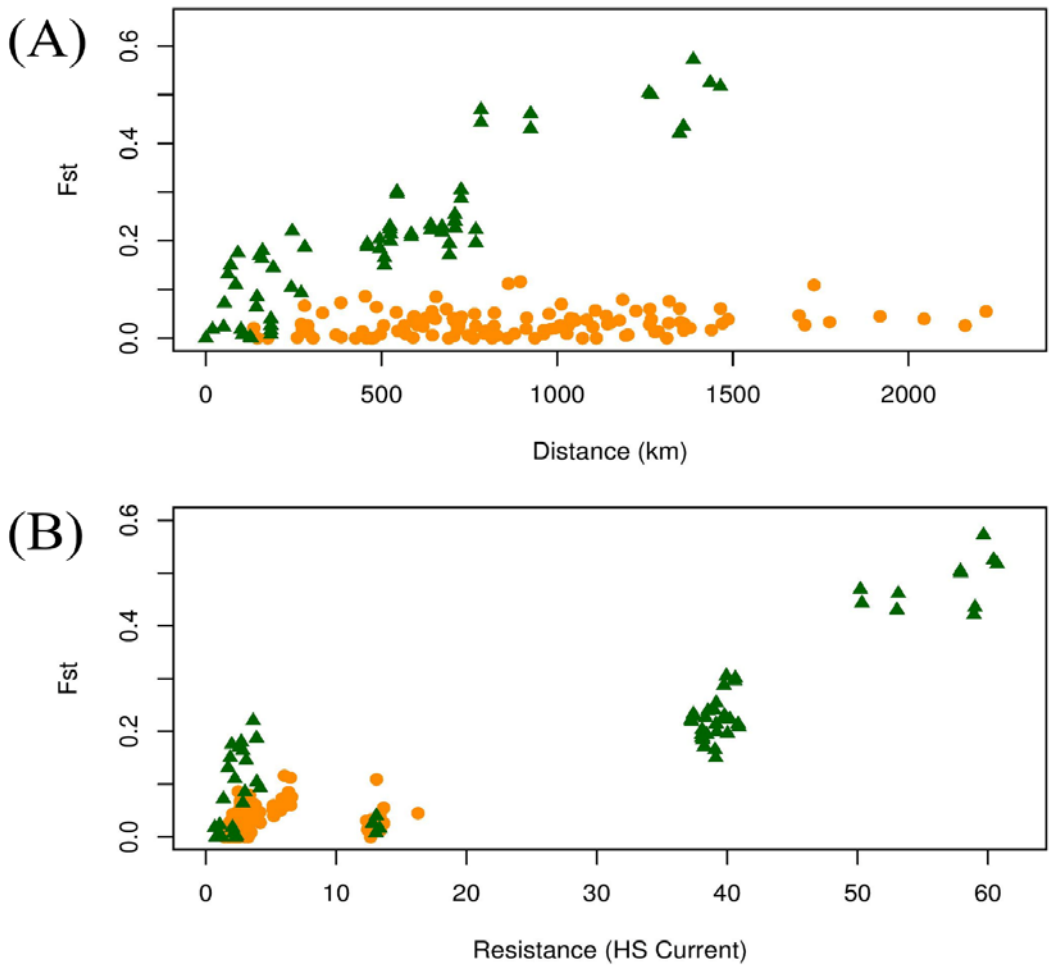

Figure 2-8. Isolation by Geographic Distance and Habitat Suitability (HS)-based Climate Resistance. Pairwise comparisons of genetic differentiation (F_{st}) as a function of (A) Geographic Distance, and (B) Contemporary estimate of *B. huntii* HS. Green triangles = Mexican populations; Orange circles = US/Canada populations.

Pleistocene climate variability (Fig. 2-2). My analyses revealed that the LGM was likely the last time *B. huntii* populations were able to disperse across the Madrean Archipelago (Figure 2-2B). Furthermore, my HS model based on LGM bioclimatic limitations revealed a degree of high HS across an elevation gradient in the lowlands of the Sierra Madre and Trans-Mexican Volcanic Belt (Fig. 2-2B). Given the high HS estimates of

low-elevation areas in Mexico during the LGM, there likely was a degree of gene flow occurring across these different mountain provinces in Mexico.

As the climate began to warm since the LGM, *B. huntii* populations likely lost a dispersal pathway in the Madrean Archipelago. Based on both contemporary HS models and occurrence records, the north edge of the Madrean Archipelago likely provides low HS for *B. huntii* populations (Fig. 2-1, Fig. 2-2). South of the Madrean Archipelago there is a more arid environment not suitable for *B. huntii*. Today, *B. huntii* populations that inhabit the Sierra Madre Oriental and Occidental, as well as the Trans-Mexican Volcanic Belt, are limited to high-elevation environments (Fig. 2-1, Fig. 2-2). Microsatellite data revealed that the *B. huntii* demes that inhabit the Sierra Madre Oriental have significantly lower population genetic diversity than the demes distributed south in the Trans-Mexican Volcanic Belt (Fig. 2-6A, 2-6B). Contemporary HS models of the Sierra Madre Oriental have less HS than the Trans-Mexican Volcanic Belt in terms of geographic space (Fig. 2-2A). The limited spatial extent of HS in the Sierra Madre Oriental deme may be a strong limiting factor of contemporary population genetic diversity. Despite low HS suitability and genetic diversity patterns in demes inhabiting the Sierra Madre Oriental and Occidental, my individual-based population assignment tests revealed the potential for this deme to be in admixture between demes located in the Intermountain West/Front Range and the deme located in the Trans-Mexican Volcanic Belt (Fig. 2-4, $K = 2$). In addition to strong evidence that *B. huntii* populations are limited to a narrow bioclimatic niche, (Fig. 2-2B, 2-2C), my data suggests that populations inhabiting the Sierra Madre Oriental and Occidental are essentially split between northern and southern populations.

Examining genetic diversity, structure, and historic HS supports my hypothesis for Pleistocene divergence of Mexican *B. huntii* populations from US/Canada populations.

Conserving evolutionary significant units of *B. huntii*

There is evidence that genetic drift associated with functional traits may have occurred since Mexican populations became isolated from northern populations. Phenotypically, *B. huntii* populations in Mexico reveal greater admixture of black setae on the anterior scutum in comparison to populations north of Mexico, which primarily exhibit an abundance of yellow setae on the thorax. Historically, diversity in bumble bee phenotypes has led to debates on what constitutes a species (Williams et al. 2014; Thorp et al. 1983; Cameron et al. 2007; Cameron & Williams 2003). As bumble bees can be difficult to rear in the laboratory, the ability to study effective mating behavior and within debated species-groups can be difficult to test with putative taxa. However, contemporary investigations with molecular data and laboratory rearing have revealed that color variability exists within species, and has the potential to be associated with regional patterns of genetic diversity and gene flow (Lozier et al. 2013; Duennes et al. 2012; Hines & Williams 2012; Owen et al. 2010; Owen et al. 1980). For example, historically, *B. melanopygus* was distinguished as two different species: *B. edwardsii* and *B. melanopygus* (Thorp et al. 1983). However, by rearing colonies it was revealed that there is potential for both color forms to be produced in a single nest (Owen et al. 2010; Owen et al. 1980). In the polymorphic *B. bifarius*, population genetic structure appears to predict differences in phenotype with a red morph primarily distributed in the Intermountain West, a black morph throughout the Sierra-Cascade Crest and Olympic

Mountains, and the emergence of a second red morph on Vancouver Island and San Juan Archipelago (Lozier et al. 2013; Koch et al. 2012).

Preliminary analyses of 16S ribosomal RNA and Cytochrome c oxidase I suggest that *B. huntii* populations in Mexico are conspecific with populations throughout the Intermountain West/Front Range (Koch unpublished data). However, given the genetic structure and diversity of Mexican populations and their unique bioclimatic niche, I suggest that habitat conservation prioritize the limited areas of HS to conserve the Mexican *B. huntii* populations which may be at risk. Globally, bumble bees have been found to be in decline at broad and narrow spatial scales (Cameron et al. 2011; Goulson et al. 2008). In the USA, Canada, and the UK, there is substantial evidence that pathogens with broad host ranges, land-use intensification, and contemporary climate change are facilitating rapid local extirpation in several bumble bee species (Goulson et al. 2008; Cameron et al. 2011; Grixti et al. 2009). As Mexican populations of *B. huntii* have a narrow HS niche (Figure 2-3, Figure 2- 4), priority should be placed by conservation organizations on documenting the needs of populations distributed throughout the Sierra Madre Oriental and Occidental, and Trans-Mexican Volcanic Belt (Cameron et al. 2010).

Since the collapse of commercial *B. occidentalis* populations due to pathogen outbreaks in a rearing facility in the 1990s, western North America has been without an endemic bumble bee pollinator. Given recent federal and state restrictions on the importation of bumble bees from foreign and domestic distributors, there remains a need to commercialize a new bumble bee endemic to western North America. The Hunt bumble bee, *B. huntii*, has been recognized to fulfill the commercial agriculture niche left vacant by *B. occidentalis*. Its broad geographic distribution, large colony sizes, low

pathogen susceptibility in the US, and ability to effectively pollinate hot house crops makes it an ideal pollinator for domestication. However, based on my results, I suggest a degree of caution be made prior to the distribution of *B. huntii*. Since bumble bees have the capacity to distribute diseases across the globe (Sachman-Ruiz et al. 2015; Goka et al. 2000; Goka et al. 2013), attention should be paid to the effect of diseases like *Nosema bombi* (Microsporidia: Nosematidae) and *Crithidia bombi* (Kinetoplastea: Trypanosomatidae) on endemic *B. huntii* populations (Goulson & Hughes 2015; Cordes et al. 2012; Cameron et al. 2011). Properly documenting the evolutionary history of *B. huntii* and the capacity for the species to transmit diseases across conspecific and heterospecific bumble bees will ensure stable commercial populations for the future.

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CHAPTER 3

MULTIGENE PHYLOGENY AND MICROSATELLITE GENOTYPES ILLUMINATES AN ENIGMATIC BUMBLE BEE SPECIES COMPLEX[3]

Abstract

Effective biodiversity conservation is hampered by a lack of consensus on what constitutes a distinct species. Disagreement on species delimitation may stem from cryptic coloration patterns and geographic convergence of phenotypes across phylogenetically distinct clades. The *B. fervidus* species-group, which includes *B. fervidus* and *B. californicus*, has been the center of debate, because multiple field guides and taxonomic keys disagree on the legitimacy of *B. californicus* as a species. In this study I used three fragments of the mitochondrial genome (Cytochrome c oxidase subunit I, 12s rRNA, and 16s rRNA) to infer a phylogeny using Bayesian analyses for 64 specimens from the *B. fervidus* species-group that are widely distributed throughout the contiguous U.S.A. and exhibit a wide range of setal color phenotypes. I also genotyped 382 specimens at 13 microsatellite loci to estimate species assignment using an admixture model with a Bayesian clustering program. In addition to assessing the genetic lineages and species status of the *B. fervidus* species complex, I examined wing morphometric characters and bioclimatic niches to test for differences associated with species-assigned genotypes. My genetic, morphometric, and ecological data suggest that *B. californicus* and *B. fervidus* are legitimate species, supporting the results of contemporary systematic investigations of the genus *Bombus*. Furthermore, I make the unexpected discoveries that

[3] This chapter is co-authored with Juanita Rodriguez, James Pitts, and James Strange. Permission has been granted by the required coauthors for this research to be included in my dissertation (Appendix A). This chapter is formatted for *Molecular Phylogenetics and Evolution.*

the dark phenotype typically associated with *B. californicus* is also found within the *B. fervidus* clade, and that the yellow phenotype typically associated with *B. fervidus* is found within the *B. californicus* clade. The recovered lineages and associated phenotypes correspond to the geographic and environmental distribution of *B. californicus* and *B. fervidus*, supporting historic observations that both species inhabit relatively different bioclimatic niches. Inferring relationships with a diverse suite of techniques is a useful pathway to evaluate biodiversity in the context of their evolutionary history and ecological niche.

Introduction

Biodiversity is rapidly declining on a global scale primarily due to resource extraction activities associated with human growth and expansion. It is estimated that the contemporary extinction rate is 1,000 times higher than what has been experienced prior to the global effects of humanity's economic and colonial activities (De Vos et al., 2015). A major impediment to the effective conservation of biodiversity includes the lack of consensus among scientists and conservation practitioners on the taxonomic resolution appropriate to a conservation or management goal. Without an operational unit that takes into account the ecology and evolutionary history of a species, efforts to promote species conservation will remain daunting (Crandall et al., 2000). Although insects dominate the biosphere in species diversity and abundance, they pose some of the greatest challenges to conservation biologists, as they are not well studied compared to vertebrates (Gaston, 1991). Considering the diverse array of ecosystems services provided by insects (Losey and Vaughan, 2006), it remains of great importance to understand the evolutionary history and ecological requirements of the planet's most species rich Order (Gaston,

1991). However, it is also critical that investigations and prioritization of species conservation continue to be dependent on holistic investigations rather than single methods of species delimitation and categorization (Cameron et al., 2010; Crandall et al., 2000; Erwin, 1991). Combining data associated with a species natural history, ecology, and evolutionary history will provide a robust, science-based conservation framework that uses a species' biology when designing management and mitigation strategies.

Bumble bees (Hymenoptera: Apidae, *Bombus*) are one of the most important native pollinators of North America, contributing to the ecosystem services required by wild and economically important flowering species (Kremen et al., 2002; Velthuis and Van Doorn, 2006). They are dominant in the northern hemisphere, and are specifically abundant in alpine and temperate ecosystems (Hines, 2008; Williams, 2007). Their abundant setae, large body sizes, and nesting biology enable them to forage and persist from early spring to late fall, thereby enabling them to pollinate a diversity of wild flowering plants (Goulson, 2003; Heinrich, 2004). In addition to the service they deliver to wild land plants, bumble bees have been commercialized to supplement and enhance the pollination of agricultural crops, namely tomatoes, berries, and pumpkins (Velthuis and Van Doorn, 2006). Unlike the perennial colonies of the European honey bee (*Apis mellifera*), bumble bee colonies are annual, where males and female worker offspring expire at the end of the colony life cycle. Mated gynes (queens) go into a state of torpor in hibernacula and then re-emerge in the early spring to be the sole foundresses of new colonies (Heinrich, 2004). Bumble bees have been revealed to be far superior to honey bees as pollinators of some commercial crops, specifically those cultivated in glass houses (Strange, 2015; Velthuis and Van Doorn, 2006). Furthermore, wild bumble bee

populations have also been found to enhance crop productivity through effective pollination (Jha and Kremen, 2013; Kleijn et al., 2015; Kremen et al., 2002). However, the global decline of both wild and managed bumble bee populations due to disease, pesticides, urbanization, and agricultural intensification have prompted state, national, and international efforts to document the diversity and distribution of these iconic bee fauna (Cameron et al., 2011; Sydney Cameron et al., 2010; Williams and Osborne, 2009).

Concurrent efforts to conserve bumble bees are dependent on recognizing operational units, whether they are species, taxonomic, evolutionary, or otherwise (Cameron et al., 2010; Crandall et al., 2000; Moritz, 1994). These units have been useful in unveiling local biotic and abiotic factors that are specific to unique evolutionary lineages of cryptic species (Agapow et al., 2004; Crandall et al., 2000). Effective bumble bee conservation will require an understanding of their ecology, distribution, and evolutionary history (Cameron et al., 2010; Crandall et al., 2000). Due to spatial convergence of aposematic setal coloration patterns, bumble bees have proven to be notoriously difficult to identify to species by both novice and seasoned taxonomist (Duennes et al., 2012; Hines and Williams, 2012; Koch et al., 2012; Stephen, 1957; Thorp et al., 1983; Williams et al., 2014). The dependence on setal coloration patterns to delineate between closely related taxa has caused debate on the species status of many of these taxa (Stephen, 1957; Thorp et al., 1983; Williams et al., 2014). Contemporary phylogenetic investigations using both single and multiple genetic loci, as well as taxonomic studies, have resolved some cryptic species complexes throughout multiple bumble bee subgenera (Cameron and Williams, 2003; Duennes et al., 2012; Hines and Williams, 2012). Specifically, it has been demonstrated with a single gene, Cytochrome *c*

oxidase I (COI), that bumble bees converging on identical aposematic coloration patterns have been found to be separate species (Carolan et al., 2012; Murray et al., 2008; Scriven et al., 2015; Williams et al., 2012). However, a lack of COI variation between species has also been detected, leading some authors to synonymize two historically recognized species (Hines, 2008; Williams et al., 2014).

In this study I examine the evolutionary history and ecology of the *Bombus fervidus* species-group, which contains two species: the nominal sister taxon *B. fervidus* (Fabricius, 1798) and *B. californicus* Smith, 1954. These species belong to the globally distributed subgenus *Thoracobombus* (Cameron et al., 2007; Cameron and Williams, 2003). Studies on the foraging ecology of bumble bees have found species in *Thoracobombus* to be dominant foragers and pollinators on plants with flowers with long corollas (Inouye, 1980; Williams et al., 2009). The decline of flowering plants with long corollas due to urbanization and agricultural intensification has been implicated in the decline of European *Thoracobombus* (Goulson et al., 2008, 2005). Additionally, *B. californicus* and *B. fervidus* have been found to be declining in abundance in both wild and urban environments of North America, relative to historic population abundance estimates (Colla and Packer, 2008; Grixti et al., 2009; McFrederick and LeBuhn, 2006). Another alarming trend that has been recently observed at a national scale in the United States includes increased disease detection in wild populations of *B. fervidus* and *B. pensylvanicus* (Cameron et al., 2011; Gillespie, 2010).

Bombus californicus and *B. fervidus* have been recognized to be legitimate species, based on historic and contemporary investigations with taxonomic and comprehensive phylogenetic tools (Cameron et al., 2007; Cameron and Williams, 2003;

Stephen, 1957; Thorp et al., 1983). However, the lack of strong divergence in COI and convergence of setal coloration patterns between *B. californicus* and *B. fervidus* have been suggested as evidence that they are conspecific (Milliron, 1973; Williams et al., 2014). *Bombus californicus* is distributed from the Pacific Coast of North America, east to the Black Hills of South Dakota (Koch et al., 2012; Stephen, 1957; Thorp et al., 1983). Unlike *B. californicus*, which is distributed across a broad latitudinal gradient relative to the longitudinal range, *B. fervidus* has a transcontinental distribution, from the Pacific Coast to the northeastern United States (Koch et al., 2012; Mitchell, 1962; Thorp et al., 1983; Williams et al., 2014). While both species are sympatric in portions of their range in western North America, Hobbs (1966) suggested that *B. californicus* and *B. fervidus* differ in nesting habitats, with *B. californicus* nesting in wooded areas and the foothills of southern Alberta, and *B. fervidus* primarily found to be in the prairies (Thorp et al., 1983).

Multiple taxonomic investigations of the two bumble bee species have agreed on one central idea: they are nearly impossible to separate morphologically (Cameron and Williams, 2003; Franklin, 1913; Stephen, 1957; Thorp et al., 1983; Williams et al., 2014). In regards to distinguishing between the *B. californicus consanguineus* and *B. fervidus*, W.P. Stephen stated in *Bumble Bees of Western North America*, “There are no morphological features in either species by which they can be distinguished, and separation is made exclusively on color pattern” (Stephen, 1957). In regards to distinguishing *B. californicus* (in the strict sense) from *B. fervidus*, he went on to write, “The species is close morphologically to *B. fervidus* (Fabr.) and is impossible to separate structurally from that species.” (Stephen, 1957). Finally, W.P. Stephen quoted Franklin

(1913), "*californicus* and *fervidus* may eventually prove to be subspecies of a single species". Twenty-six years later, R. Thorp led the writing of *Bumble Bees and Cuckoo Bumble Bees of California (Hymenoptera: Apidae)*, and expressed a similar sentiment for the lack of variability (outside of setal color) between *B. californicus* and *B. fervidus* (Thorp et al., 1983). However, he stated that there were distinct ecological differences between *B. californicus* and *B. fervidus* when sympatric, showing no signs of intergradation. At present, there is no biological evidence that *B. californicus* and *B. fervidus* have the capacity to breed in the wild, despite historic reports that initially proposed this hypothesis (Milliron, 1973). Cameron et al. (2007) in a global systematic survey of bumble bees that inferred a phylogeny based on 5 genetic loci, found that *B. californicus* and *B. fervidus* formed two distinct clades. However, given that their sampling consisted of a single exemplar of each species, Williams et al. (2014) challenged that the lack of morphological differences and COI divergence between the two species is evidence that *B. californicus* and *B. fervidus* are conspecific.

My goal in this study is to test the hypothesis that *B. fervidus* and *B. californicus* are conspecific. I use bioclimatic data, wing morphometric analyses, and data from neutral and adaptive genetic loci to examine their species boundaries. I first infer a phylogeny with three mitochondrial loci: COI, 12s RNA, and 16s RNA, with specimens distributed across a broad geographic range and exhibiting diverse setae phenotypes (Fig. 3-1). Next, I expand my genetic sampling effort of specimens and genotype populations using neutral microsatellite loci to examine potential hybridization and species assignment. I predict that neutral microsatellite loci will identify introgression between *B. californicus* and *B. fervidus* when they are sympatric. Finally, I measure wing characters

and estimate bioclimatic niches to determine if they can discriminate between the putative species based on the results of the genotypes and recovered lineages proposed by the Bayesian phylogeny.

MATERIALS AND METHODS

Taxon sampling and examination

Williams et al. (2014), who synonymized *B. californicus* with *B. fervidus* based on morphology-based taxonomy and the species barcoding gene (*i.e.*, COI), identified several different phenotypes associated with *B. fervidus* queens and workers. In this study I included a total of 320 specimens associated with the *B. fervidus* species complex, including the contested *B. californicus*. I made an effort include a diversity of phenotypes associated with the *B. californicus* and *B. fervidus* species complex (Fig. 3-1). Exemplars of *B. weisi* (*Thoracobombus*) and *B. insularis* (*Psithyrus*) were selected as outgroup taxa based on recent *Bombus* phylogenies (Cameron et al. 2007; Cameron & Williams 2003). In-group taxa, exclusive to females were sampled throughout a major portion of their range in North America. I recorded setal color pattern data and locality information associated with queen and worker caste. I categorized specimens into four broad phenotype groups (Fig. 3-1). Assignment of setal color patterns to specimens follow the schematic diagram presented in Williams et al. (2014) and Koch et al. (2012). In addition to phenotypes, I assigned specimens to putative species following Thorp et al. (1983), Koch et al. (2012), and Stephen (1957). Collection data associated with specimens used for this study have been digitized and deposited in the United States National Pollinating Insect Collection at Utah State University in Logan, Utah, U.S.A.

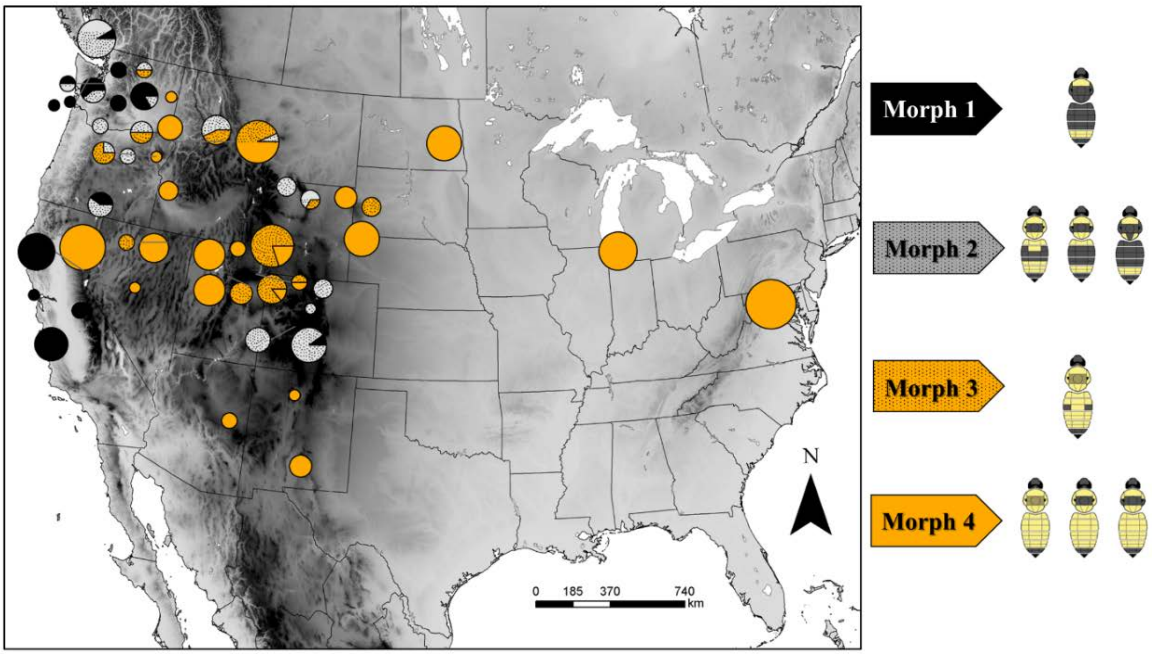

Fig. 3-1 Distribution of the major phenotypes associated with *B. fervidus* and the putative *B. californicus* in the United States. The size of each circle represents the number of specimens associated with each locality. The color of each pie slice represents the proportion of specimens exhibiting one of four phenotype classes. Phenotype (*i.e.*, morph) diagrams modified from Williams et al. (2014).

DNA extraction, amplification, and gene sequencing

I extracted genomic DNA from the mid-leg of specimens using a modified Chelex 100™ protocol following Strange et al. (2009). DNA extracted in this manner was primarily used for microsatellite genotyping (*i.e.*, Fragment Analysis), and was not especially successful when used in PCR aimed at amplifying sequences >500 base pairs. As such, I also extracted genomic DNA using the Roche High Pure Template Preparation Kit (Roche Diagnostics GmbH, Germany) to obtain high quality genomic DNA extractions suitable for downstream DNA sequencing.

In 64 specimens, I amplified three mitochondrial gene fragments: 489 aligned nucleotides of 16S rRNA, 369 aligned nucleotides of 12S rRNA, and 900 nucleotides of COI. PCR conditions and primers followed the recommendations of the published

literature (Bertsch et al., 2005; Cameron et al., 1992; Cameron and Williams, 2003; Tanaka et al., 2001). Briefly, PCR was carried out in 25 µL reaction volume, containing approximately 3 µL of extracted DNA, 1x Promega (Madison, WI) reaction buffer, 0.6 mM dNTP mixture, 10 µM primer, 5 units Taq polymerase (Promega, Madison, WI) and the $MgCl_2$ concentration was adjusted to 1.4 mM. 16S rRNA fragments were amplified with the primers 875-16S1F and 875-16S1R described in Cameron et al. (1992) at 50°/70°C annealing and elongation temperatures, respectively. 12S rRNA fragments were amplified with the primers 12Sa-5' and 12-SLR-5' described in Cameron & Williams (2003) at 48°/70°C annealing and elongation temperatures, respectively. Finally, COI was amplified with the forward primer 5'-ATAATTTTTTTTATAGTTATA-3' and the reverse primer 5'-GATATTAATCCTAAAAAATGTTGAGG-3' described in Bertsch et al. (2005) from Tanaka et al. (2001) at 45°/60°C annealing and elongation temperatures, respectively. Sequencing reactions were performed for both forward and reverse DNA strands (http://etonbio.com). I edited and assembled reads, and aligned the DNA sequences with Geneious v8 (http://geneious.com, Kearse et al. 2012).

Phylogenetic Analysis

The mitochondrial genes were examined separately and combined into a single partitioned dataset (1758 nucleotides) to infer a phylogeny with a Bayesian likelihood-based approach. Models of molecular evolution for each mitochondrial locus and codon position (COI) were first investigated with PartitionFinder v1.0.1 (Lanfear et al., 2012). I implemented the model HKY+Gamma for 12S and 16S, HKY+I for COI first codon position, F81 for COI second codon position and HKY for COI third codon position. The Bayesian single-gene and concatenated phylogenies were estimated with MrBayes v3.2.1

(Ronquist et al., 2012) using two independent runs with three heated chains and one cold chain each. The MCMC chains were run for 10 million generations with sampling every 1000 generations. Convergence diagnostics were evaluated with Tracer v1.5. Ten-percent of samples were discarded as burn-in. Trees were visualized in FigTree v1.4.0 (Rambaut, 2014).

Microsatellite genotyping

A total of 373 bumble bees across 53 field sites were screened at 13 microsatellite loci documented in the literature: BL15, B124, BTERN01, BT28, BT10, B96, BTMS0066, B126, BTMS0062, BTERN02, BTMS0086, BTMS0044 and BTMS0059 (Estoup et al., 1996, 1995; Stolle et al., 2009). PCR were performed in final volumes of 10 μL, containing approximately 1 μL of extracted DNA, 1x Promega (Madison, WI) reaction buffer, 0.6 mM dNTP mixture, 0.2–0.4 μM primer, 0.001 mg BSA, 0.4 units Taq polymerase (Promega, Madison, WI) and the $MgCl_2$ concentration was adjusted to 1.4 mM. The PCR conditions for both multiplex reactions were one 3:30 min cycle at 95°C, 30 cycles of 95°C for 30 s, annealing temperature 55/58°C for 1:15 min, 72°C for 45 s and a final extension period of 15 min at 72°C. The DNA amplifications were performed with fluorescent 5’ dye-labeled primers (6-FAM, NED, VIC, or PET) and separated on an Applied Biosystems 3730xl automatic sequencer at the Center for Integrated Biology at Utah State University (Logan, UT). The allele sizes were scored manually using GENEIOUS version 8 (Kearse et al., 2012). Because I was potentially working with two different species in my study, I elected to use a universal bin set when scoring alleles for all specimens. This approach ensured that alleles were being consistently called with the appropriate microsatellites motifs with no *a priori* assumptions of species identity. My

method did not yield any ambiguous allele calls nor did I observe any “bin creep” (Amos et al., 2007), suggesting that the genotypes discovered in this study were suitable for downstream analyses.

Population genetic structure analysis

A Bayesian clustering method implemented in STRUCTURE v 2.3.4 was employed to assign individuals to ‘populations’ (Pritchard et al., 2000). This method ensured that I did not base my species identifications on the setal color phenotype the specimen displayed (Fig. 3-1). I predicted that specimens that were grouped together based on microsatellite genotypes composed distinct genetic lineages separate from specimens in other predicted groups. The utilization of the STRUCTURE algorithm in this way has been found to be useful in identifying distinct genetic lineages in other studies of bumble bees with cryptic phenotypes and evolutionary histories (Duennes et al., 2012).

I used the admixture model in STRUCTURE, which assumes that individuals comprise *K* unknown genetic lineages, to which an individual can be fractionally assigned. This allowed me to group specimens based on their genotype without prior delineation to a population or species. In this case, the inferred “population” represents a genetic lineage and would illuminate any contemporary admixture of genes. The alternative to the admixture model would be to set my modelling scheme to “no admixture” which would assume that populations are discrete, where genotypes were assigned to a lineage in full (*i.e.*, no fractional assignment). As I am testing whether *B. californicus* and *B. fervidus* were conspecific with gene flow among populations, incorporating admixture into my modelling framework would allow for fractional

assignment to *K* population(s). Furthermore, the admixture model would allow us to detect if any hybridization at the microsatellite loci between the two species was evident in areas where the two color forms are sympatric. I set the admixture model to run with 20,000 burn-in steps and 100,000 samples, with 10 iterations for each *K*, where *K* ranged from 1 to 10. Testing a wide range of *K* ensured that I did not bias my assignment of genotypes to only one or two species. To determine the optimal *K* (*i.e.,* populations/species or genetic lineages), the distributions of the probability of the data (*ln P(D)*) and ΔK, as described by Earl and von Holdt (2012) and Evanno and vonHoldt (2012) were visualized with the web-based software program STRUCTURE HARVESTER (Earl and von Holdt, 2012). To account for multimodality associated with individual STRUCTURE runs, I averaged each individual's admixture proportions over the 10 replicates for the best *K* using CLUMPP v1.1.2 (Jakobsson and Rosenberg, 2007). In addition to STRUCTURE analyses, I combined the 13 microsatellite loci into a principal components analysis to determine if significant clustering of similar genotypes could be inferred.

After determining the appropriate 'species' assignments and number of *K* lineages, the probability of null alleles was estimated with the software program MicroChecker (van Oosterhout et al., 2004). I then estimated pairwise linkage disequilibrium (LD) and deviations from Hardy-Weinberg equilibrium (HWE) across populations and loci with the web-based software program GENEPOP v 4.0.10 using default parameters (Raymond and Rousset, 1995). Based on the lineages inferred by STRUCTURE, I performed an analysis of molecular variance (AMOVA) to test for differences in genetic fixation with Arlequin 3.5 (Excoffier et al., 2005). I then tested for

a correlation between pairwise estimates of fixation based on allele frequencies with geographic distance (Isolation by Distance) within the lineages inferred from the STRUCTURE analysis with GeneAlEx 6.5 (Peakall and Smouse, 2012).

Measuring wing morphometric characteristics

The right forewing of 103 genotyped female worker specimens was removed at the wing base with a pair of forceps and compressed between two glass microscope slides. These specimens represent the diversity of phenoptypes associated with *B. californicus* and *B. fervidus*, and were broadly distributed throughout North America. Digital photos of each forewing were taken with the Keyence Digital Microscope VHX-500F (Keyence Corp., Itasca, IL). Coordinates for 19 vein junctions were measured with Adobe Photoshop CS2 (Adobe, San Jose, CA), following Ruttner (1988) to obtain 28 wing characters (See Kozumus et al. (2011) for a full description of the 28 wing characters). Wing angles, lengths, and indices were calculated with custom scripts written by the first author with the Python 2.7 computing language (Python Software Foundation 2010). While I initially used color phenotype to identify the specimens to species (*B. californicus* or *B. fervidus*), I ultimately relied on the results of the microsatellite genotypes and sequence data in species identifications. To determine whether the wing morphometric data were normally distributed, I visually inspected the data with histograms and conducted Shapiro-Wilk tests for normality. My preliminary analysis found that 13 of the 28 wing characters did not follow a normal distribution. I elected to conduct Wilcoxon rank sum tests to determine whether *B. californicus* and *B. fervidus* could be separated on wing characteristics. Informative wing characteristics identified by the Wilcoxon rank sum tests ($P < 0.05$) were combined with principal components

analysis and plotted to examine potential clustering patterns of *B. californicus* and *B. fervidus*. Finally, a linear discriminant analysis with a jackknife prediction (*i.e.*, leave one out) was performed to test the accuracy of the informative wing morphometric characters in differentiating between the two species. Statistical tests were conducted with R v3.3.0 (R Development Core Team 2012).

Estimating bioclimatic niche

To examine the bioclimatic niche space inhabited by the two species, I amassed a large dataset of georeferenced occurrence records available on the Global Biodiversity Information Facility data repository (http://gbif.org). I only retained occurrence records of *B. californicus* and *B. fervidus* that were congruent with range maps described in Thorp et al. (1983), Stephen (1957), and a recent habitat suitability model described in Williams et al. (2014). My assumption is that these two species were identified by following the taxonomic keys from Thorp et al. (1983), Stephen (1957), Koch et al. (2012), and Mitchell (1962). After data cleaning, I retained 290 unique occurrence records for *B. californicus* and 1133 unique occurrence records for *B. fervidus* (Appendix C1). The disproportionate availability of unique georeferenced records is likely attributed to the broader geographic distribution of *B. fervidus* and the greater collection intensity typically associated with the eastern United States, in comparison to *B. californicus*. Furthermore, *B. californicus* appears to be a relatively uncommon species, as suggested by a recent contemporary survey of bumble bees in the United States (Koch et al. submitted).

I aggregated the spatial data of both species with the 19 WorldClim bioclimatic variables and altitude (Hijmans et al., 2005) with ArcGIS v10.0 (ESRI 2012). The

bioclimatic variables represent monthly, seasonal, and annual temperature and precipitation phenomena, and have been a useful tool in estimating habitat suitability models for a diversity of species. The bioclimatic variables include: Annual Mean Temperature (BIO1), Mean Diurnal Range (BIO2), Isothermality (BIO3), Isothermality (BIO4), Max Temperature of Warmest Month (BIO5), Min Temperature of Coldest Month (BIO6), Temperature Annual Range (BIO7), Mean Temperature of Wettest Quarter (BIO8), Mean Temperature of Driest Quarter (BIO9), Mean Temperature of Warmest Quarter (BIO10), Mean Temperature of Coldest Quarter (BIO11), Annual Precipitation (BIO12), Precipitation of Wettest Month (BIO13), Precipitation of Driest Month (BIO14), Precipitation Seasonality (BIO15), Precipitation of Wettest Quarter (BIO16), Precipitation of Driest Quarter (BIO17), Precipitation of Warmest Quarter (BIO18), Precipitation of Coldest Quarter (BIO19). To reduce redundancy in the variables used to examine bioclimatic niche, a Pearson Correlation Coefficient Test was implemented to determine which variables were highly correlated ($r > 0.75$). Only one variable from a pair of highly correlated variables was retained for further analyses, with preference given to variables that captured seasonal trends in precipitation and temperature (Lozier et al., 2013). As in my analysis of wing morphometric data, I combined all the remaining bioclimatic variables into a PCA and plotted the first two principal components against each other to examine clustering patterns in bioclimatic niche of *B. californicus* and *B. fervidus*.

RESULTS

Phylogenetic analysis

Our inferred phylogeny based on the concatenated gene sequences recovered two distinct monophyletic groups with strong support (Bayesian Posterior Probability, BPP = 1.0) (Fig. 3-2A). Specimens predominantly associated with a dark phenotype composed the monophyletic *B. californicus* clade **(b),** which was sister to the monophyletic "*B. fervidus"* clade **(c)** (Fig. 3-2A). Single gene investigations revealed similar topologies to the full evidenced set but with lower support for *B. californicus* and *B. fervidus*, specifically, $BPP_{COI} = 0.89$, $BPP_{12s} = 0.84$, and $BPP_{16s} = 0.86$. All genes contributed to the inferred Bayesian phylogeny and were retained in all analyses. Examination of sequence divergence between the *B. californicus* and *B. fervidus* clades **(a)**, revealed the COI gene to have 861 identical sites (95.7%) with an average sequence divergence of 1.67% between species; 16s revealed 473 identical sites (97.1%) with an average sequence divergence of 1.66%; and 12s revealed 348 identical sites (94.8%) with an average sequence divergence of 5.04%. Taxonomic descriptions of *B. californicus* by Smith (1859) and *B. fervidus* by Fabricius (1798) did not capture the phenotype (setal color) variability associated with lineages inferred in my well supported phylogeny. Specifically, while setal color variability has been documented in both species, taxonomic keys by Thorp et al. (1983), Stephen (1956), Mitchell (1962), Koch et al. (2012), and others do not account for the variability and convergence presented in this genetic study. *Bombus fervidus* in particular has been regarded as less phenotypically variable than *B. californicus* (Thorp et al., 1983), however, I detected a Coastal/South Sierra California clade **(i)** that exhibits a dark setae phenotype (Fig. 3-2A). Within the

polyphyletic Intermountain West + Pacific Northwest **(h)** clade I detected specimens of *B. californicus* with yellow setae (Fig. 3-2A), showing no signs of admixed black setae on the dorsal regions of the terga two and three of the metasoma, which is typically attributed to *B. californicus consanguineus* (Stephen, 1957).

Within the respective *B. californicus* and *B. fervidus* clades I found a degree of support for geographic structuring across lineages (Fig. 3-2A). Specifically, within the *B. californicus* clade **(b)** I found strong support (BPP = 1.0) for a Rocky Mountain & Black Hills clade **(g)** as sister to the populations distributed in the Intermountain West + Pacific Northwest and the Pacific West **(d)**. An exception was a South Dakota specimen (CusterSD, DD13197) found within the Intermountain West clade, but it was preceded by a node with poor support (BPP = 0.71) **(d)**. Within the *B. fervidus* clade **(c)** I found strong support for the Coastal/South Sierra California clade **(i)** as sister to a lineage that comprises specimens distributed from North Sierra California + Intermountain West to Eastern USA **(e)**. Within clade **(e)** I found low support (BPP = 0.61) for the sister relationship between the North Sierra California and the lineage that comprises the Intermountain West to Eastern USA.

Microsatellite genotyping and population genetic structure analysis

Microsatellite genotypes corroborate the existence of two monophyletic groups within the *B. fervidus-californicus* species complex (Fig. 3-2B). STRUCTURE analysis of the available genotypes revealed two major clusters within the *B. californicus* + *B. fervidus* clade **(a)** (Fig. 3-2A). The estimate of the optimal cluster is based on a STRUCTURE HARVESTER analysis that found the highest log likelihood of the inferred models of K to occur at $K = 2$ (Table 3-1; Mean $LnP(K|2) = -14577.2$).

Significantly less explanatory power was gained by additional clusters (ΔK = 954.68) (Evanno et al. 2005) (Table 3-1). Furthermore, at six localities in my study I found relatively sympatric populations of both species as evidenced by distinct genotypes (Fig. 3-4A) and the inferred phylogeny (Fig. 3-2A). Principal components analysis estimated 202 principal components for the 13 genetic loci used in my study. Principal component 1 explained 4% of the variance in the genotype data and principal component 2 explaining 6% of the variance in the genotype data (Fig. 3-3B). While the number of principal components is large, visual inspection principal components 1 plotted against principal components 2 revealed obvious clusters associated with the genotype assignments inferred from the STRUCTURE and analyses (Fig. 3-2B). AMOVA results found that 14.66% of the genetic variation was partitioned among the two major genetic clusters (*B. fervidus* and *B. californicus*), 14.10% among individuals within populations, and 71.24% among individuals within sites (Table 3-2). Overall F_{ST} among populations is 0.15 ($P < 0.001$) and F_{IS} is 0.17 ($P < 0.001$).

To determine HWE and LD associated across populations within each clade, I first separated out individuals based on the STRUCTURE cluster assignment. After partitioning the specimens by genetic clusters, I employed MICRO-CHECKER to determine if any loci by population combinations exhibited evidence of null alleles or stuttering. From my analyses of population within the "*B. californicus*" clade I elected to remove BTMS0044 as it was found to be in LD with BTERN02. Finally, BL15 and B124 did not amplify in several *B. fervidus* populations and were not used in any further analyses. After the removal of problematic loci, I retained the following 8 loci for further analyses with *B. californicus*: BT10, B96, BTERN02, B124, BL15, BT28, BTMS0086,

BTMS0066, and the following 8 loci with *B. fervidus*: B126, BT10, B96, BTERN02, BTERN01, BTMS0044, BT28, BTMS0066.

Isolation by distance within clades

Across the *B. fervidus* lineage I detected a strong effect of geographic distance on patterns of allelic fixation (Mantel Tests, $r = 0.39$, $P = 0.03$), with estimates of pair-wise linearized F_{ST} ranging from 0 to 0.26 (Fig. 3-3A). I also detected a strong effect of geographic distance on patterns of allelic fixation within the *B. californicus* lineage (Mantel Tests, $r = 0.56$, $P = 0.01$), with estimates of pairwise linearized F_{ST} ranging from 0 to 0.53 (Fig. 3-3B).

Wing Characteristics

I found significant differences in the median value of 8 out of the 28 wing characteristics measured (Table 3-3). I found that the Dumb-bell index, and the following six angles: D7, N23, B3, B4, J16, H12, and G7 had means that significantly differed for the *B. californicus* and *B. fervidus* clades. The remaining 20 wing characteristics were not significantly different between the lineages. Combining the informative wing characteristics into a principal components analysis found seven components to explain the variance in the data, with principal component 1 explaining 40% of the variance in the data and principal component 2 explaining 22% of the variance in the data (Fig. 3-5). Finally a linear discriminant analysis with a jackknife prediction using the data associated with the eight wing characteristics was able to accurately assign *B. californicus* and *B. fervidus* for 93% and 61% of the records, respectively.

Fig. 3-2 (A) Bayesian phylogeny of *B. fervidus* and *B. californicus* lineages inferred using the fragments of three mitochondrial genes: cytochrome c oxidase I + 12s rRNA+ 16s rRNA. Values proceeding each node is the Bayesian posterior probability. The scale bar indicates branch lengths in expected substitutions per site. The phenotype group that each specimen belongs to is mapped out with a corresponding shape and color. Phenotype Group 1 = Black hexagon, Phenotype Group 2 = Black triangle, Phenotype Group 3 = Orange circle, Phenotype Group 4 = Orange heart. Outgroups = *B. weisi* (*Thoracobombus*) and *B. insularis* (*Psythirus*), with the branch length of the latter species truncated. Bold lowercase letters refer to the clades associated with a nodes preceding each lineages' geographic distribution. (B) Genetic lineage assignment based on a Bayesian analysis of 13 microsatellite loci implemented in STRUCTURE assuming K = 2. Each horizontal bar represents a single specimen's microsatellite genotype, where each color represents a fractional assignment to one of two genetic lineages. Colors of each fractional genotype correspond to the text color of the specimens mapped on the Bayesian phylogeny (A).

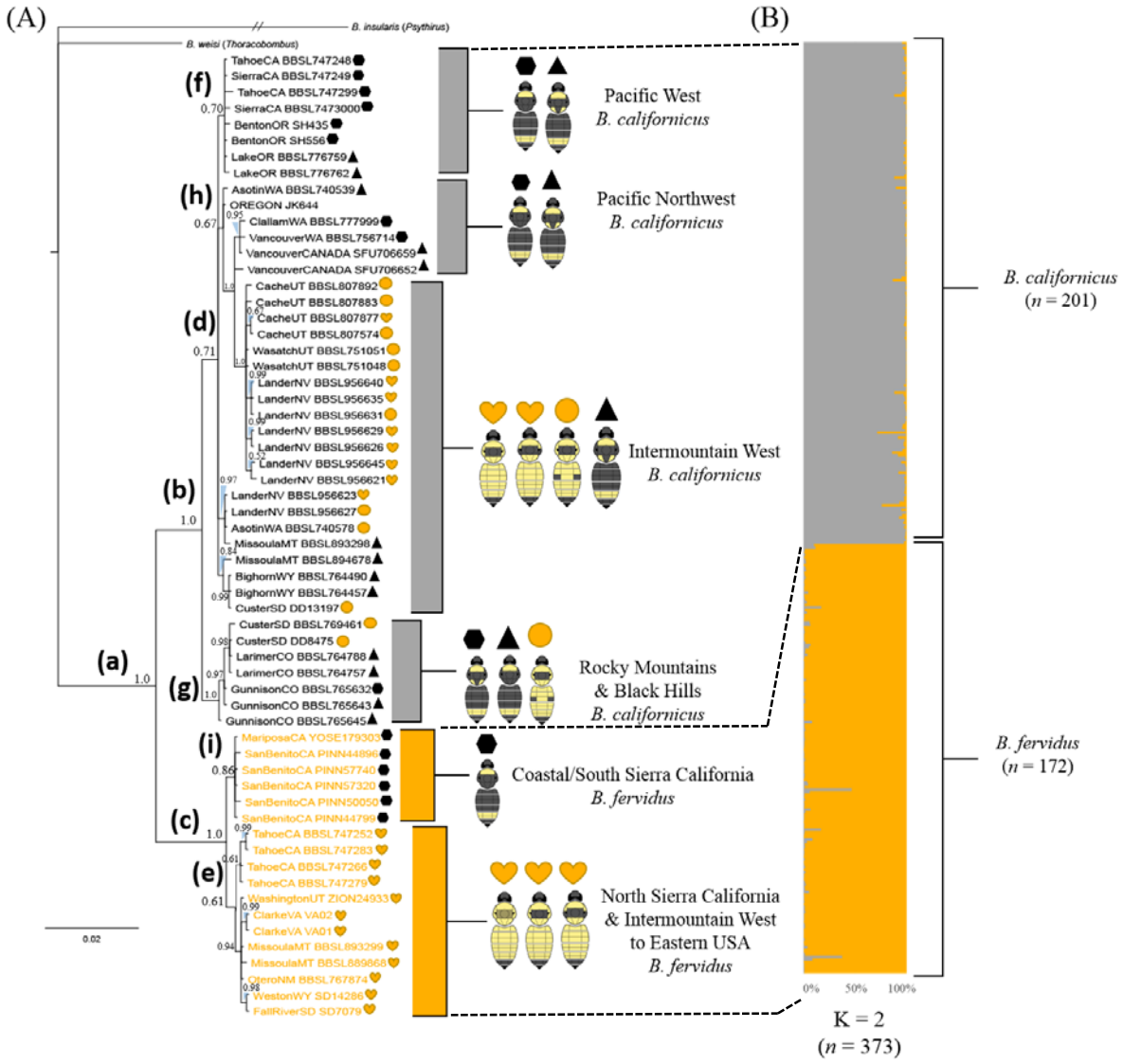
(A)
(B)
B. insularis (Psythirus)
B. weisi (Thoracobombus)
(f)
(h)
(d)
(b)
(a)
(g)
(i)
(c)
(e)
0.02
Pacific West
B. californicus
Pacific Northwest
B. californicus
Intermountain West
B. californicus
Rocky Mountains
& Black Hills
B. californicus
Coastal/South Sierra California
B. fervidus
North Sierra California
& Intermountain West
to Eastern USA
B. fervidus
B. californicus
(n = 201)
B. fervidus
(n = 172)
0%
50%
100%
K = 2
(n = 373)

Table 3-1 Table of four probabilities of model fit implemented with the Evanno method associated with different values of *K* (i.e., clusters) based on 13 microsatellites implemented in STRUCTURE Harvester. Bold text represents the indices that suggests the value of *K* that best predicts the microsatellite genotypes assigned in the STRUCTURE analysis.

K	**Reps**	**Mean *LnP(K)***	***Ln'(K)***	***|Ln''(K)|***	***Δ K***
1	10	-15870.1	-	-	-
2	**10**	**-14577.2**	**1292.85**	**865.67**	**954.6799**
3	10	-14150	427.18	208.34	80.19935
4	10	-13931.2	218.84	31.18	0.906039
5	10	-13743.5	187.66	38.67	0.474636
6	10	-13594.5	148.99	99.93	1.567087
7	10	-13545.5	49.06	8.32	0.101161
8	10	-13488.1	57.38	99.03	0.945423
9	10	-13529.7	-41.65	146.24	0.348091
10	10	-13425.2	104.59	-	-

Table 3-2 Results of Analysis of Molecular Variance (AMOVA) for *B. californicus and B. fervidus* (n = 330), based on allele frequencies of 13 loci.

Source of Variation	***df***	**Sum of Squares**	**Variance Components**	**% variation**
Among populations	1	201.56	0.55	14.66
Among individuals within populations	356	1340.11	0.54	14.10
Within Individuals	358	965.50	2.70	71.24
Total	715	2507.173	3.79	100

F_{IS} = 0.17, F_{ST} = 0.15, F_{IT} = 0.29, (all $p < 0.001$)

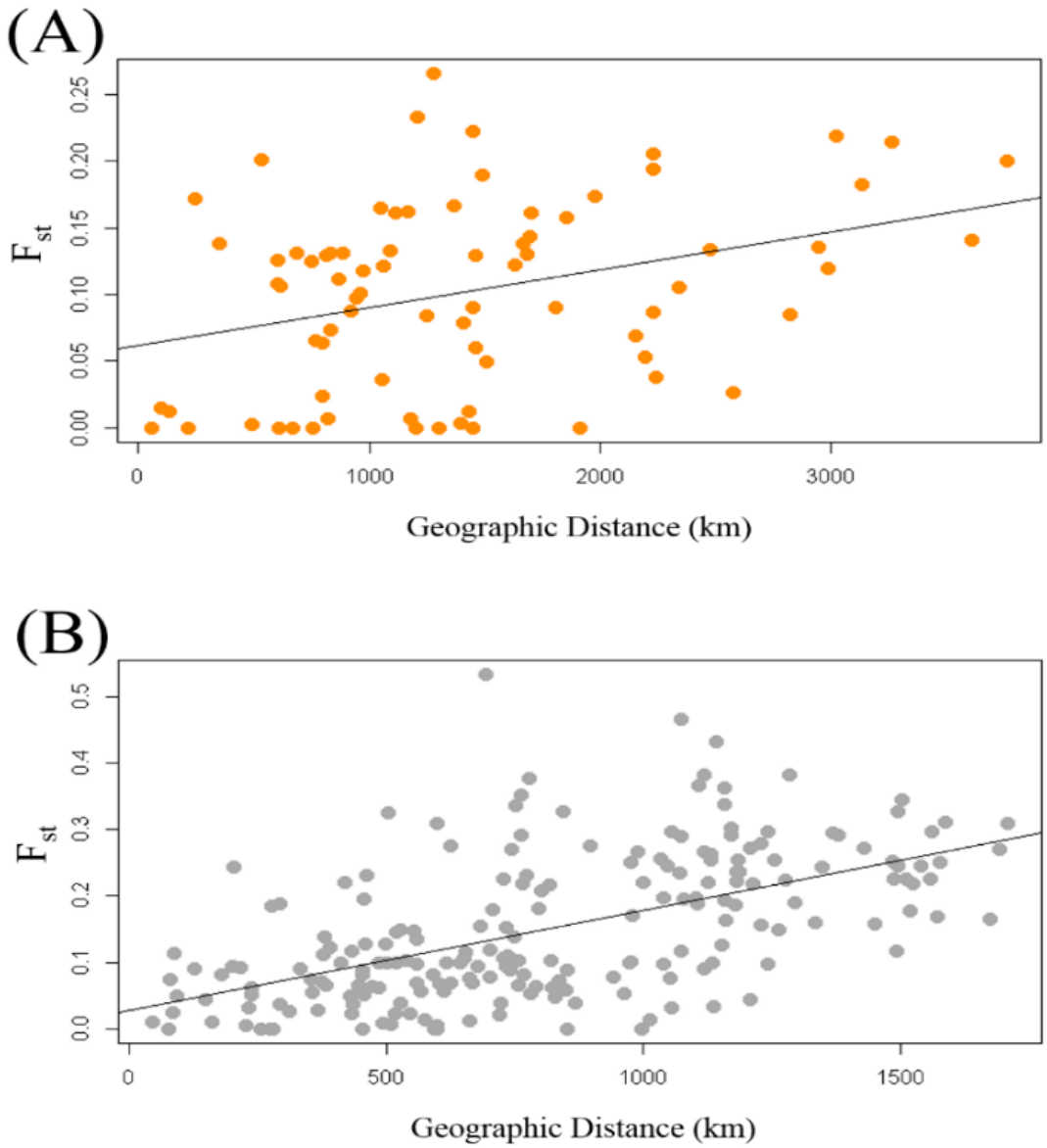

Fig. 3-3 (A) Isolation by Distance Plot: Linearized F_{ST} between pairs of *Bombus fervidus* demes compared to geographic distance. (B) Isolation by Distance Plot: Linearized F_{ST} between pairs of B. californicus demes compared to geographic distance.

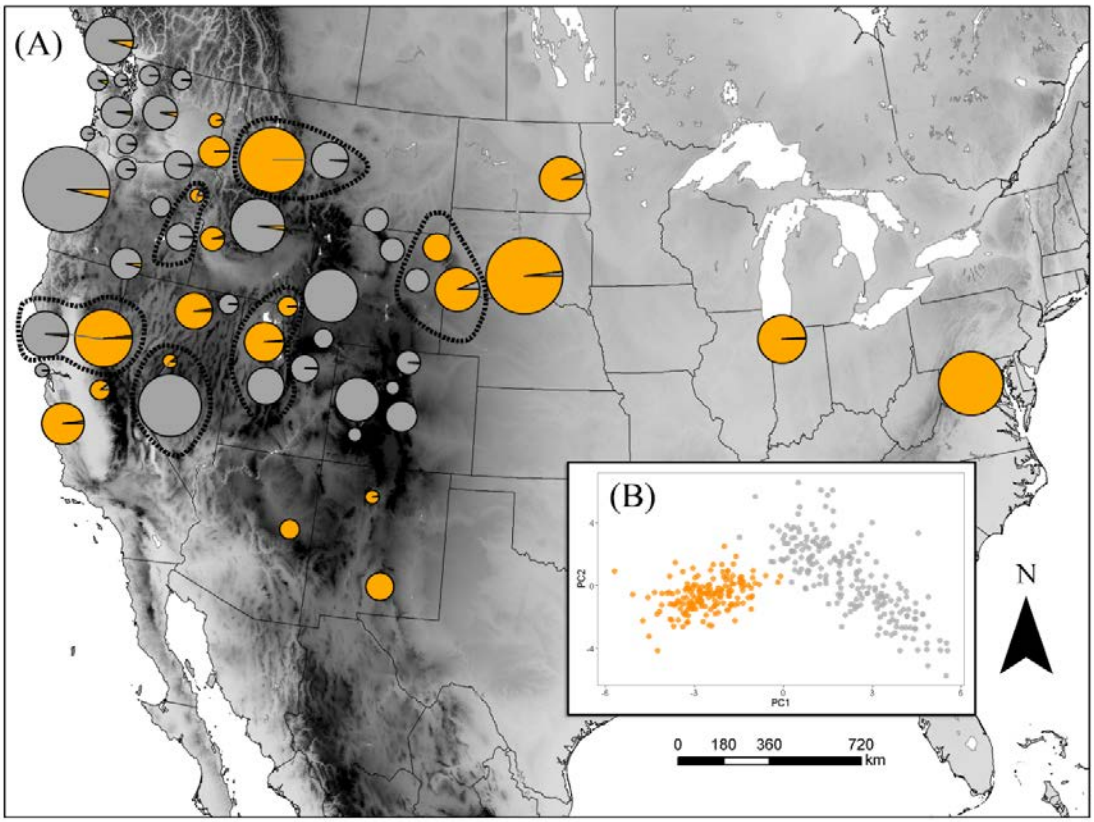

Fig. 3-4 (A) Spatial distribution of K = 2 genetic lineages, *B. californicus* (gray circles) and *B. fervidus* (orange circle) inferred from a Bayesian analysis of 13 microsatellite loci implemented in STRUCTURE. The size of each circle represents the number of specimens genotyped per locality. Fractional genotypes are averaged across specimens within each lineage (see Fig. 3-1B for individual genotype assignment to a lineage). Demes enclosed by a black dotted ellipse represent localities where *B. californicus* and *B. fervidus* are geographically sympatric. (B) Principal component analysis of 13 microsatellite loci shared between *B. californicus* (gray points) and *B. fervidus* (orange points).

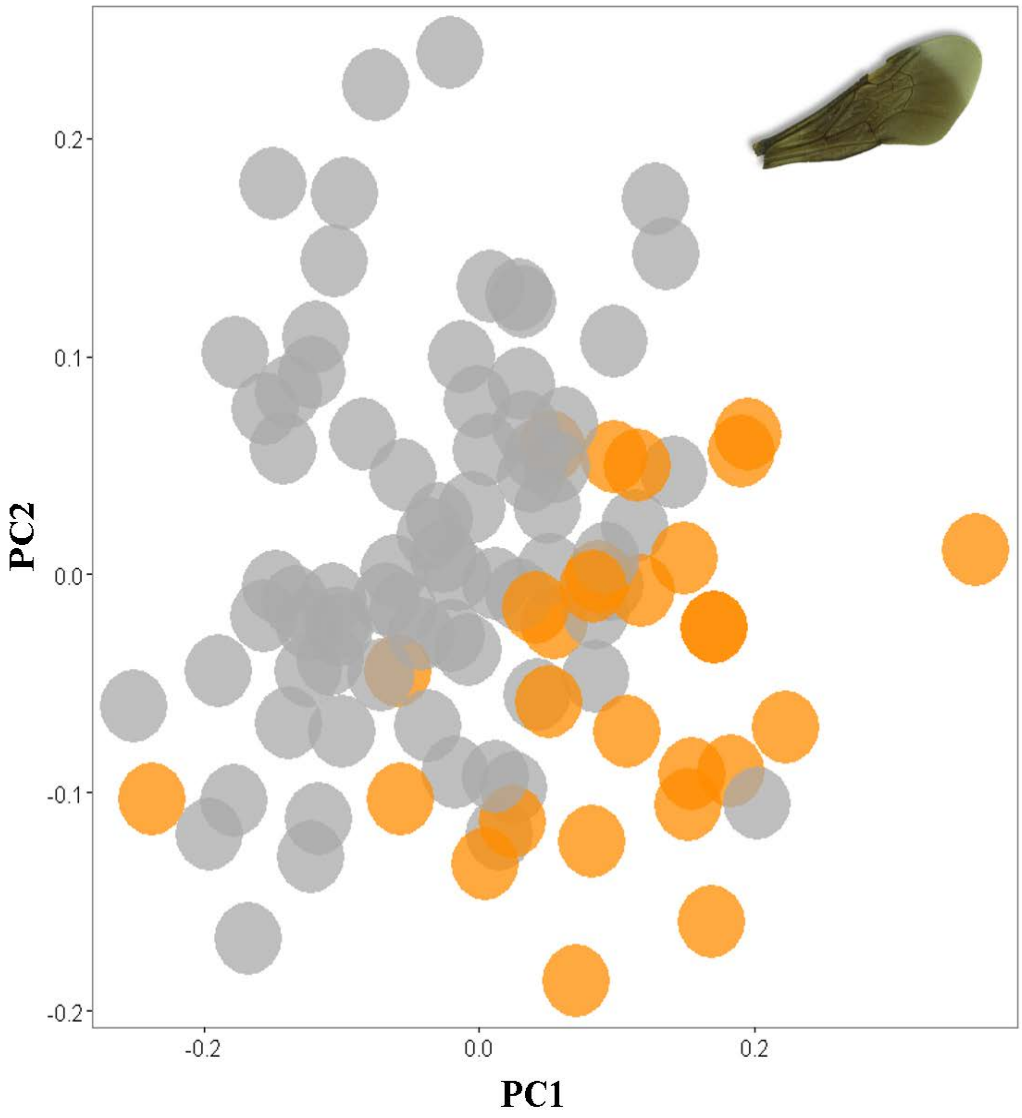

Fig. 3-5 Differences in seven informative forewing venation characteristics based on principle component analysis between *B. californicus* (dark gray points) and *B. fervidus* (orange points). See Table 3-3 for the wing characteristics and Ruttner (1988) for an extensive description of the characteristics.

Table 3-3 Wing estimates and statistical significance ($P < 0.05$) of 28 forewing venation and shape characteristics calculated by Kruskal-Wallis Test. Significant differences in the median of a wing characteristics between specimens associated with the "*B. californicus*" and "*B. fervidus*" lineage are highlighted in bold.

Characteristic	**W**	***P*-value**
Dumb-bell (DBI)	**464**	**< 0.001**
D7	**493**	**< 0.001**
J16	**1472**	**< 0.001**
G7	**1645**	**< 0.001**
H12	**999**	**< 0.001**
N23	**590**	**0.000659**
B3	**608**	**0.001066**
B4	**778**	**0.04417**
O26	852	0.1432
Pre-Cubital (PCI)	874	0.1933
K19	894	0.2491
E9	902	0.2742
B	961	0.5118
Area6	980	0.6064
C	1066	0.9115
A1	1073	0.8675
Radial Field	1074	0.8646
Inner Length	1105	0.6862
L13	1148	0.4699
Q21	1188	0.3081
J10	1192	0.2942
Cubital (CI)	1218	0.2144
A	1220	0.209
A4	1221	0.2063
Inner Width	1224	0.1984
M17	1238	0.1646
D	1253	0.1333
G18	1310	0.05441

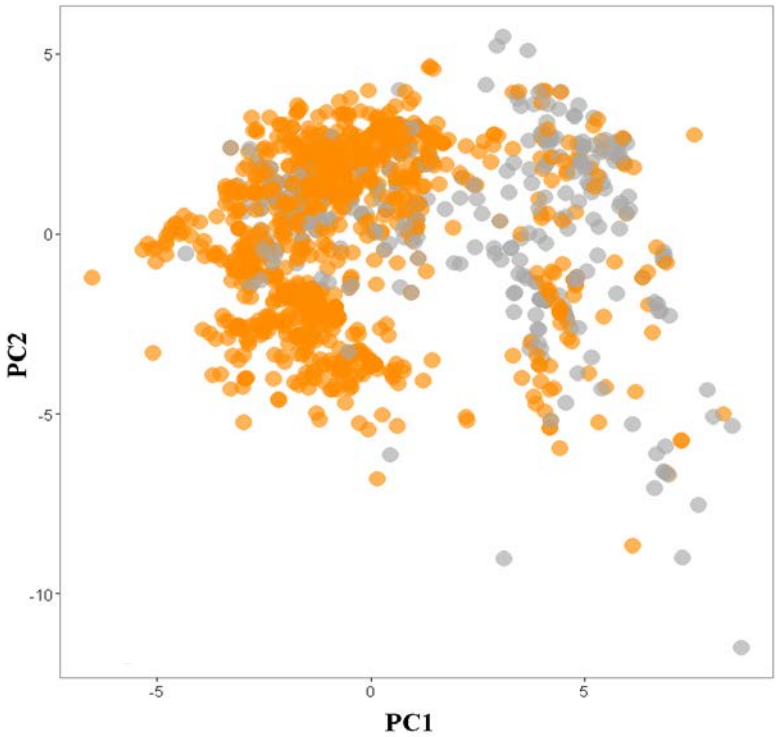

Fig. 3-6 Differences in 9 bioclimatic variables (temperature and precipitation) based on principle component analysis for georeferenced localities of *B. californicus* (dark gray points) and *B. fervidus* (orange points).

Estimating bioclimatic niche

Aggregating the 1,423 locality records of *B. californicus* and *B. fervidus* with the WorldClim bioclimatic dataset revealed high correlation coefficients ($r > 0.75$) between precipitation and temperature variables. After removing one variable from a pair of highly correlated variables, I retained seven of the 19 bioclimatic variables and altitude for further examination of bioclimatic niche: Mean Temperature of Coldest Quarter (BIO 11), Precipitation of Driest Month (BIO 14), Precipitation of Warmest Quarter (BIO 18), Precipitation of Coldest Quarter (BIO 19), Mean Diurnal Range (BIO 2), Mean Temperature of Wettest Quarter (BIO 8). Combing the seven bioclimatic variables and altitude into a principal components analysis found eight principal components, with principal components 1 and 2 accounting for 34% and 26% (PC1 + PC2 = 60%) of the

variance observed in the data (Fig. 3-6). I detected a degree of bioclimatic clustering associated with the distribution of *B. fervidus* and *B. californicus*, suggesting that the species do occupy different bioclimatic niches, despite their geographic overlap (Fig. 3-4A). However, I suggest caution with interpreting this result of differentiated bioclimatic niches. Given that there is a degree of ambiguity in species identities in some areas of western North America due to phenotype color convergence it is highly probable that the georeferenced specimens are associated with the wrong species identities. It is possible that the estimates of bioclimatic niche differentiation presented here are conservative, because those likely misidentifications may explain some of the ambiguity in the results of this analysis.

DISCUSSION

Globally, there are only about 250 species of *Bombus* (Cameron et al., 2007). Bumble bees are typically regarded as well studied relative to other Hymenoptera given that they represent the only extant genus in the tribe Bombini (Apidae), particularly in North America. In my study I infered the existence two well-supported lineages composed of convergent phenotypes associated with *B. californicus* and *B. fervidus* (Fig. 3-2A). Cluster assignment of 13 microsatellite loci corroborates the results of the inferred phylogeny, specifically, that two distinct genetic lineages are present in areas where the species are broadly sympatric (Fig. 3-2B, Fig. 3-4A). Examining wing morphology between the genetic lineages found seven informative wing characteristics, which may be useful in future endeavors in species identification (Fig. 3-5, Table 3-3). Finally, an examination of bioclimatic niche occupancy reveals a degree of clustering associated with the distributions of *B. californicus* and *B. fervidus* (Fig. 3-6). However, overlap was

also observed, suggesting that microclimate variability, phenology, and perhaps foraging preferences may drive niche partitioning when the species are in sympatry.

Phylogeny and population genetic structure

The recent and rapid diversification within the *californicus-fervidus* species-group was likely driven by climate change and glacial oscillations associated with the late Pleistocene (Duennes et al., 2012; Wilson and Pitts, 2011, 2010). Simple pairwise examination of the average levels of divergence across COI between the two species is 1.67%. The observed level of divergence is below the 2% level that is often considered reflective of species status (Williams et al. 2014). This suggests that divergence from a common ancestor likely occurred less than ~1 million years ago based on estimates of mitochondrial divergence with respect to time (Brower, 1994). However, in addition to COI, I considered that distinct 16s and 12s haplotypes are characteristic of individuals associated with either the *B. californicus* or *B. fervidus* lineage despite convergent phenotype color patterns (Fig. 3-1, Fig. 3-4A).

My study has revealed that convergent phenotypes between two sister taxa of the *Thoracobombus* have formed discrete lineages, and occur in sympatry (Fig. 3-4A). Uncertainty regarding *B. californicus* as specifically distinct from *B. fervidus* is well documented in the literature (Stephen, 1957; Thorp et al., 1983; Williams et al., 2014). The primary argument that favors the two species as conspecific is that no morphological characteristic has been observed that can delineate the two, with the exception of setal color. However, it is well understood that bumble bees display a diversity of color patterns, and have converged on regional phenotypes, likely to maintain Müllerian mimetic complexes (Hines and Williams, 2012; Plowright and Owen, 1980; Williams,

2007). In addition, Hobbs (1966) observed habitat differences between *B. californicus* and *B. fervidus* in nesting preferences in southern Alberta, Canada. My results suggest that the two species occupy different bioclimatic niches, despite the degree of overlap observed in their western distribution (Fig. 3-4A). Finally, Cameron et al. (2007) and Cameron and Williams (2002) found that *B. californicus* and *B. fervidus* were well-supported sister taxa based on a suite of nuclear and mitochondrial loci.

This current study rejects the hypothesis that they are a single species despite phenotypic convergence, as sympatric populations are found to be reproductively isolated, with no evidence for hybridization (Figs. 3-2B, 3-4A, and 3-4B). However, I agree with Williams et al. (2014) that the contemporary phenotype characteristics used to discriminate between *B. californicus* and *B. fervidus* are not entirely useful in certain parts of the respective species ranges. For example based on the available data in this study, I found that specimens, which would be identified as *B. californicus* in southern California, are, in fact, dark phenotypes of individuals within the *B. fervidus* lineage (Fig. 3-2A, clade **(i)**). Furthermore, individuals distributed in the Toiyabe Mountain Range in Nevada and the Bear River Mountain Range in Utah that were identified as *B. fervidus* based on the absence of black setae on the dorsum of the metasoma (Thorp et al. 1983) were found within the *B. californicus* lineage (Fig. 3-2A, clade **(d)**). Given the results of my study, setal color patterns appear to be of limited taxonomic use in some areas of these species' ranges. Despite the degree of phenotype crypsis associated with the appropriate genetic lineages, I was able to correctly assign 89% of the *B. californicus* specimens used in my study to the *B. californicus* clade with microsatellite genotypes (Fig. 3-2A, 3-2B, clade **(b)**). With *B. fervidus*, I was able to associate 93% of the

specimens to the *B. fervidus* clade with microsatellite genotypes based on currently recognized phenotypes of the species-group (Fig. 3-2A, 3-2B, clade **(c)**) As such, species assignment to either *B. californicus* or *B. fervidus* based on known phenotypes is possible in some areas of North America (Fig. 3-1, 3-4A).

Wing morphology in species delimitation

Due to the convergence of phenotypes and morphological similarity associated with *B. californicus* and *B. fervidus* populations in my study, I predict that future identifications of the two species will be difficult. However, I have found evidence that species may be differentiated based on wing morphometric characteristics (Fig. 3-5) (Table 3-3). Specifically I have found that seven wing angles and one wing index has the potential to differentiate between specimens of *B. californicus* and *B. fervidus*. Wing morphometric characteristics has been a useful identification tool in both honey bees and bumble bees, however further work is needed across more specimens and populations to determine the effectiveness and significance of these methods (Kozmus et al., 2011; Owen et al., 2010; Ruttner, 1988). With a molecular basis of species differentiation, perhaps previously rejected morphological characters can be used to differentiate the species.

Conservation implications and future work

Bumble bees are well regarded for their value in agricultural ecosystems as they are efficient pollinators of a diversity of crops (Kremen et al., 2002; Strange, 2015; Velthuis and Van Doorn, 2006). However, there is global concern for bumble bee decline due to economic activities associated with human growth and expansion, namely the shuffling of Hymenopteran disease due to movement of bumble bee and honey bee

colonies to meet pollination demands, as well as increased urbanization and agricultural intensification (Cameron et al., 2011; Cordes et al., 2012; Goka et al., 2000; Sachman-Ruiz et al., 2015). *Bombus fervidus* in particular has been associated with decline at regional scales (Colla and Packer, 2008), and has been found to be highly susceptible to a suite of pathogens (Kissinger et al., 2011). Despite its co-distribution with *B. fervidus* throughout western North America, *B. californicus* does not appear to be associated with high levels of pathogen incidence (Cordes et al., 2012; Kissinger et al., 2011).

A prevailing hypothesis associated with bumble bee decline includes the introduction of novel pathogens or pathogen strains (Cameron et al., 2011; Cameron et al., 2010). Given the differences in pathogen prevalence between *B. californicus* and *B. fervidus*, I suggest that researchers treat the two species differently in the context of conservation, ecology, and evolution. My results show that the two lineages are distinct and inhabit different bioclimatic niches when they are sympatric (Fig. 3-6). Given the pronounced genetic and ecological differences in the species, treating them as separate will allow for a more robust assessment of their conservation needs. Despite the inability to identify the individuals to species based on current taxonomy, there is potential for alternative, non-destructive ways to ensure correct species identification. Specifically, I found that microsatellite genotypes have the capacity to differentiate species, even when they are sympatric (Figs. 3-2B, 3-4). While I advocate for synoptic collections to be done when conducting ecological research, I have found that taking a tarsal clipping from the mid-leg for DNA extraction and subsequent genotyping is possible, which avoids sacrificing the individual, allowing it to continue with its contribution to the nest

economy (Holehouse et al., 2003). Also, immobilizing an individual on ice and taking a photograph of its forewing might be found useful if molecular resources are not available.

An understanding of the evolutionary processes associated with the formation of a species is required in conservation biology (Agapow et al., 2004; Crandall et al., 2000; Erwin, 1991). In this study I demonstrate that populations that compose the *B. californicus* and *B. fervidus* lineages are cryptic, yet form well supported clades. To synonymize the *B. californicus* with *B. fervidus* based on the inability to identify them will likely obscure the host-pathogen dynamics associated with the species, hindering progressive action on their conservation and management.

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CHAPTER 4

MÜLLERIAN MIMICRY STRUCTURES LOCAL BUMBLE BEE COMMUNITIES ACROSS AN ELEVATION GRADIENT[4]

Introduction

The coexistence of closely related taxa in an ecological community has long intrigued evolutionary biologists and ecologists. Resource and habitat partitioning have been implicated to be important mechanisms driving the diversity and abundance of species that coexist within a community (Schoener 1974; Ranta and Lundberg 1980; Inouye 1980). However, phenotypic variation, particularly when expressed by Müllerian and automimicry has also been found to structure animal communities across a mosaic of environments (Brown and Benson 1974; Pfennig et al. 2001; Beatty et al. 2004; Alexandrou et al. 2011). Müllerian mimicry is defined as the phenomenon where noxious and harmful species converge on an aposematic color body pattern, whereas Batesian mimicry is the phenomenon where non-noxious species converge on an aposematic color body pattern of a noxious species (Stiles 1979; Plowright and Owen 1980; Mallet and Joron 1999; Sherratt 2008). Automimicry or interspecific mimicry is similar to Batesian mimicry, with the exception that the mimetic advantage is gained by a member of the same species. By sharing vivid and typically contrasting body colors, animals participating in a mimicry complex are able to protect themselves from predation.

The spatial convergence of similarly colored organisms has long captivated evolutionary scientists including Alfred Russel Wallace and Charles Darwin. Müeller's

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[4] This chapter is co-authored with Jaime Florez, Terry Griswold, James Pitts, and James Strange. Permission has been granted by the required coauthors for this research to be included in my dissertation (Appendix A). This chapter is formatted for *Evolutionary Ecology*.

theory of mimicry in promoting the fitness of the mimics was presented by Darwin in his 4th edition of *The Origin of Species* as evidence of the power of natural selection in stimulating the evolution of organisms (Mallet and Joron 1999; Sherratt 2008). Examples of Müllerian mimicry complexes in insects have been documented in *Heliconius* butterflies (Benson 1972; Brown and Benson 1974), *Psorthaspis* spider wasps (Rodriguez et al. 2014), diurnal *Dasymutilla* velvet ants (Wilson et al. 2012; Wilson et al. 2013) and bumble bees (Hymenoptera: Apidae, *Bombus*) (Stiles 1979; Plowright and Owen 1980; Williams 2007; Hines 2008). The evolutionary success of these mimicry complexes is dependent on local geographic and ecological pressure for organisms to train potential predators with their aposematic coloration patterns.

Müller's theory is contingent on the ability of predators to learn and distinguish between palatable and non-palatable prey items (Sherratt 2008). Experimental studies have found that naïve predators are unlikely to attack and consume prey items that were painfully combative or distasteful during an initial encounter (Brower et al. 1960; Brown and Benson 1974; Pinheiro 2003; Finkbeiner et al. 2014). By training potential predators of their distastefulness, inflammatory, or noxious chemical compositions, animals belonging to Müllerian mimicry complexes are able to avoid extirpation. As natural selection operates on populations at local spatial and temporal scales, the maintenance and evolutionary success of Müllerian mimicry complexes is dependent on co-located organisms sharing a similar appearance (*e.g.* body size, body shape, body color) and ecological niche. For example, while phylogenetically distant, both *Dasymutilla* and *Psorthaspis* wasps converge on strikingly similar aposematic body color patterns throughout North America (Wilson et al. 2012; Rodriguez et al. 2014). However, they are

distinct ecologically, as reproductive females of *Dasymutilla* are documented to predate upon bees and wasps to rear offspring, while *Psorthaspis* predate upon spiders to rear offspring (Norden et al. 2003; Rodriguez et al. 2014). However, even though their resource niches differ to a great extent, both Hymenopterans are likely exposed to similar predators, including frogs, lizards, and mammals (Schmidt and Blum 1977). Through the convergence of regional aposematic coloration patterns, species of both genera are likely participating in exclusive Müllerian mimicry rings (Wilson et al. 2012).

Like *Psorthaspis* and *Dasymutilla* wasps, convergence of unique setal coloration patterns across bumble bee (Hymenoptera: Apidae, *Bombus*) distributions are well documented (Plowright and Owen 1980; Williams 2007). Bumble bees are notorious for their cryptic setal coloration patterns, and are well known to locally converge on setal color patterns with other phylogenetically distant bumble bee taxa (Stephen 1957). Variability in setal color across bumble bees has been suggested to promote thermoregulation, crypsis, and warning mimicry (reviewed in Williams 2007). While latitude appears to be a strong predictor of the some 38 color pattern groups across all bumble bee fauna, elevation as a predictor of local color pattern groups has been suggested, but never formally tested (Franklin 1912; Milliron 1971; Plowright and Owen 1980; Williams 2007). In areas west of the Sierra-Cascade Crest of North America, bumble bees are found to converge on dark setal coloration patterns, whereas as bumble bees found across the intermountain west are predominantly red and orange in setal coloration patterns (Thorp et al. 1983; Williams 2007; Koch et al. 2012). However, convergence of red or black phenotypes is not exclusive to a geographic area, and there is a degree of variability found across species. Even more interesting is the fact that bumble

bee species with broad geographic ranges have populations that appear to converge on regional setal color patterns that are often shared with other non-related bumble bee species (Duennes et al. 2012; Lozier et al. 2013).

In addition to setal color, proboscis or tongue length is another morphological trait associated with bumble bee abundance and diversity (Heinrich 1976; Ranta and Lundberg 1980; Inouye 1980; Johnson 1986). Bumble bees depend on nectar and pollen from flowering plants for growth and development. Floral resources are collected by adult female bumble bees and brought back to the nest to feed their developing sisters, the offspring of their mother. To avoid competition for floral resources, it has been suggested that bumble bee communities are differentiated by proboscis-length, body size, and phenology (Ranta and Lundberg 1980; Inouye 1980). Bumble bees with short proboscises prefer to forage more intensely on flowers with short corollas, whereas bumble bees with long proboscises tend to forage more on flowers with long corollas (Inouye 1980). While there is disagreement as to how many species comprise a functional trait class within a community (Ranta and Lundberg 1980; Ranta and Tiainen 1982; Goulson and Darvill 2004), and whether the pattern holds across continents (Goulson and Darvill 2004), resource partitioning based on proboscis length within bumble bee communities continues to be an observable phenomenon at local geographic scales (Harmon-Threatt and Ackerly 2013).

In this study I examine the role of Müllerian mimicry, species richness, and proboscis length in predicting the structure and composition of bumble bee communities across an elevation gradient in the Sierra Nevada of California, USA. My primary objective is to test the hypothesis that bumble bees that converge on similar color-

banding patterns will be co-distributed more frequently together than bumble bees that exhibit an alternative color-banding pattern. I predict that bumble bee communities will be partitioned across an elevation gradient based on their participation in a Müllerian mimicry ring. My second objective is to examine the spatial distribution of species as it relates to their proboscis length class. I predict that bumble bee functional trait classes will be evenly distributed across study sites in the Sierra Nevada, and that species richness will increase with elevation. The representation of bumble bee species with different proboscis lengths in a community would support the prevailing hypothesis that co-existence of bumble bees is governed partly by functional (*i.e.*, proboscis length) trait divergence within mimicry complexes.

Methods

I focused my study of bumble bee Müllerian mimicry in Yosemite National Park (hereafter referred to as Yosemite) which encompasses a cross section of the Sierra Nevada, a range that forms a north-south spine in California, USA. Yosemite National Park is one of the oldest federally protected national parks in the USA (established in 1890), and has experienced limited human impact, relative to adjacent areas. Bumble bee communities were surveyed within 1 ha plots across the Merced River drainage of Yosemite, spanning an elevation gradient from 300 to 3900 m (984 -12800 ft.) across three consecutive years (2004 to 2006). Some low-elevation plots were not located in Yosemite, but rather in the adjacent foothills of the Central Valley. Opportunistic sampling outside of Yosemite provided the opportunity to survey taxa that are exclusive to low-elevation habitats (Thorp et al. 1983). The habitats associated with each plot ranged from oak woodland and chaparral to alpine meadows and scree. Each plot was

systematically surveyed biweekly by two collectors using two techniques: pan traps and insect netting (Cane et al. 2000; Roulston et al. 2007). Blue, yellow, and white pan traps were set in an array at each plot and filled with soapy water to attract and euthanize passing bumble bees (Roulston et al. 2007). Pan traps were deployed for 6 hour time intervals whereas netting was done twice during the survey event, once each in the morning and afternoon for 0.5 hours within the plot. It is well established in the literature that bumble bee phenology is variable across species (Thorp et al. 1983; Koch et al. 2012). Thus, sampling effort was conducted throughout the entire flight season of bumble bees, from late March to late August (Thorp et al. 1983), ensuring that all species present in the Yosemite area were detected (Appendix D1 and D2). The majority of the bumble bees netted were collected on flowering plants, while some were caught flying through the plot, or were found on a non-flowering plant substrate (*i.e.*, rock, soil). While netting for bumble bees, collectors surveyed all male and worker bumble bees, but made an effort to avoid queen bumble bees as they represent the sole effective female reproductive unit of the current or potential colony.

Species identification and trait classification

Bumble bees were identified to species with dichotomous keys found in Thorp et al. (1983). Bumble bee species were classified based on the presence of red setae on their body (Table 4-1, Fig. 4-1). The participation of a bumble bee species in a particular mimicry complex is well documented (Plowright and Owen 1980; Williams 2007), which is why I elected to not conduct human perception tests as in other recent studies (Wilson et al. 2012; Rodriguez et al. 2014). My broad setal coloration assignment identified two distinct Müllerian mimicry rings in Yosemite National Park: (1) Bumble bees with strong

black and yellow setal banding and (2) Bumble bees with red setae, particularly on the terminal end of the metasoma (Fig. 4-1). Proboscis length categories for female bumble bee workers were taken from the literature to summarize the following functional trait classes: short, medium, medium-long, and long proboscis (Table 4-1) (Koch et al. 2012; Williams et al. 2014). I excluded males and queens from the functional trait class analysis as queens typically have larger probosces than workers, and males are not recognized as avid pollen foragers or as pollinators (Inouye 1980).

Fig. 4-1 Representative community of bumble bee species participating in Müllerian mimicry and automimicry complexes in Yosemite National Park, California: (A) B. vosnesenskii female (Photo Credit: CC M. Layne on Flickr), (B) *B. vosnesenskii* male (Photo Credit: CC J.J. Kehoe on Flickr, (C) *B. californicus* female (Photo Credit: CC A. Redling on Flickr), (D) *B. californicus* male (Photo Credit: L. Dahlberg on DiscoverLife), (E) *B. vandykei* female (Photo Credit: J. Strange, USDA-ARS), (F) *B. vandykei* male (Photo Credit: H. Wisch on DiscoverLife) , (G) *B. centralis* female (Photo Credit: L/ Lewis, USDA-ARS), (H) *B. mixtus* female (Photo Credit: Don Rolfs), (I) *B. balteatus* female (Photo Credit: CC D. Wilson on BugGuide), (J) *B. sylvicola* female (Photo Credit: CC D. Wilson on BugGuide), (K) *B. morrisoni* female (Photo Credit: J. Strange, USDA-ARS), (L) *B. melanopygus* female (Photo Credit: CC K. Schneider on Flickr). The *B. vosnesenskii* (B) and *B. californicus* males are automimics, participating in the A,C,D mimicry ring as they does not have the capacity to sting and is therefore not harmful to predators. CC = Creative Commons.

Table 4-1 Classification and relative abundance of 15 bumble bee species detected in Yosemite National Park by setal color and tongue length.

Species	Red Present	Tongue Length Class	Raw Abundance			Relative Abundance
			2004	2005	2006	
B. balteatus	Yes	long	12	6	12	0.67%
B. bifarius	No	medium	362	113	206	15.22%
*B. californicus**	No	long	9	29	22	1.34%
B. centralis	Yes	medium-long	40	1	4	1.01%
B. crotchii	Yes	short	1	1	0	0.04%
B. fernaldae	No	cuckoo	11	3	6	0.45%
B. flavifrons	No	long	32	19	30	1.81%
B. insularis	No	cuckoo	58	222	181	10.31%
B. melanopygus	No	medium	86	70	121	6.19%
B. mixtus	Yes	medium	49	16	42	2.39%
B. morrisoni	No	short	2	2	1	0.11%
B. rufocinctus	Yes	short	1	1	1	0.07%
B. sylvicola	Yes	medium	257	58	81	8.85%
B. vandykei	No	medium-long	108	117	123	7.78%
B. vosnesenskii	No	medium	556	966	423	43.48%
Unidentified			6	1	5	0.27%
Grand Total			1590	1625	1258	

*(= *B. fervidus*, in part)

Community and statistical analysis

Due to differences in cumulative abundance across plots in the three-year survey I elected to remove plots with less than 10 individuals from any further analyses of species abundance and diversity. I first tested for colinearity between the predictor variables: elevation, year, latitude, and longitude with the Pearson Correlation Coefficient test. I estimated species diversity with the Shannon Weiner Index and evenness with the Pielou Evenness Index. Next, given the high variability in abundance across plots and inability to capture all species present in a plot, I randomly sampled 10 specimens per plot using rarefaction. Rarefaction is a useful method for estimating species richness based on sampling effort (*i.e.*, sample size). I tested for the effect of geography and time on diversity, evenness, and rarified species richness with a Generalized Linear Model (GLM) with a normal distribution. Visual inspections of the residuals vs. fitted values were made to ensure homoscedasticity of the data. I test for the significance of the target model relative to the null model with an ANOVA with significance set at the $P < 0.05$ level for all comparisons.

I next tested the hypothesis that variation in setal color pattern is structured across an elevation gradient. Unlike my previous GLM, I used the negative binomial distribution as a link function. I controlled for over dispersion of the predicted values (*i.e.*, Residual Deviance >> degrees of freedom) with the function *glm.binomial.disp()* in the *dispmod* package in R v3.142 (R Development Core Team 2014). Rather than report an *F-statistic* and *p*-value as in my previous GLMs with a Gaussian link, I instead report the '% Deviance Decrease' (%DD) of the target model relative to the null model (Table 4-2). Higher percentages of %DD suggest better fit of the model with the proposed predictor variable (*e.g.*, elevation) relative to the null model. The response variable of

these GLMs is the proportion of bumble bees with red setae on the dorsum of the abdomen and the explanatory variables are elevation and latitude (Table 4-1). Following this same modeling strategy I also investigate the effect of elevation on the different proboscis trait classes. Finally, I incorporate the most significant proboscis functional trait as a predictor variable to observe its effect on the distribution of setal coloration in bumble bee communities (Table 4-2). All statistical analyses and data visualizations were produced with the R v3.142 (R Development Core Team 2014). Species richness and diversity were estimated with functions defined in the *vegan* package in R (Dixon 2003).

Results

In this three year study a total of 4463 specimens ($\bar{x}$ = 1,590, S.E. = 115) representing 15 species were detected across an elevation gradient starting at 300 m above sea level (asl) and ending at 3900 m asl (Fig. 4-2A). The abundance of different bumble bee species detected across plots was highly variable, with *B. vosnesenskii* representing 43% of the total bumble bees detected across all years (Table 4-1, Fig. 4-2A). The least abundant species in this study constituted less than 0.01% of the surveyed fauna: *B. crotchii*, *B. rufocinctus*, and *B. morrisoni*. While *B. vosnesenskii* represented the most abundant bumble bee without red setae, *B. sylvicola* was the most abundant bumble bee with red setae (9% of the total bumble bees) and was predominantly found at high-elevation plots (Table 4-1, Fig. 4-2A, 2B) (Appendix D1 and D2). At plots > 3000 m asl, *B. vosnesenskii* accounted for 0.02% of the bumble bee individuals detected across plots and years (Fig. 4-2A). Despite intensive collection efforts, 17% of the plots had nine or less bumble bee individuals detected and were removed before further statistical analyses.

Removing these plots enabled a robust use of rarefaction techniques in estimating species richness. After this removal, a total of 3735

Table 4-2 Model performance evaluating (A) the proportion of bumble bees with red setae by elevation, latitude, or proboscis length and (B) the proportion of bumble bees belonging to a proboscis trait class by elevation. Model performance estimated as % Deviance Decrease from the Null model.

Model Category	**% Deviance Decrease**
A. Models Predicting Red Setae	
Red~ Elevation	64.38
Red~ Latitude	3.01
Red~ Elevation + Latitude	64.68
Red~ Short Proboscis	1.21
Red~ Medium Proboscis	6.02
Red~ Medium/Long Proboscis	8.21
Red~ Long Proboscis	11.67
Red~ Cuckoo	5.78
Red~ Elevation + Long Proboscis	65.32
B. Models Predicting Proboscis Length	
Short Proboscis~ Elevation	0.4
Medium Proboscis~ Elevation	19
Medium/Long Proboscis~ Elevation	28.96
Long Proboscis~ Elevation	12.97
Cuckoo~ Elevation	7.41

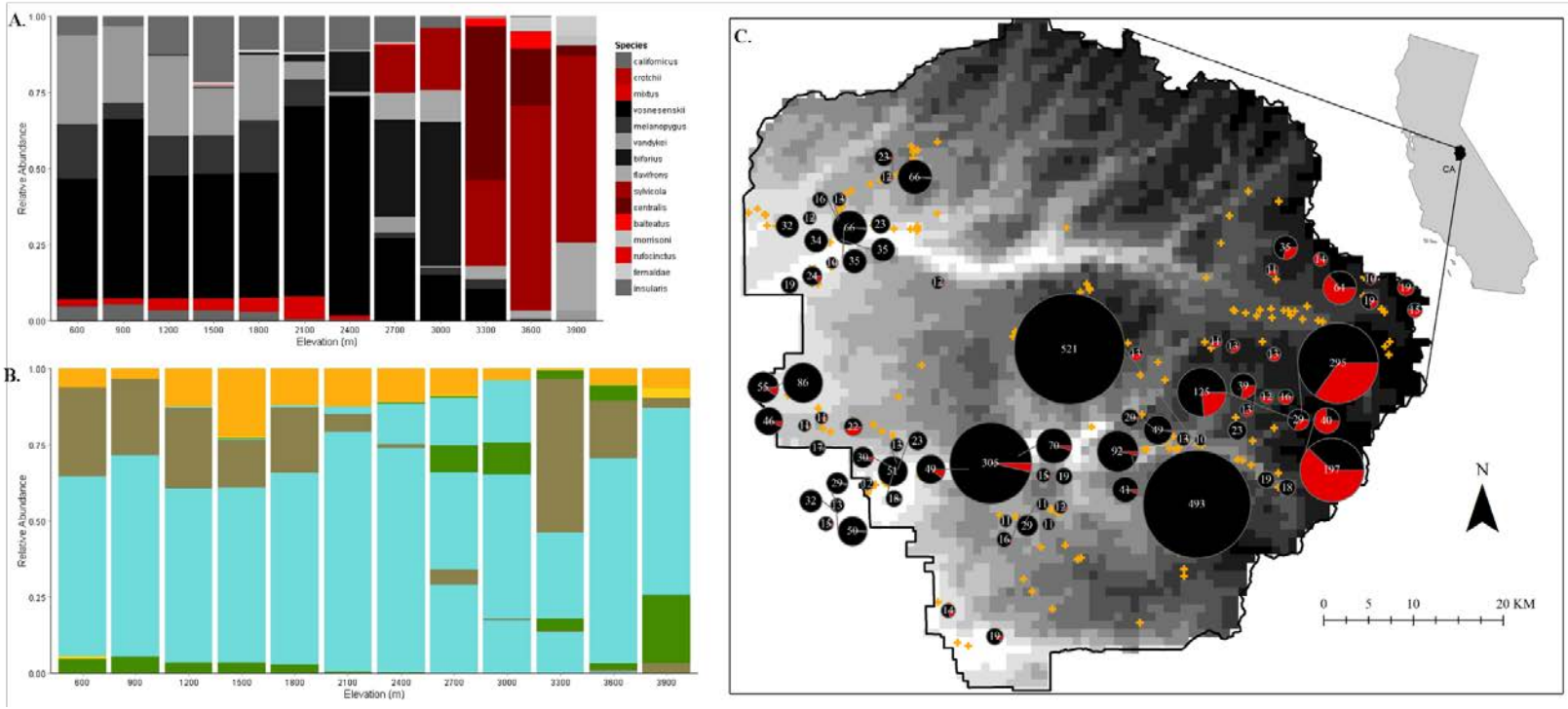

Fig. 4-2 Distribution of bumble bee species pooled across an elevation gradient in Yosemite National Park, California. (A) Species with red setae are represented by the red color ramp while species with no red setae are represented by the gray color ramp. (B) Species-grouped by proboscis length: Yellow = Short, Blue = Medium, Brown = Medium/Long, Green = Long, Orange = Cuckoo. (C) The spatial distribution of survey sites (pie diagrams) by setal color pattern (red = bumble bees with red setae, black = bumble bees without red setae). The size of pie diagram represents the number of specimens detected at the field site. The orange cross-hairs represent sites with nine or less bumble bee specimens detected. Background shading represents increasing elevation.

specimens remained (83% of the total data). I determined the probability of colinearity between the geographic and temporal predictor variables (*i.e.*, elevation, latitude, longitude, and year) and found no significant correlation between variables, except for between elevation and longitude (Pearson's Correlation Coefficient, $r = 0.86$, $P < 0.001$). As the design of this study targeted the effect of elevation on bumble bee community structure, I selected elevation over longitude in constructing the predictive models (GLMs) of diversity and color variability (Fig. 4-1). Given that the Sierra Nevada range runs from north to south in North America (Fig. 4-2C), it makes sense that longitude and elevation would be correlated and possess geographic information representative of the scale in my study.

Elevation, year, and latitude, were not significant predictors of species diversity, evenness, or rarified species richness (GLM-Diversity: $F = 1.08$, $P = 0.35$; GLM-Rarified Species Richness: $F = 1.35$, $P = 0.26$; GLM-Evenness: $F = 2.07$, $P = 0.09$) (Fig. 4-3A-3C). I found a significant and positive correlation between observed species richness and rarefied species (Pearson's Correlation Coefficient Test: $R = 0.66$, $P < 0.001$) (Fig. 4-3D), even though I were unable to reach asymptote in species richness across the majority of field sites with my study design (Fig. 4-4).

As I found no significant differences in species richness and evenness across plots (Fig. 4-3), I elected to pool the plots together across all three years to investigate the effect of elevation and latitude on color variability (Fig. 4-2A). Pooling plots across years provided a more comprehensive dataset to test my hypotheses and capture species with extremely low abundances like *B. crotchii*, *B. rufocinctus*, and *B. morrisoni* (Table 4-1,

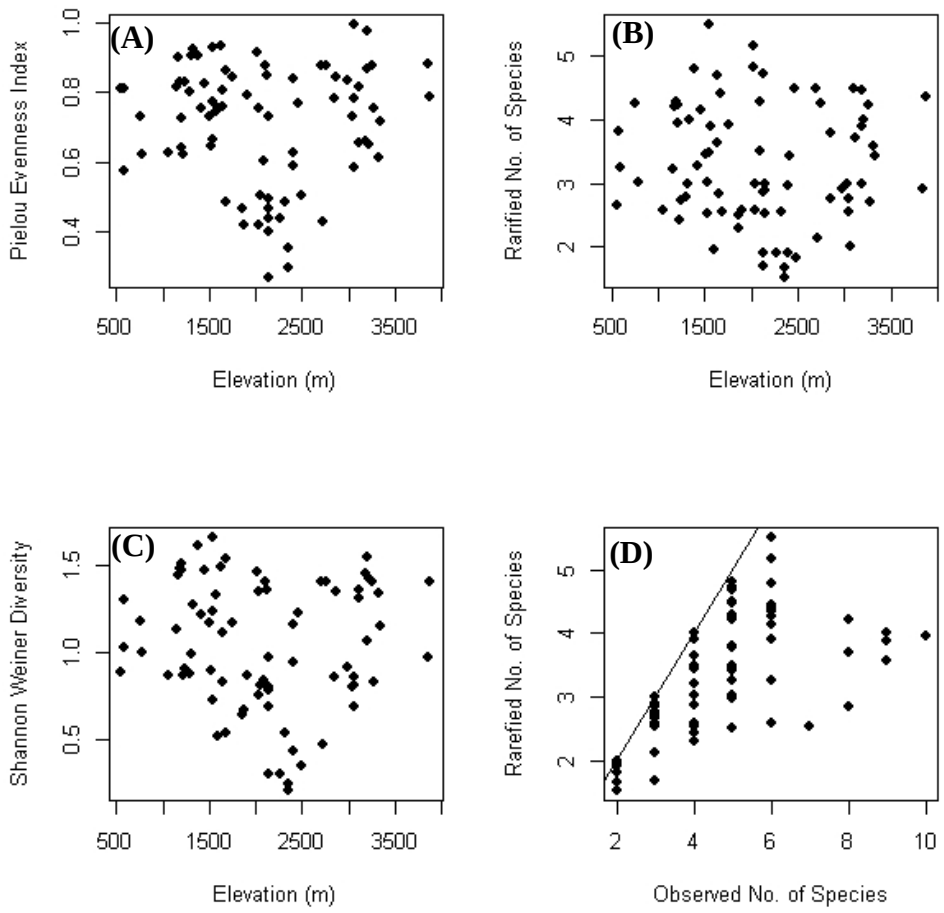

Fig. 4-3 Plot of the realtionship between (A) Pielou's species evenness with elevation, (B) Shannon Wiener diversity with elevation, (C) Rarified species richness with elevaiton, and (D) Rarified species richness with observed species richness.

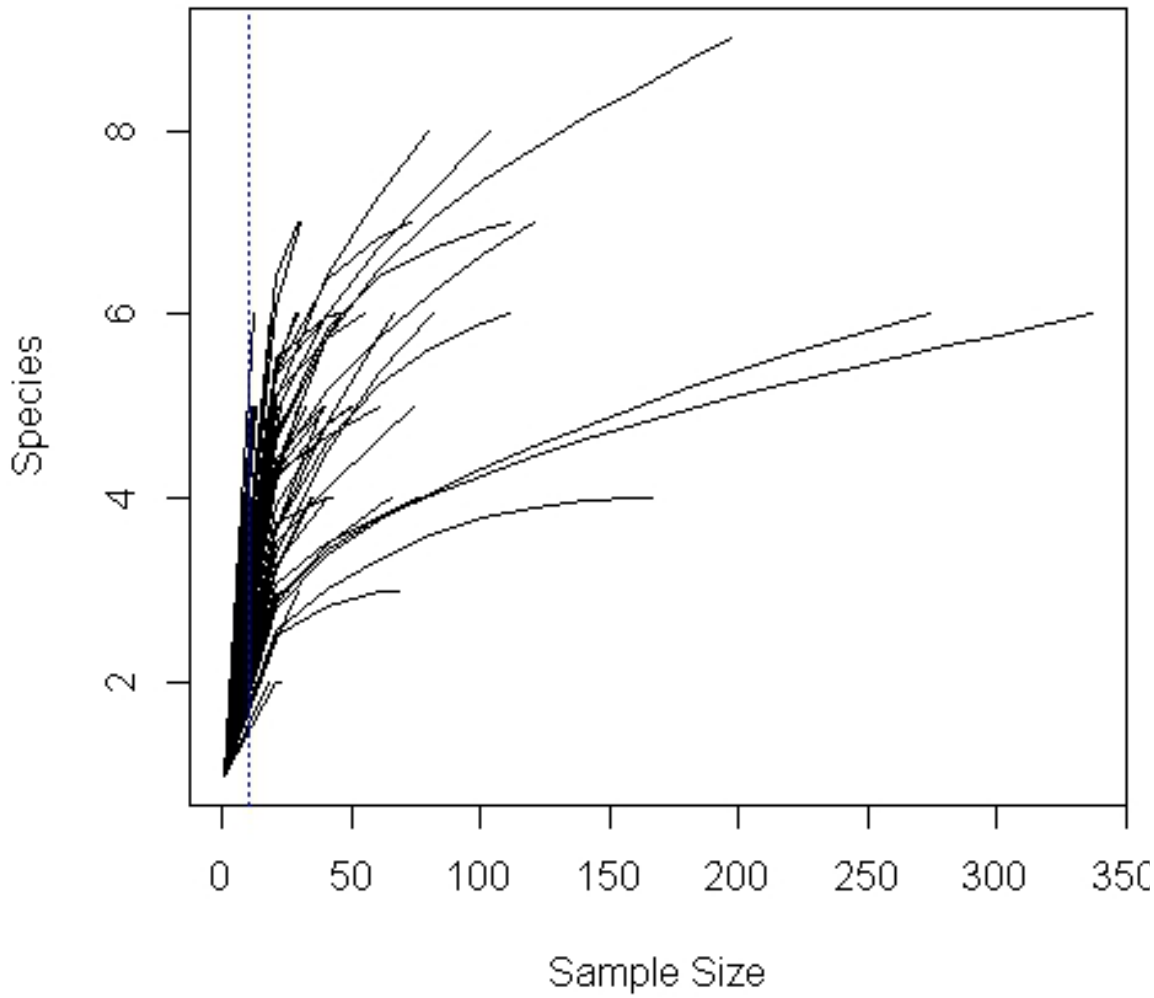

Fig. 4-4 Rarefaction curves for each plot across years in Yosemite National Park. Dashed vertical line represents *n* = 10, the random number of specimens used in rarefaction analysis.

Fig. 4-2A). I found a significant and positive relationship between the proportion of bumble bees with red setae and elevation (Red~ Elevation: %DD from Null Model = 64.38%; Elevation Coefficient = 1.72e-03, P < 0.001) (Table 4-2, Fig. 4-5). When latitude was included as predictor variable with elevation I found no further predictive power in the variable (Red~ Elevation + Latitude: %DD from Null Model = 64.38%; Elevation Coefficient = 1.72e-03, *P* < 0.001; Latitude Coefficient = 4.43e01, *P* = 0.76) (Table 4-2). Testing for the effect of proboscis length on the proportion of bumble bees

with red setae exhibited lower %DD relative to the model exclusive to Elevation as a predictor variable (*i.e.*, Red~ Elevation) (Table 4-2). Furthermore, testing for the effect of elevation on the proportions of bumble bees belonging to different proboscis trait classes did not result in large %DD from the null model (%DD Range: 0.4 - 28.96%) as found in the Red~ Elevation model (Fig. 4-5).

Discussion

In this study I examined the role of Müllerian mimicry in predicting the structure and composition of bumble bee communities across an elevation gradient in the Sierra Nevada of California, USA. I found that high-elevation communities were composed of bumble bees with significantly more red setae than low-elevation communities, a result consistent with mimetic-evolution hypotheses generated well over a century ago (Franklin 1912; Milliron 1971; Plowright and Owen 1980). Furthermore, I found that bumble bees with black and yellow banding were substantially more numerous than bumble bees with red setae, particularly *B. vosnesenskii*. I also found that communities across an elevation gradient were represented by one or more species of each functional proboscis trait class, suggesting that bumble bee species overlap in the utilization of floral resources (Fig. 4-2A). The results of my study provide evidence that community structure of closely-related taxa that share similar resources and habitats are likely modulated by Müllerian mimicry rings.

Müllerian mimicry as explanation for the regional convergence of aposematic coloration patterns of different bumble bee species has been proposed by numerous authors (Thorp et al. 1983; Prys-Jones et al. 1987; Goulson 2003; Heinrich 2004). Specifically, it is the convergence of aposematic coloration patterns that has been

suggested to be a mechanism that enables species to avoid predation (Plowright and Owen 1980; Williams 2007). For a Müllerian mimicry ring to be successful, potential predators must be trained to distinguish between palatable and unpalatable prey. An experimental study by Brower et al. (1960)

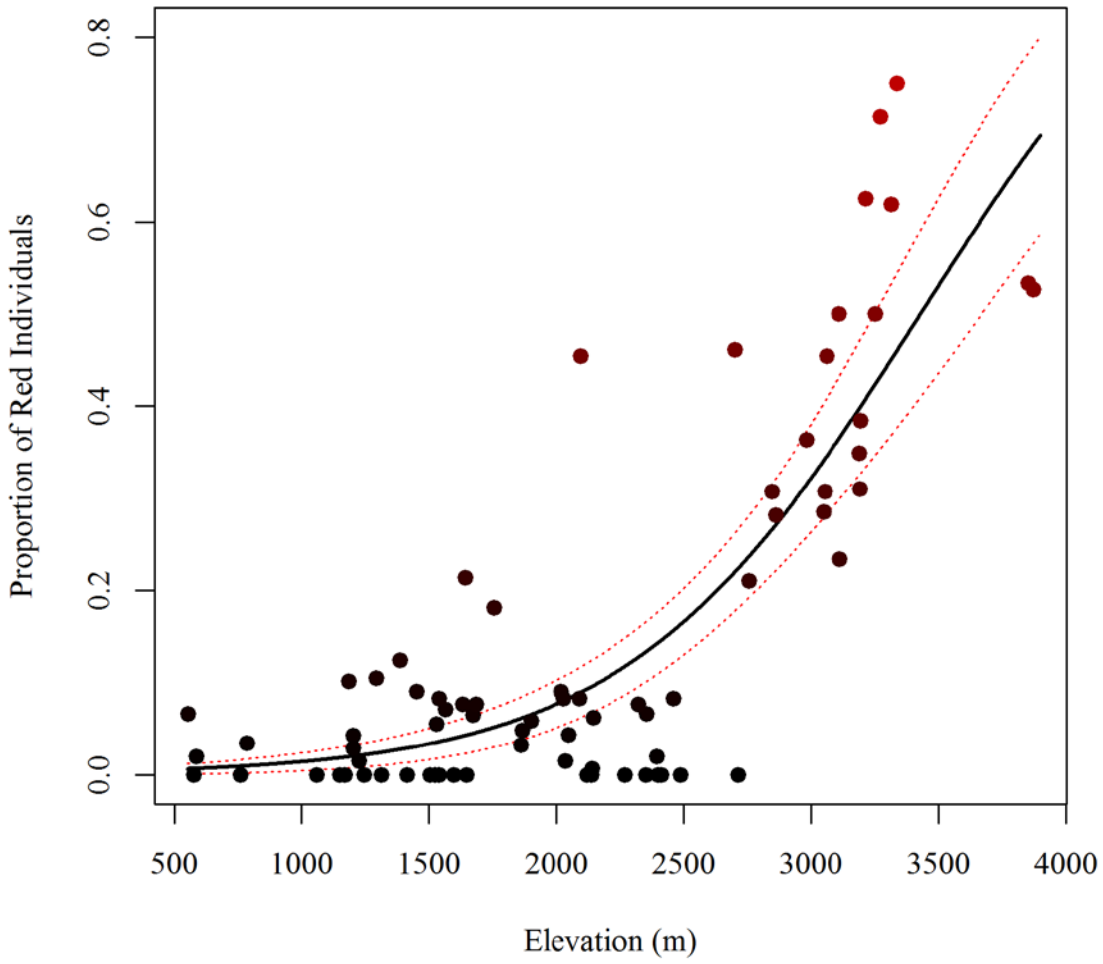

Fig. 4-5 The proportion of bumble bees with red setae across an elevation gradient in Yosemite National Park, California. The black line represents the model fit and the dashed lines represent the 95% confidence intervals of the model. The black to red color gradient of the points represents the relative abundance of bumble bees in each field site representing the red phenotype.

found that toads would avoid eating the bumble bee *B. pensylvanicus* (=*Bremus americanorum*) and its Batesian mimic, *Mallota bautias* (Diptera: Syrphidae) after being stung in the initial trial by *B. pensylvanicus*. In their study, they also found that a naïve toad would readily eat *B. pensylvanicus* and the Batesian mimic if the stinger was first removed from *B. pensylvanicus* in the initial trial. Their experiment provides evidence that the experience of being stung will likely elicit avoidance of a bumble bee and its mimics in a vertebrate predator. Among predatory insects, a dragonfly species, *Aeshna grandis* (Odonata: Aeshnidae) was observed to avoid palatable prey (Dipteran) when they physically represented the aposematic and venomous wasp species, *Vespula norwegica* (Hymenoptera: Vespidae) (Kauppinen and Mappes 2003). The authors found that when they painted dipterans yellow and black, *A. grandis* avoided them more frequently than individuals that were not painted or were painted solely yellow or black. The significance of yellow and black coloration of animals as an effective warning coloration pattern as a signal of being distasteful, noxious, or poisonous has a long and rich history of observation and hypothesis testing (Poulton 1890; Schuler and Hesse 1985; Pfennig et al. 2001; Kauppinen and Mappes 2003). In my study, the most abundant bumble bees are black and yellow banded and are co-located, which supports the hypothesis that aposematic body coloration patterns is an effective strategy in warning predators.

Mimicry as a phenomenon to evade predation has been investigated for a diversity of fauna. In a study on the spatial abundance of kingsnakes, *Lampropeltis triangulum elapsoides* (Squamata: Colubridae) a Batesian mimic of coral snakes, *Micrurus fulvius*, (Squamata: Elapidae), it was found that the number of predator attacks on kingsnakes when in sympatry with their noxious models was significantly lower than when the

kingsnake was in allopatry with the noxious model (Pfennig et al. 2001). In the same study, it was found that decreasing abundance of the noxious model was correlated with an increase in the number of predatory attacks on the Batesian mimic. While Pfennig et al. (2001) was unable to determine why the Batesian mimic occurred outside of the range of the noxious model, they were able to conclude that the abundance of the noxious model has likely contributed to the persistence of the non-noxious mimic. In the case of bumble bees, *B. vosnesenskii* is the most abundant bumble bee up ~3000 m in my study. Bumble bees that converge on the coloration pattern of *B. vosnesenskii* (Fig. 4-1) are predominantly co-located in my study. Furthermore, the males of *B. vosnesenskii* are excellent mimics (automimicry) of female *B. vosnesenskii* while *B. vandykei* males are not (Fig. 4-1G). However, *B. vandykei* males may be a Batesian mimic of females of another species, *B. morrisoni* (Fig. 4-1L), which co-occurs with *B. vandykei* at mid-elevation (Fig. 4-2A). Further investigation on the effect of male coloration pattern on predation risk may elucidate why some bumble bee species may be less abundant than others. It is possible that the abundance of some bumble bee species may be the result of males not converging to the regional mimicry pattern (automimicry or Batesian mimicry) and experiencing increased predation (Kauppinen and Mappes 2003). In addition to automimicry, an alternative hypothesis to the variability in male setal color includes thermoregulation (Stiles 1979; Williams 2007). Bumble bee males typically emerge late during colony growth and do not collect pollen and nectar as do female bumble bees. Unlike female workers, males may not return to the nest, and are known to rest on flowers and leaves overnight. The adaptation towards having longer and brighter setae in

male bumble bees may be a selective advantage in persisting during cold nights associated with high-elevation and latitude environments (Stiles 1979; Williams 2007).

Proboscis length has also been inferred to be an important factor in shaping bumble bee communities. In my study I did not a find a significant effect of elevation on the distribution of bumble bee proboscis lengths (Fig. 4-2B, Table 4-2). This result was expected as previous studies of sympatric populations are composed of species representing a diversity of different proboscis lengths. This hypothesis is predicated on the partitioning of floral resources across a bumble bee community. I also did not detect any significant trend in species richness or evenness across the elevation gradient. The only significant pattern I observed in my study was the relationship between bumble bee aposematic color patterns across an elevation gradient. While I did not directly test for the effect of aposematic coloration patterns on predation, historic experiments on insects with contrasting coloration patterns support my hypothesis that Müllerian mimicry has structured bumble bee communities in Yosemite (Kauppinen and Mappes 2003). Alternative hypotheses for the distribution of color patterns I observed in Yosemite may be attributed to crypsis or thermoregulation (Stiles 1979; Williams 2007). However, while carefully crafted arguments have been made to support these hypotheses, experimental evidence for these proposals are lacking and should be investigated in future studies.

This survey of bumble bees in the Sierra Nevada represent one of the largest elevation gradients (range: 3600 m or 11,811 ft.) studied in the context of bumble bee community composition and turnover. Given the dramatic differences in habitat types across the elevation gradient, I have likely detected species that are specific to a plant

community type or thermal tolerance (Stiles 1979; Thorp et al. 1983; Ploquin et al. 2013). *Bombus crotchii*, for example, is primarily found at low-elevation sites (Thorp et al. 1983), whereas *B. balteatus* and *B. sylvicola* are alpine specialists (Koch et al. 2012, Koch et al. submitted). The latter two species, along with *B. bifarius*, have been found to exhibit regional color variability, which supports my hypothesis that local selection of aposematic coloration patterns likely structure bumble bee communities. In turn, the results of my study support the theory that Müllerian mimicry is a significant selective force that has shaped the contemporary distribution of the some 250 bumble bee species distributed worldwide.

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CHAPTER 5

RANGE EXTENSION OF TWO BUMBLE BEE SPECIES (HYMENOPTERA: APIDAE) INTO OLYMPIC NATIONAL PARK[5]

ABSTRACT

Bumble bees (Hymenoptera: Apidae, *Bombus*) are cold adapted insects, important in providing ecosystem services to wild and cultivated flowering plants. Recent expeditions into the wilderness regions of Olympic National Park, USA discovered undocumented populations of two bumble bee species: *Bombus sylvicola* and *B. vandykei*. Application of species distribution models with range-wide locality records identified the Olympic Mountains to have high habitat suitability for *B. sylvicola* and low habitat suitability for *B. vandykei*. My results suggest that Olympic National Park is a habitat island for *B. sylvicola*, isolated from the relatively contiguous distribution of the species in the Cascade and Sierra Nevada mountain ranges. As bumble bees are sensitive to environmental change, this discovery will likely stimulate conservation-oriented investigations on these charismatic pollinators on the Olympic Peninsula and throughout the Pacific Northwest.

INTRODUCTION

The majority of bumble bee species (Hymenoptera: Apidae, *Bombus*) are primitively eusocial insects that depend on pollen and nectar from flowering plants for development and survival. Although there are several arid- and tropical-adapted species, bumble bees are the most diverse in alpine and temperate environments with highest species diversity found in the mountains of central China (Hines 2008). Within the past

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decade several bumble bee species have declined at local and regional spatial scales across the planet (Cameron et al. 2011, Goulson et al. 2008). In the western USA, it is likely that *Bombus franklini* may already be extinct, while a closely related species, *B. occidentalis* has rarely been detected west of the Sierra Nevada and Cascade Mountains in the last decade (Cameron et al. 2011, Kevan 2008). Despite contemporary sightings of *B. occidentalis* within the city of Seattle, the Olympic Peninsula, and throughout the states of Washington and Idaho, abundances of *B. occidentalis* across its distribution in the Pacific Northwest continue to be low (Doughton 2013, Rhoades et al. submitted).

Recent surveys of bumble bees in the Olympic Mountains in Olympic National Park in the state of Washington, USA discovered the presence of two bumble bee species poorly documented from the mountain range (Figure 5-1). The forest bumble bee, *B. sylvicola*, is distributed primarily in high-elevation (> 1000 m) and high latitude environments (Thorp et al. 1983). The species is found in the Rocky, Sierra Nevada, and Cascade Mountain ranges, is common throughout Alaska as far north as the Arctic Ocean (Figure 5-1a) (Koch et al. 2012, Thorp et al. 1983), and is sparsely distributed as far east as Labrador in Newfoundland, Canada (GBIF 2014). The only specimen of *B. sylvicola* on the Olympic Peninsula was collected more than 90 years ago by P.G. Putnam near Mt. Elinor, outside of Olympic National Park (Figure 5-1a) (US NPID 2014). This record of *B. sylvicola* was only recently discovered during a recent digitization effort of museum specimens. It was never included in any taxonomic keys of western bumble bees, nor was the Olympic Peninsula historically identified as a possible region suitable for *B. sylvicola* habitation (Koch et al. 2012, Stephen 1957, Thorp et al. 1983). While a recent comprehensive field guide of North American bumble bees identifies the Olympic

Peninsula as suitable *B. sylvicola* habitat, it does not document an occurrence record of the species on the Olympic Peninsula in the included range maps (Williams et al. 2014).

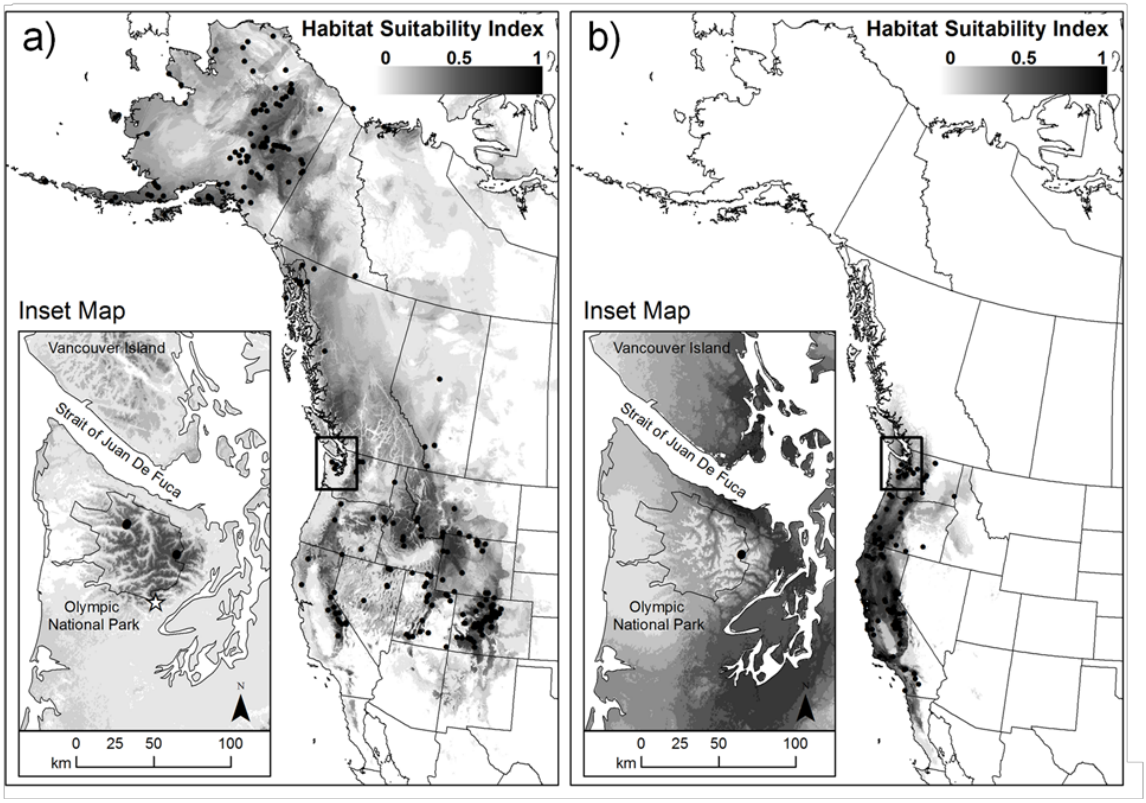

Figure 5-1. The geographic distribution of (a) *B. sylvicola* and (b) *B. vandykei* across western North America and the Olympic Peninsula. Black points represent occurrence records. The white star in the inset map of 1a represents the location of a *B. sylvicola* collected by P.G. Putnam near Mt. Elinor in 1922. Grayscale shading on the maps represents habitat suitability (HS) with dark shading representing high HS and light shading representing low HS.

The van Dyke bumble bee, *B. vandykei*, was also discovered in the Olympic Mountains. Like *B. sylvicola*, *B. vandykei* was not recorded from the Olympic Peninsula in Thorp et al. (1983), although Williams et al. (2014) document *B. vandykei* in the Puget Lowlands ecoregion (U.S. Environmental Protection Agency 2013) near Lake Cushman (Ascher and Pickering 2015) and Olympia, Washington, only recently discovered by Koch (2011). These localities are approximately 50 and 100 km from my contemporary

sightings within Olympic National Park, which represent a new ecoregion (the High Olympics ecoregion) and habitat for this bumble bee species.

In this paper I summarize the abundance and distribution of *B. sylvicola* and *B. vandykei* detected in my field survey of Olympic National Park. I also estimate the distribution of habitat suitability for both species throughout western North America with species distribution models (SDMs). Finally, I briefly discuss the value of rigorous wilderness surveys of insect taxa, and the utility of presence-only SDMs in predicting the distribution of uncommon species in unsurveyed areas within a species' predicted geographic range.

METHODS

Surveys were conducted on 27 of August 2010, 15 – 17 of July 2013, and 6 – 7of August 2014 along wilderness trails and minor roads in Olympic National Park (Table 5-1). Sites were surveyed by teams of individuals using random and opportunistic net collections of bumble bees in approximately 0.5 ha plots following methods described in Cameron et al. (2011). Collectors used aerial insect nets to survey bumble bees foraging on flowers for approximately 90 minutes per site. Bees were placed into 20 mL plastic vials, immobilized on ice for ~5 min, then identified in the field to species. Field species identifications follow Stephen (1957), Thorp et al. (1983), and Koch et al. (2012). All specimens collected in 2010 and 2014 were retained and curated into the National Pollinating Insect Collection in Logan, Utah. A voucher representing males and female workers from each site was retained to confirm species identifications in the 2013 survey. Most specimens were released after the 2013 survey event, with a tarsal clipping of each individual collected and retained in 95% ethanol for subsequent molecular confirmation of identification.

Bumble bees are notoriously difficult to identify due to cryptic and convergent setal coloration patterns across taxa (Thorp et al. 1983). I confirmed species identities of *B. sylvicola* and *B. vandykei* by sequencing a region of the Cytochrome c oxidase subunit I gene (COI) in mtDNA. COI has proven to be a useful gene in distinguishing between cryptic insect species, including bumble bees (Williams et al. 2012). I used this popular 'species barcoding method' to avoid confusing *B. sylvicola* for the morphologically similar and more common *B. melanopygus* (Stephen 1957).

Total DNA was extracted from the mid-leg of the field-collected specimens using a 5% Chelex100^{TM} (BioRad, Hercules, CA) extraction protocol following Strange et al. (2009). Reference specimens of *B. vandykei* were collected from Okanogan County, WA and Jackson County, OR (GenBank accession numbers: KJ845648 and KJ845649, respectively). For the reference *B. vandykei* specimens, I extracted total DNA with a Roche High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany). A reference specimen of *B. sylvicola* was not needed as approximately 45 partial COI sequences are published in the genetic sequence database GenBank and were available for comparison. PCR was used to amplify a 1064 base-pair region of the COI gene with the primer pair: forward 5'-ATAATTTTTTTTATAGTATAC-3' and reverse 5'GATATTAATCCTAAAAAATGTTGAG-3' described in Tanaka et al. (2001). The total 25 µL reaction volume consisted of 1 – 2 µL of template DNA (~200 ng/uL), 1x Promega (Madison, WI) reaction buffer, 0.6 mM dNTP mixture, 0.2 µM of each primer, 0.2 units Taq Polymerase (Promega, Madison, WI), 2 mM of $MgCl_2$ (Promega, Madison, WI), and molecular grade H_2O to final volume. The PCR conditions follow Tanaka et al. (2001). PCR products were confirmed on a 1.4% agarose gel and sequenced with an

Applied Biosystems 3730xl automatic sequencer. Sequences were aligned and analyzed with Geneious v.7 (Biomatters, http://www.geneious.com/).

To estimate the geographic distribution of habitat suitability (HS), SDMs were constructed under the principle of maximum entropy with MaxEnt v3.3.1 (Phillips and Dudík 2008). Similar species distribution modelling approaches are described in Williams et al. (2014) and Cameron et al. (2011). In the MaxEnt framework HS is constrained between 0 and 1 where values closer to 0 represent low HS, and values closer to 1 represent high HS. SDMs were developed by aggregating range-wide georeferenced distribution records with a suite of informative bioclimatic variables. Occurrence records of *B. sylvicola* and *B. vandykei* were queried from the Global Biodiversity Information Facility (GBIF 2014). Ten averaged contemporary bioclimatic (1950 – 2000) variables were downloaded from the WorldClim database (http://worldclim.org; Hijmans et al. 2005) at a spatial resolution of 30 arc seconds (~1 km). The variables were chosen to reflect seasonal variation in temperature and precipitation across the species' ranges. The variables include the following: Mean Temperature of Warmest Quarter, Precipitation of Seasonality, Precipitation of Wettest Quarter, Precipitation of Driest Quarter, Precipitation of Warmest Quarter, Mean Diurnal Range, Isothermality, Temperature Annual Range, Mean Temperature of Wettest Quarter, and Mean Temperature of Driest Quarter. Spatial data processing and visualization of the SDMs was executed in ArcGIS 10.1 (ESRI 2012). SDMs were averaged over 50 replicates with each replicate cross-validated with a 10-fold partition of the data. Models were evaluated with the area under the curve statistic (AUC), where AUC values closer to 1 indicate good model fit and values closer to 0 indicate poor model fit.

RESULTS

Between 2010 and 2014 I surveyed 15 areas in Olympic National Park. *Bombus sylvicola* was detected in six surveyed field sites, all at elevations above 1150 m, near the upper limit of the forested zone (Table 5-1). The majority of the specimens collected in the Olympic Mountains were female workers (n = 96), with four queens also observed at the highest sites. I surveyed bees foraging on a variety of native flowering plants including *Lupinus latifolius*, *Phyllodoce empetriformis*, and *Vaccinium* spp. *Bombus vandykei* was detected in two survey sites, three specimens at a high-elevation site (1421 m), and one specimen at a site slightly lower (1177 m). Bees were collected foraging on *Cirsium* sp. and *L. latifolius*. Throughout most of its range *B. vandykei* can easily be mistaken for the abundant *B. vosnesenskii* due to convergence in color banding patterns; however, the phenotype collected in my survey did not reflect the typical dark setae phenotype (Thorp et al. 1983). Unlike the commonly observed *B. vandykei* phenotype, which has black setae on tergum 1 and tergum 2 (Koch et al. 2012), the specimen collected in my survey had admixture of yellow setae on the lateral margins of tergum 1 and tergum 2. This less common phenotype was documented in Thorp et al. (1983) from the north Cascade Mountains, but was never observed in any other portions of the species range. Voucher specimens are housed at the USDA National Pollinating Insect Collection in Logan, UT.

The final aligned COI fragment for *B. vandykei* and *B. sylvicola* was 939 base-pairs (GenBank accession numbers: KJ845647 and KJ845650, respectively). However, I reduced the *B. sylvicola* COI fragment size for the final analysis to 436 base-pairs due to limited overlap with the published COI fragments in GenBank (Accession number: JX833556). Pairwise comparisons of the COI sequences between the field-caught and

reference *B. sylvicola* and *B. vandykei* specimens found 0.009% and 0% differences in nucleotide substitutions, respectively. The genetic data in this study further confirm that the specimens detected in Olympic National Park are *B. sylvicola* and *B. vandykei*.

Table 5-1. Summary of abundance and distribution of *B. sylvicola* and *B. vandykei* detected in 2010, 2013, and 2014 standardized bumble bee survey in Olympic National. *All day effort (> 2 hours) to survey bumble bees along a road and trail.

Species	Year	Location	Latitude	Longitude	Elevation	Survey	W	Q	M
sylvicola	*2010*	Royal Lake Tr.	47.8478	-123.2161	1402m	1.5h	9	1	10
sylvicola	*2013*	Heart Lake	47.9109	-123.7330	1460m	1.5h	10	0	0
sylvicola	*2013*	Lower Royal Basin	47.8391	-123.2113	1421m	1.5h	2	0	0
sylvicola	*2013*	Royal Basin R.S.	47.8330	-123.2112	1564m	1.5h	8	4	0
sylvicola	*2013*	Lower Bridge Creek	47.9241	-123.7334	1163m	2.0h	8	0	0
sylvicola	*2014*	Obstruction Point Rd./Grand Lake Tr.	47.9681	-123.4805	1458-1896m	-*	59	0	27
vandykei	*2014*	Lower Royal Basin	47.8391	-123.2113	1421m	1.5h	3	0	0
vandykei	*2014*	Royal Basin Tr.	47.8591	-123.2028	177m	-*	1	0	0

DISCUSSION

Our rediscovery of *B. sylvicola* and detection of *B. vandykei* populations in the Olympic Mountains underscores two important aspects of understanding species ranges. The first is the value of thorough investigations into historic collections and the subsequent digitization of these specimens, and the second is the value of surveying insects along wilderness trails, away from major transportation corridors. Furthermore,

given the concerted effort across multiple natural history collections to digitize specimens, it is also of great importance that data are publically available for wide dissemination (Cameron et al. 2010). Many biodiversity inventories of insects and other taxa are collected near roadsides or other relatively accessible localities (Dennis and Thomas 2000, Graham et al. 2004), due perhaps to the high monetary and physical costs of surveying remote areas (Funk et al. 2005). The impacts of this particular collecting bias are well documented, and can lead to poorly delineated range maps, inaccurate prediction of biodiversity hotspots, and knowledge gaps for entire habitat types and geographic areas (Newbold 2010). My study conforms to other work demonstrating that surveys in wilderness areas have the capacity to generate new knowledge about uncommon or unknown species (Messinger 2006, Wilson et al. 2010).

The records documenting the distribution of *B. vandykei* in western Washington by Williams et al. (2014) were only collected within the past 7 years by co-authors J. Koch and J. Strange (Cameron et al. 2011), highlighting the value of contemporary surveys for bumble bees in the United States (Cameron et al. 2010). Considering that the legacy of bumble bee research in the western US spans a century, and that bumble bees are highly important pollinators and are in decline (Cameron et al. 2011), the recent discovery of *B. sylvicola* and *B. vandykei* in Olympic National Park is important to conservation biologists and park managers alike (Cameron et al. 2010).

The species information gathered from the Olympic Mountains in Olympic National Park will be of great use to future efforts in bumble bee conservation. Because *B. sylvicola* is distributed primarily in high-elevation habitat, and the Olympic Mountains are separated from other mountain ranges (e.g. Cascade Range and Vancouver Island

Ranges) by regions with low habitat suitability (Figure 5-1), it is likely that the Olympic Mountain population has reduced opportunities for gene flow to other populations (Lozier et al. 2013). To test this hypothesis, future work is needed to estimate the genetic diversity of the Olympic Mountains *B. sylvicola* population and to examine the degree of contemporary gene flow into adjacent populations.

In this study I demonstrate the utility of SDMs to guide surveying efforts of bumble bee species that are at the edge or outside of their documented distribution. Unlike range and extent maps commonly used in taxonomic keys and field guides, greater precision in surveying decisions can be made with SDMs. Increased chance for success in surveying uncommon species with SDMs is of great value in the field of conservation biology, especially in the context of a changing climate (Bartomeus et al. 2011, Williams et al. 2009) and restricted budgets. It is not particularly surprising that the Olympic Peninsula has not been generally identified as a region with suitable *B. sylvicola* habitat (but see Williams et al. 2014). Bumble bees are notorious for being misidentified, even by the most seasoned taxonomists, and it is possible that *B. sylvicola* has been misidentified as *B. melanopygus* in the region (Stephen et al. 1957). In the case of *B. vandykei*, the Olympic Mountain population appears to truly be at the edge of the species' bioclimatic niche (low HS), and may be under different selection pressures, as suggested by a locally distributed phenotype of another bumble bee species, *B. bifarius*, found in the San Juan Islands (Lozier et al. 2013). However, to confirm misidentifications and test species concepts, an investigation of the specimens used in previous taxonomic studies from the area would need to be conducted.

Bumble bees are model insects to support the conservation of wild lands as they are easily recognizable by the general public and citizen scientists (Koch et al. 2012), are obligate pollinators of native flowering plants, and are well understood to be important ecosystem service providers (Kearns et al. 1998). National Parks provide an opportunity to study bees in environments with low human impacts, free from the confounding effects of immediate urbanization and agricultural intensification (Winfree et al. 2009). Bumble bee declines also serve as a warning to the effects of global climate change (Bartomeus et al. 2011), and are likely to be exacerbated by increases in temperature and variable precipitation patterns over the next 80 years in the Pacific Northwest and beyond (Mote and Salathé 2010). Thus, Olympic National Park is an important natural area for the investigation of the impacts of global change on bumble bee biology and stability of pollination services.

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CHAPTER 6

PATTERNS OF POPULATION GENETIC DIFFERENTIATION ACROSS BUMBLE BEE COMMUNITIES IN THE PACIFIC NORTHWEST [6]

Abstract

Multiple bumble bee species are declining due to a diversity of anthropogenic activities. However, little research has been conducted to examine underlying population genetic diversity and structure of wild, putatively non-threatened bumble bees. In this study, I compare average population genetic diversity across conspecific populations of four bumble bees in the Olympic and Cascade Mountains and San Juan Islands located in the western Washington, USA using microsatellite loci. I found that bumble bee populations distributed in the Olympic Mountains and San Juan Islands had significantly less genetic diversity than conspecific populations distributed in the Cascade Mountains. Testing for the effect of isolation by geographic distance (IBD) with linearized F_{st} found that the alpine specialist *Bombus sylvicola* exhibited significant IBD across subpopulations. In contrast, my assessment of both *B. melanopygus* and *B. flavifrons*, two species that are broadly distributed across an elevation gradient, did not find a signature of IBD, a result further corroborated by Bayesian *a priori* population assignment tests. Finally, *B. mixtus*, also a broadly distributed species in North America exhibited significant IBD across subpopulations, with an Island and west Cascade population assigned to a distinct lineage with respect to populations detected in the Olympic and Cascade Mountains. My results suggest that bumble bees distributed in the Olympic

[6] This chapter is co-authored with Chris Looney, Walter Sheppard, and James Strange. Permission has been granted by the required coauthors for this research to be included in my dissertation (Appendix A). This chapter is formatted for *Conservation Genetics.*

Mountains represent a distinct genetic lineage relative to the Cascade Mountains, with *B. sylvicola* likely experiencing the greatest degree of genetic drift relative to *B. flavifrons*, *B. melanopygus*, and *B. mixtus*. Given the geographic isolation of the Olympic Mountains from the Cascade Mountains, I speculate that the alpine specialist, *B. sylvicola* will be most at-risk for habitat loss due to effects of global climate change in the Pacific Northwest.

Introduction

Examining the relationship between global change and the distribution of genetic diversity among conspecific populations is an underlying goal in biodiversity studies (Hoffmann and Willi 2008; Manel and Holderegger 2013). Genetic diversity is the raw material of evolution, a reflection of neutral and adaptive processes facilitating the fitness and ultimate survival of a population or species. Maintenance of genetic diversity is strongly driven in part by dispersal-mediated gene flow among populations (Kokko and López-Sepulcre 2006). In fact, signatures of adaptation to a novel environment may be observed in the patterns of genetic diversity within a population. Failure to adapt may result in the extinction of the population, and a loss of genetic diversity for the species (Erwin 1991; Moritz 1994). The geographic distribution and ecological niche of a species have been found to strongly influence patterns of genetic diversity and gene flow observed across a diversity of taxa (Lozier et al. 2013). In the context of global change, some species may be at risk of reduced gene flow across populations due to decreased opportunities for migrants to disperse across favorable habitats (Kokko and López-Sepulcre 2006; Norberg et al. 2012).

Bumble bees (Hymenoptera: Apidae, *Bombus*) are a temperate-adapted genus of primitively eusocial insects that are dependent on a variety of floral resources for the pollen and nectar on which they feed (Goulson 2003). They provide 'ecosystem services' as pollinators of both wild and economically important flowering plants, second only to the European honey bee in agricultural importance (Delaplane and Mayer 2000; Kleijn et al. 2015). It is estimated that there are 250 different bumble bee species worldwide, 30 of which are distributed in the western US (Koch et al. 2012; Williams et al. 2014). Community composition of bumble bees strongly varies across latitude and elevation gradients both functionally and taxonomically (Hoffmann and Sgrò 2011; Bartomeus et al. 2011; Miller-Struttmann et al. 2015). Many species that overlap in their geographic distribution also co-exist in communities where floral resources are partitioned across taxa relative to the length of their proboscis (Inouye 1980). As bumble bees co-exist in an ecological community to varying degrees of abundance (Bowers 1985), they prove to be an excellent model in studying the differential effects of climate and topography on the distribution of genetic diversity and structure (Lozier et al. 2013; Jha 2015).

Rapid climate change since the industrial revolution has adversely affected the distribution a diversity of organisms and ecosystems (Hoffmann and Sgrò 2011; Bartomeus et al. 2011; Miller-Struttmann et al. 2015). An assessment of over a century's worth of museum records has found multiple bee species to be emerging from winter dormancy up to 10 days earlier than historic records, paralleling increases in global temperature (Bartomeus et al. 2011). Furthermore, montane distributed bumble bee species have increased population genetic differentiation relative to species distributed in basins and valleys (Lozier et al. 2011; Lozier et al. 2013), with some alpine species

experiencing rapid evolutionary change in proboscis length due to the decline of floral resources (Miller-Struttmann et al. 2015). A diversity of studies suggest that alpine adapted species may be exposed to greater extinction risk from global climate change, relative to species that have broader ecological or bioclimatic niches (Hoffmann and Willi 2008; Williams et al. 2009; Hoffmann and Sgrò 2011; Rubidge et al. 2012; Miller-Struttmann et al. 2015). However, little is known about the contemporary environmental distribution of genetic diversity in co-occurring bumble bee species, particularly in the western United States. Given the wealth of studies documenting the decline of bumble bees across the globe (Williams and Osborne 2009; Cameron et al. 2011), an assessment on the underlying genetic structure and diversity of bumble bees in the context of their distribution and ecological niche will help prioritize conservation and management needs (Cameron et al. 2010).

At least 15 bumble bee species are found in the Pacific Northwest and distributed across an elevation gradient in the Olympic and Cascade mountain ranges in western Washington (Stephen 1957; Koch et al. 2012). Thought to be a well-studied bee genus in North America (Williams et al. 2014), a recent survey of bumble bees has expanded the distribution of two climatically restricted species into remote high-elevation regions of the Olympic Mountains (Chapter 5). In the Pacific Northwest bumble bees can be found in a diversity of habitats including high alpine meadows, along the Pacific coastline, and in human-altered landscapes (Strange et al. 2013). However, in the next 80 years the Pacific Northwest is projected to incur rates of warming by up 1°C per decade and a 1-2% increase in annual precipitation, likely facilitating wetter autumns and drier summers and winters (Mote and Salathé 2010). The complexities of the Pacific Northwest

landscape and climate coupled with the diversity of bumble bees that inhabit the region provide an opportunity to test the differential effects of environmental heterogeneity on population genetic structure.

This study examines population genetic diversity and structure across populations of four bumble bee species distributed throughout the Pacific Northwest, *Bombus sylvicola*, *B. mixtus*, *B. melanopygus*, and *B. flavifrons* (Figure 6-1). I specifically investigated populations distributed in two mountain provinces, the Olympic Mountains and the Cascade Mountains, and the San Juan Islands of the Puget Sound in Washington. All four bumble bees are of subgenus *Pyrobombus*, and are broadly distributed across western North America (Cameron et al. 2007; Koch et al. 2012). *Bombus sylvicola* is primarily an alpine specialist bumble bee and is rarely detected at low elevations (< 1100 m) in the southern extent of their distribution in North America (*i.e.*, south of US-Canadian border) (Thorp et al. 1983). However, in the northern extent of their distribution, *B. sylvicola* has been detected at low-elevation sites (*i.e.*, north of US-Canadian border and Alaska), with its most eastern extent in Labrador in Newfoundland, Canada (Koch and Strange 2012; Pampell et al. 2015). The remaining species, *B. mixtus*, *B. melanopygus*, and *B. flavifrons* are found across a broad elevation gradient (sea level to >1100 m) and overlap in their geographic distribution in western North America (Koch et al. 2012). I hypothesize that the alpine specialist, *B. sylvicola* will exhibit increased subpopulation differentiation (F_{ST}) relative to species across a broad elevation gradient (Lozier et al. 2011, 2013). However, given the degree of isolation of the Olympic Mountains from the Cascade Mountains (Figure 6-1), I further predict that bumble bees distributed in the Olympics will exhibit decreased population genetic diversity relative to

conspecific populations in the Cascade Mountains. Finally, I hypothesize that bumble bee populations that are distributed in the San Juan Islands will also exhibit reduced genetic diversity relative to mainland populations, a phenomenon that has been reported in other bumble bee species endemic to the area (Lozier et al. 2011; Lozier et al. 2013).

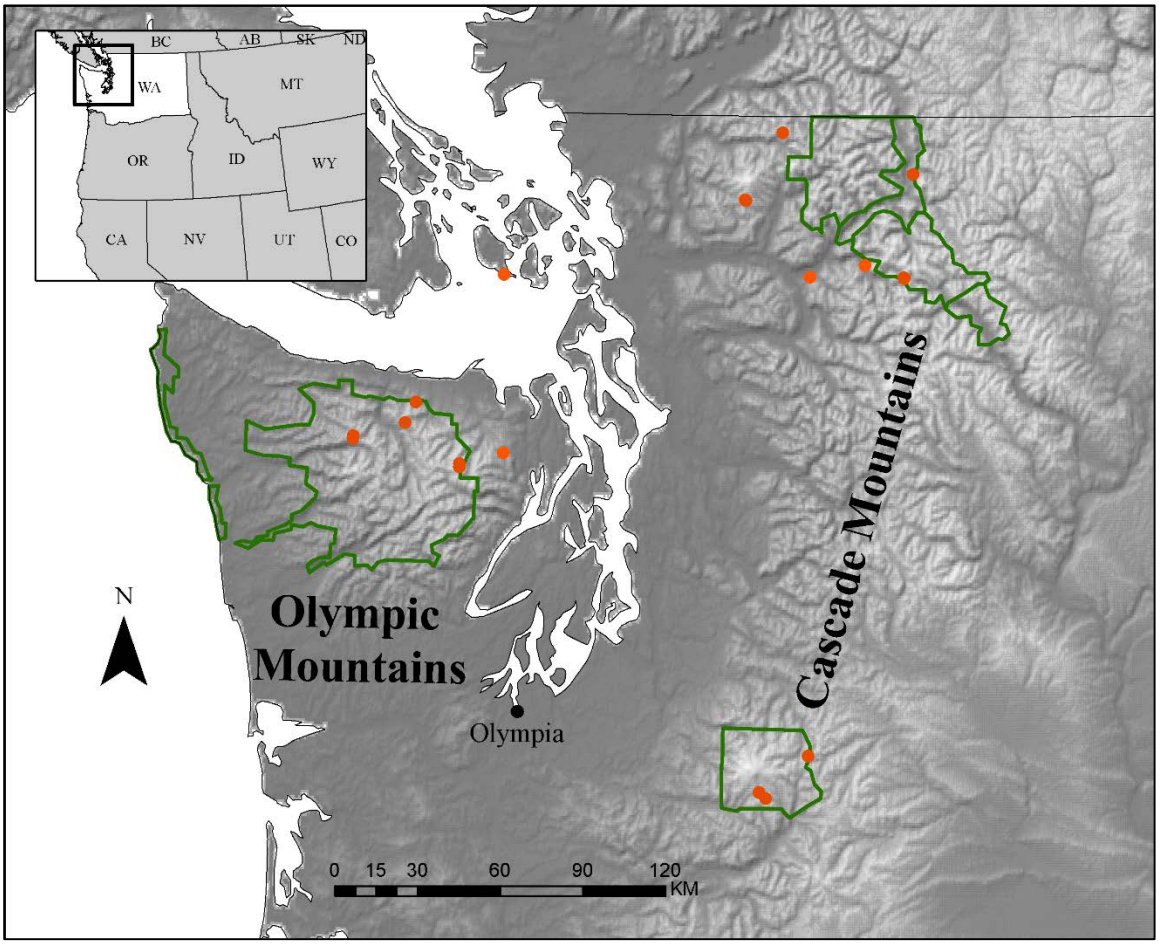

Figure 6-1. Distribution of survey sites in the Pacific Northwest. Orange points represent the survey sites. Green outlines represent federally protected areas (U.S. National Parks). Clockwise, from the top, North Cascades National Park, Mount Rainier National Park, and Olympic National Park. Gray scaling in the background represents elevation, with lighter shading representing high elevation, and darker shading representing low elevation.

Materials and Methods

Field methods

In the late summer months of 2013 and 2014 (July - August) 38 different localities were surveyed for wild bumble bees throughout the Pacific Northwest (Strange et al. 2013) (Table 6-1). Majority of the bumble bee collections were made within the boundaries of seven National Parks and Monuments: Olympic National Park, North Cascades National Park, Mount Rainier National Parks, Ebey's Landing National Monument, San Juan Islands National Monument, Lewis and Clark National Historical Park, and Fort Vancouver National Historic Site (Table 6-1). Additional field sites adjacent to these federally protected areas were explored to achieve a robust sampling of the bumble bee species targeted in this study. Sites were surveyed by teams of individuals using random net collections of bumble bees at plots of approximately 0.5 ha following Cameron et al. (2011). These sites varied in floral density and accessibility of off trail sampling. To standardize collection effort, surveys were timed and collections were standardized to 1.5 collector hours per site when feasible. Collectors surveyed with aerial insect nets (30 cm diameter) and collected bumble bees foraging on flowers directly into 20 mL plastic vials. The vials were placed on ice for 10-15 minutes until surveys were complete and the bees were immobilized by the cold. Upon completion of the survey period, the bumble bees were sexed and preliminarily identified to species following Thorp et al. (1983) and Koch et al. (2012). While the specimens were immobilized, I non-lethally sampled DNA from the bumble bees by removing a mid-leg from each individual (Holehouse et al. 2003). The mid-legs were individually stored in 95% ethanol for downstream DNA analysis to verify the species identity. At each site, a worker and

male of each captured species were sacrificed and retained as voucher specimens. All queens were released after legs were sampled. However, in 2014, all surveyed bees were sacrificed and retained as voucher specimens and for use in DNA analysis. Each survey event was associated with a unique locality description and georeferenced with a Garmin GPSmap 60CS. Voucher specimens were pinned and associated with a unique barcode ID and curated into the USDA-ARS National Pollinating Insect Collection in Logan, UT.

DNA extraction, amplification, and genotyping

Total DNA was extracted from the mid-leg of female bumble bees with a modified Chelex protocol following Strange et al. (2009) and genotyped with the following 15 variable DNA microsatellite markers: BL13, BTERN02, BTMS0044, BTMS0059, BTMS0062, BTMS0066, BTMS0086, B124, B96, BT10, BT28, BT30, BTERN01, BTMS0052 and BTMS0081; all loci were identified from the literature (Estoup et al. 1995, Reber-Funk et al. 2006, Stolle et al. 2009). These markers were selected not only for their utility in verifying the species with field identifications, but also for utility in studying population divergence and genetic diversity within populations (Cameron et al. 2011, Lozier et al. 2011). Multiplex polymerase chain reactions (PCR) were performed in final volumes of 10 μL, containing approximately 1-3 μL of extracted DNA, 1x Promega (Madison, WI) reaction buffer, 0.6 mM dNTP mixture, 0.2–0.4 μM primer, 0.001 mg BSA, 0.4 units Taq polymerase (Promega, Madison, WI) and the $MgCl_2$ concentration was adjusted to 1.4 mM. The PCR conditions for both multiplex reactions were one 3:30 min cycle at 95°C, 30 cycles of 95°C for 30 s, annealing temperature 55/58°C for 1:15 min, 72°C for 45 s and a final extension period of 15 min at 72°C. The DNA amplifications were performed with fluorescent 5’ dye-labeled primers

(6-FAM, NED, VIC, or PET) and separated on an Applied Biosystems 3730xl automatic sequencer at the Center for Integrated Biology at Utah State University. The allele sizes were scored manually using the microsatellite plugin v1.4 in Geneious v8 (Kearse et al. 2012), and analyzed with Micro-checker v 2.2.3 (van Oosterhout et al. 2004) to examine the possibility for stutter, null, or dropped alleles across loci of each species.

Population genetic analysis

To avoid pseudo-replication within sampling locations, full-siblings were first assigned to colonies using a full maximum likelihood approach with Colony v2.0 (Jones and Wang 2010). In the colony-assignment exercise, I set the genotyping error rate to 0.005, based on error rates documented in previous studies (Lozier et al. 2011) and the sex-determination system to “haplodiploid”. If full-siblings were detected in the colony assignment tests (≥ 95% genotype similarity), I randomly retained only one representative from each family using a coin-toss. The removal of full-siblings and individuals with < 50% amplified genotypes resulted in an average (± S.E.) population size of 15.55 ± 5.79 *B. sylvicola* (171 individuals across 11 sites), 9.42 ± 3.05 *B. melanopygus* (66 individuals across 7 sites), 10.89 ± 1.92 *B. flavifrons* (196 individuals across 18 sites), and 9.89 ± 4.77 *B. mixtus* (89 individuals across 9 sites).

The genotype data for all populations by species were tested for deviations from Hardy-Weinberg (HWE) and linkage disequilibria (LD) using the Markov chain method implemented with Genepop v1.2 (Raymond and Roussett 1995). Differentiation between conspecific populations were estimated and tested for significance with pairwise F_{ST}, which were transformed to $F_{ST}/1\text{-}F_{ST}$ with Genalex 6.5 (Peakall and Smouse 2012). In my study I treated localities as subpopulation for each species, and examined isolation by

distance (IBD) patterns by calculating pairwise geographic distances between conspecific subpopulations and using the associated pairwise $F_{ST}/1\text{-}F_{ST}$ values to conduct Mantel tests. In an attempt to identify distinct lineages that might be useful in classifying evolutionary (ESU) or management significant units (MSU) across the four bumble bees in the Pacific Northwest (Erwin 1991; Moritz 1994; Crandall et al. 2000), I implemented a Bayesian clustering algorithm in the program Structure v2.3.3 (Pritchard et al. 2000). I elected to use the admixture model in Structure, which assumes that individuals comprise *K* unknown populations, to which an individual can be assigned based on their genotype without *a priori* delineation of populations. I set the model to run with 20,000 burn-in steps and 100,000 samples, with 10 iterations for each *K*, where *K* ranged from 1 to 10. To determine the optimal *K* (*i.e.*, populations or lineages), the distributions of the probability of the data (*ln P(D)*) and ΔK, as described by (Evanno et al. 2005; Earl and vonHoldt 2012) were estimated with the web-based software program Structure Harvester 0.6.94 (Earl and vonHoldt 2012). To account for multimodality associated with individual Structure runs, I averaged each individual's admixture proportions over the 10 replicates for the best *K* using Clumpp v1.1.2 (Jakobsson and Rosenberg 2007) and spatially visualized population-assignment by average individuals within each subpopulation with pie-charts in ArcGIS v10.1 (ESRI 2012).

Effective allele diversity (AD) and expected heterozygosity (H_e) were estimated with rarefaction techniques in the *gstudio* package in R (Dyer 2012). Estimating effective allele diversity allows for a comparison of conspecific populations where the number and distribution of alleles can differ drastically from one population to the next (Weir 1990). Private allelic richness, which is a measure of genetic distinctiveness was also estimated

with rarefaction techniques with HP-Rare 1.0 (Kalinowski 2005). I tested for differences in average AD, H_e, and PAR of each the target species in this study by grouping subpopulation by their presence in three different regions of the Pacific Northwest: Olympic, Cascade, and Island (Figure 6-1). Shapiro-Wilk tests revealed that estimates of genetic diversity for all bumble bee species were normally distributed. As such, I elected to test for differences in average genetic diversity across the three regions for each species with two-sample *t*-tests.

Unless otherwise indicated, statistical analyses and indices of genetic diversity were estimated within the R statistical computing platform (R Development Core Team 2005). I specifically used the *gstudio* to estimate genetic diversity and *ggplot2* to visualize non-spatial data.

Results

Prior to population genetic analyses for all species, I needed to determine which microsatellite loci deviated from HWE or are in LD across conspecific subpopulations. Across *B. flavifrons* subpopulations I found BTERN01 and BTMS0086 to not be in HWE and BTMS0044, BTMS0062, BTMS0081, and BT30 to be in LD. Across subpopulations of *B. melanopygus* I found BTERN01 and BT10 to not be in HWE and BTMS0086 and B124 in be in LD. Across subpopulations of *B. mixtus* I found BT28 was monomorphic and BTERN02 exhibited a degree of stuttering based on Micro-checker analyses. Furthermore, I found that BTMS0086, BTMS0081, BTMS0044, and B124 to not be in HWE or was in LD. Finally, across *B. sylvicola* subpopulations BT10 was not in HWE and BTMS0066, BTMS0044, BTERN02, BTMS0059, and B124 exhibited LD.

Table 6.1 Survey sites in the Pacific Northwest where bumble bees were collected.

Location Description	Lat	Long	Day	Month	Year	Elev. (ft.)
Lake Crescent, near	48.09	-123.72	8	8	2014	580
Washington Pass	48.72	-121.84	4	8	2014	5367
Mt. Baker, meadows low	48.72	-121.84	3	8	2014	4555
Kettles Trail, near Coupeville	48.35	-121.07	6	8	2014	62
Washington Pass	48.72	-121.84	8	8	2014	5367
Cascade Pass	48.47	-121.06	5	8	2014	5371
Obstruction Point Road Low	48.04	-123.43	6	8	2014	4997
Obstruction Point Road High	47.97	-123.48	7	8	2014	6221
Grand Lake Trail	47.93	-123.33	7	8	2014	6298
Rainy Pass	48.52	-120.73	4	8	2014	4802
Mt. Baker, meadows high	48.72	-121.86	3	8	2014	5401
Burpee Hill	45.62	-122.66	3	8	2014	975
Newhalem	48.52	-120.74	4	8	2014	652
Ione, 32.73 km S	48.45	-117.33	29	8	2014	2369
Colville, 42.75 km N	48.92	-117.87	0	8	2014	2106
Royal Basin Ranger Station	47.83	-123.21	16	7	2013	5132
Lower Royal Basin	47.84	-123.21	16	7	2013	4663
Lower Bridge Creek Campsite	47.92	-123.73	16	7	2013	3816
Heart Lake	47.91	-123.73	16	7	2013	4789
Royal Basin Trail	47.86	-123.20	17	7	2013	3862
Royal Basin Parking Lot	47.88	-123.00	17	7	2013	3837
Crescent Lake, East Beach	48.09	-123.74	17	7	2013	687
Ebey's Landing	48.19	-122.71	18	7	2013	65
Sahale Arm Trail	48.47	-121.52	19	7	2013	6150
Cascade Pass	48.47	-121.06	19	7	2013	5374
English Camp	48.59	-123.15	19	7	2013	2
American Camp	48.46	-123.02	19	7	2013	124
Sibley Creek	48.51	-121.25	20	7	2013	1375
Lewis & Clark	46.12	-123.88	22	7	2013	535
West Side Road	46.78	-121.88	22	7	2013	2880
Upper Crystal Lake	46.91	-121.51	22	7	2013	5854
Fort Vancouver	45.62	-122.66	23	7	2013	127
Paradise Meadows	46.79	-121.74	23	7	2013	5258
Snow Lake	46.77	-121.71	23	7	2013	4684
Upper Crystal Lake	46.91	-121.51	23	7	2013	5854
Upper Palisades Lake	46.95	-121.59	23	7	2013	5917
Sunrise Meadows	46.91	-121.62	24	7	2013	6256
Sand Point Loop	48.15	-124.72	1	8	2013	13

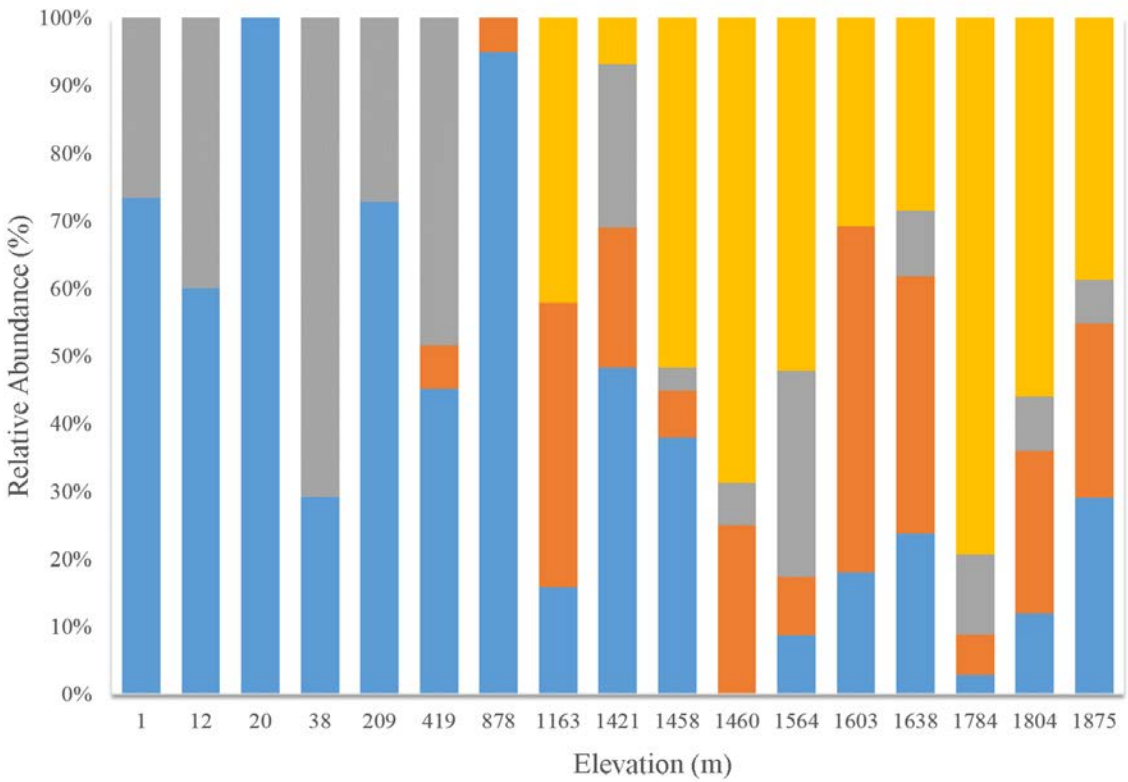

Figure 6-2. Relative abundance of four bumble bee species distributed across an elevational gradient in the Pacific Northwest. See Figure 6-1 for the spatial distribution of study sites. Yellow bars = *B. sylvicola*, Gray bars = *B. mixtus*, Orange bars = *B. melanopygus*, Blue bars = *B. flavifrons*.

Table 6-2. Microsatellite loci amplified for each species and used in the final analysis.

	B. flavifrons	*B. melanopygus*	*B. mixtus*	*B. sylvicola*
B124	x			x
BT28	x			
BT10	x		x	
BT30		x	x	x
B96	x	x	x	x
BTMS0066	x	x	x	x
BTMS0062		x	x	x
BTMS0044		x		x
BTERN01			x	x
BTERN02	x			
BL13	x	x	x	x
BTMS0059	x	x	x	
BTMS0081		x		

Patterns of allelic fixation across conspecific subpopulations varied among species and are likely associated with each species' distribution and niche across an elevation gradient in the Pacific Northwest (Fig. 6-2). I detected significant genetic differentiation relative to geographic distance (IBD) across populations of *B. sylvicola* (Mantel test: rxy = 0.42, $P = 0.02$, $r = 0.17$; Fig. 6-3A) and *B. mixtus* (Mantel test: rxy = 0.48, $P = 0.01$, $r = 0.23$; Fig. 6-3B), with subpopulations > 100 km experiencing increased levels of allelic fixation as the microsatellite loci used in the present study. I did not detect significant genetic differentiation relative to geographic distance among *B. melanopygus* (Mantel test: rxy = -0.06, $P = 0.41$, $r = 0.004$; Fig. 6-3C) and *B. flavifrons* (Mantel test: rxy = -0.06, $P = 0.37$, $r = 0.003$; Fig. 6-3D) subpopulations. The lack of genetic fixation across populations of *B. melanopygus* and *B. flavifrons* suggests that there is a high degree of gene flow across the study area in the Pacific Northwest.

Evidence for population structuring across *B. sylvicola* and *B. mixtus* was also based on the results of the Bayesian population assignment algorithm using the admixture

ancestry model. The ΔK statistic was greatest at $K = 2$, with significantly less explanatory power gained by assuming additional clusters (K) across *B. sylvicola* subpopulations (Fig. 6-4A), and $K = 3$ across *B. mixtus* populations (Fig. 6-4B) (Appendix E1). Specifically, I detected that *B. sylvicola* subpopulations distributed in the Olympic Mountains of Olympic National Park composed a distinct genetic cluster relative to subpopulations distributed in North Cascades, Mount Rainier National Park, and adjacent areas in the Cascade Mountains. I did not detect significant genetic structuring within *B. melanopygus* (Fig. 6-4C) nor *B. flavifrons* (Fig. 6-4D). For both species, the ΔK statistic was also greatest at $K = 2$, with significantly less explanatory power gained by additional clusters (Appendix E1). However, even at $K = 2$, both species lack population structuring, which is corroborated by the lack of allelic fixation ($F_{ST}/1\text{-}F_{ST}$) of subpopulations across geographic distance (Fig. 6-3C, 6-3D).

Despite differences in population genetic structure and allelic fixation observed across populations of *B. sylvicola*, *B. mixtus*, *B. melanopygus*, and *B. flavifrons*, I observed convergent patterns of genetic diversity across regionally populations. Because I found variable abundances of each species across my sampling sites (Fig. 6-2), I elected to rarefy the genotypes to estimated effective allelic diversity across conspecific subpopulations of each species (Dyer 2012). For *B. sylvicola*, rarefaction was set at $n = 5$, *B. mixtus* at $n = 5$, *B. melanopygus* at $n = 3$, and *B. flavifrons* at $n = 5$. *Bombus sylvicola* subpopulations distributed in Olympic National Park had significantly lower average allelic diversity ($\bar{x} = 2.79$) relative to subpopulations found in the National Park areas of the Cascades (*i.e.*, North Cascades + Mount Rainier) ($\bar{x} = 3.12$) (Fig. 6-5A, AD: $t(7.34) = 3.20$, $P = 0.01$)

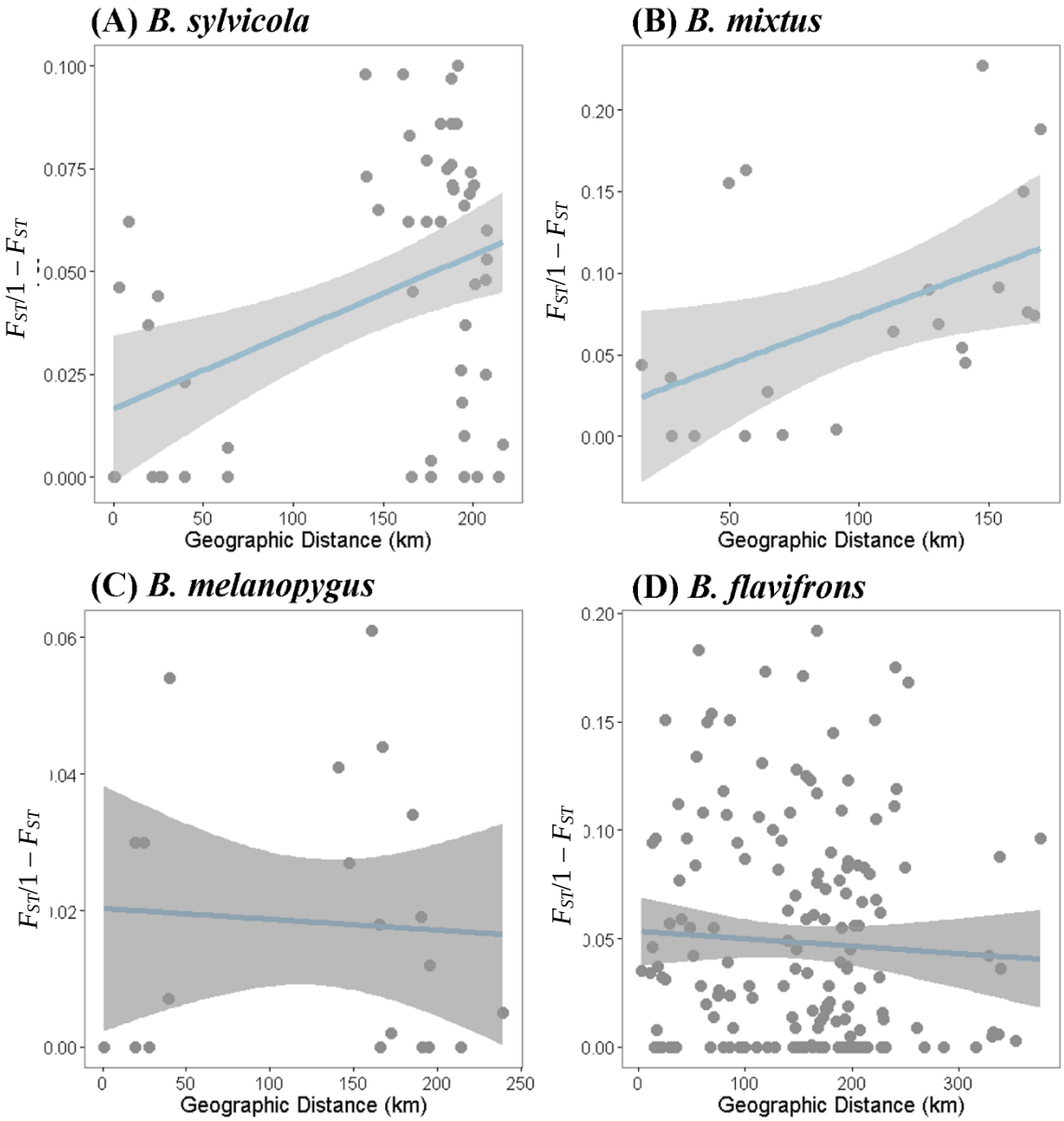

Figure 6-3. Pairwise differences in allelic fixation across subpopulations of four bumble bee (Hymenoptera: Apidae, *Bombus*) species by geographic distance in the Pacific Northwest. Gray shading represents 95% confidence of interval of the linear model drawn to fit the data. F_{ST} is represented in its linearized form ($F_{ST}/1 - F_{ST}$).

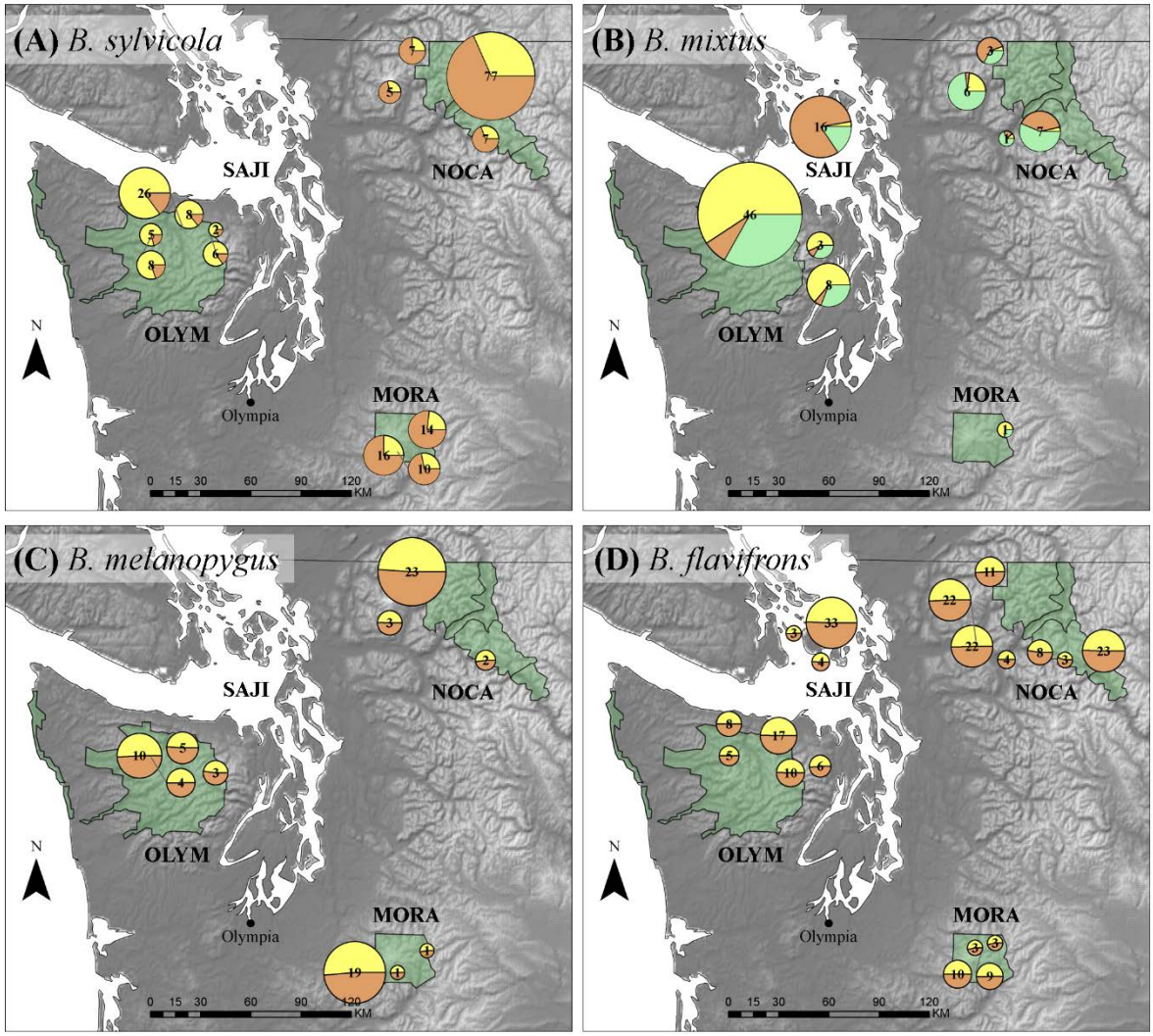

Figure 6-4. Genetic assignment of subpopulations (sampling sites) to *K* populations for four different bumble bee species (Bombus) in the Pacific Northwest. The size of the circle represents the number of individuals surveyed at each subpopulation, with the number at the center of each circle. The pie slice of each circle represents the average genetic assignment of all individuals in each subpopulation to one of *K* populations. Green polygons represent federally protected areas (U.S. National Parks and Recreation Areas). OLYM = Olympic National park, NOCA = North Cascades National Park, MORA = Mt. Rainier National Park, and SAJI = San Juan Islands National Historic Park and Orcas Island. Gray scaling in the background represents elevation, with lighter shading representing high elevation, and darker shading representing low elevation.

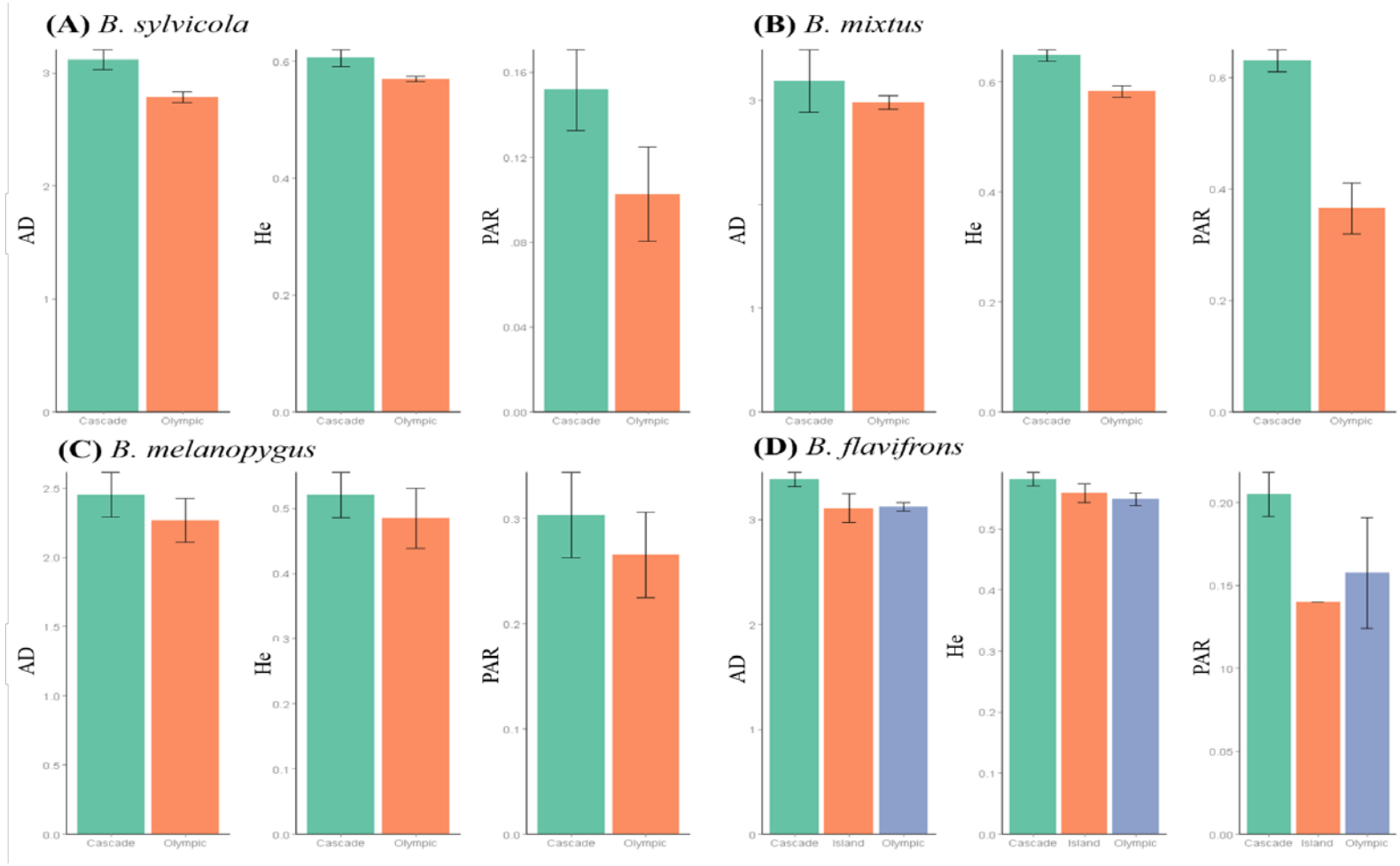

Figure 6-5. Average (±SE) effective allelic diversity (AD), expected heterozygosity (H_e), and private allelic richness (PAR) comparisons across four bumble bees distributed in different regions in the Pacific Northwest.

I did not detect a significant difference in average expected Heterozygosity (H_e: t(6.14) = 2.38, P = 0.053) or private allelic richness (PAR: t(6.14) = 2.38, P = 0.053) between Olympic National Park and Cascades (t(6.84) = 1.68, P = 0.14) (Fig. 6-5A).

Average allelic diversity and private allelic richness between *B. mixtus* subpopulations distributed in the Olympic National Park and Cascades were not significantly different (Fig. 6-5B, AD: t(1.09) = 0.67, P = 0.62; PAR: t(1.38) = 5.38, P = 0.07). However, there was a significant difference in average expected Heterozygosity between subpopulations in Olympic National Park ($\bar{x}$ = 0.58) and those distributed in the Cascades ($\bar{x}$ = 0.65) (Fig. 6-5B, H_e: t(1.99) = 4.44, P = 0.05). While *B. mixtus* was detected in the San Juan Islands, I was unable to genotype bees from more than one locality (*i.e.*, subpopulation) to include in this current analysis (Fig. 6-4B). I did not detect a significant difference in average allelic diversity (AD: t(4.75) = 0.82, P = 0.45), expected Heterozygosity (H_e: t(4.75) = 0.82, P = 0.45), and private allelic richness (PAR: t(4.80) = 0.67, P = 0.53) across *B. melanopygus* subpopulations distributed in the Olympic National Park and Cascades (Fig. 6-4C). Finally, I detected significant differences in average allelic diversity between *B. flavifrons* subpopulations distributed in the Olympic National Park ($\bar{x}$ = 3.12) and Cascades ($\bar{x}$ = 3.38) (AD: t(7.55) = 3.34, P = 0.01), but not in average expected heterozygosity (H_e: t(7.70) = 2.13, P = 0.06) nor private allelic richness (PAR: t(3.98) = 1.33, P = 0.26) (Fig. 6-4D). My analysis found no significant difference in average allelic diversity, heterozygosity, or private allelic richness between subpopulations in the San Juan Islands and the Olympic National Park area (all P > 0.64) (Fig 6-5D). I found significantly higher private allelic richness in the Cascades ($\bar{x}$ = 0.21) relative to San Juan Island subpopulations ($\bar{x}$ = 0.14) (PAR: t(5) = -

4.87, P = 0.005). Finally, there was no significant difference in expected heterozygosity (H_e: t(2.27) = -1.21, P = 0.34) or allelic diversity (AD: t(1.55) = -1.81, P = 0.25) between Island and Cascade subpopulations (Fig 6-5D).

Discussion

This study found that genetic differentiation across populations were significant in *B. sylvicola* and *B. mixtus* but not in *B. flavifrons* nor *B. melanopygus*. I discovered that the population of *B. sylvicola* in the Olympic Mountains composed a distinct genetic lineage relative to the population in the Cascade Mountains. Significant genetic fixation across widely distributed subpopulations suggest that relatively little gene flow occurs in *B. sylvicola* and *B. mixtus* among isolated habitats of the Pacific Northwest. While patterns of genetic fixation differed between bumble bee species, I discovered a general trend of reduced effective allelic diversity in Olympic Mountains relative to conspecific subpopulations found in the Cascade Mountains. Differences in effective allelic diversity are likely a product of each species' respective phylogeography and the glaciation history of the Pacific Northwest (Hewitt 2000). With the exception of *B. mixtus*, I did not detect any significant differences in expected heterozygosity, suggesting that subpopulations are not suffering from any significant recent inbreeding.

Previous work on bumble bees distributed in western North America has found that montane species are associated with high levels of genetic fixation across populations, predicated by their bioclimatic distribution (Lozier et al. 2013). Furthermore, bumble bees with broad geographic and environmental distributions are found to lack a signature of isolation by distance, suggesting that individuals experience relatively few barriers to gene flow (Cameron et al. 2011). However, populations of bumble bees found

on offshore islands are associated with high levels of fixation relative to adjacent populations, likely serving as significant barrier to bumble bee dispersal, and ultimately limiting gene flow (Lozier et al. 2011; Jha 2015). The results of my study agree with the central theme that habitat fragmentation and heterogeneity are effective filters of gene flow across conspecific bee populations (Suni and Brosi 2011; Lozier et al. 2013; Jha 2015). However, I did discover that patterns of genetic fixation and structure can be predicted by a species' distribution across an elevation gradient, with alpine specialists and uncommon species associated with increased genetic fixation across geographic and environmental distance.

Bumble bees are primitively eusocial insects that form annual colonies, with all members of the colony, except for the recently mated reproductive queen expiring in the late summer and early autumn of each year (late July - early September) (Heinrich 2004). Recently mated queens of some European bumble bee species have been found to disperse 3 - 5 km from their nest, likely facilitating the patterns of genetic fixation observed across subpopulations of bumble bees in my study (Lepais et al. 2010). However, little is known about the capacity for males to disperse, and future work should examine the effect of male dispersal on the population genetic structure of conspecific bumble bees. Ultimately, the patterns of genetic diversity observed in female workers are driven by the location of the nest their mother founded earlier in the spring/summer (Rao and Strange 2012). In my study, I found that populations of *B. sylvicol*a were associated with high levels of genetic fixation across geographic distances, a pattern not detected in *B. melanopygus* and *B. flavifrons*. Patterns of strong genetic fixation among subpopulations of *B. sylvicola* in the Pacific Northwest are similar to patterns observed

with a co-distributed species, *B. bifarius* in the region (Lozier et al. 2013). Furthermore, island *B. bifarius* are phenotypically distinct relative to those found on the mainland, and have been suggested to be additional subspecies or a closely related species of *B. bifarius* (Stephen 1957; Lozier et al. 2013). Given that *B. sylvicola* is primarily restricted to high-elevation habitats, it is not surprising that I found subpopulations in the Olympic Mountains to be a genetically distinct lineage relative to subpopulations found in the Cascade Mountains. Both *B. melanopygus* and *B. flavifrons* are broadly distributed across an elevation gradient, and are likely less inhibited by natural and human-mediated barriers to gene flow.

In the context of rapid global climate change, bumble bees have been found to respond to changes in floral abundance and onset of earlier spring by adjusting their foraging behavior and emerging earlier in the spring (Bartomeus et al. 2011; Miller-Struttmann et al. 2015). Species that appear most sensitive to change include species that emerge early in the year (Bartomeus et al. 2011), or are distributed primarily in alpine ecosystems (Miller-Struttmann et al. 2015). Both *B. flavifrons* and *B. melanopygus* are broadly distributed species that inhabit a diversity of habitats across an elevation gradient. Furthermore, both species have been documented to have larger nest sizes, and more reproductive offspring relative to *B. mixtus* and *B. sylvicola* (Hobbs 1967; Macfarlane et al. 1994). Population genetic data for both species suggest that gene flow is relatively high across populations. Furthermore, both species emerge relatively early in the spring, especially *B. melanopygus* (Macfarlane et al. 1994), and may have the capacity to adjust their emergence to changes in early spring temperatures (Thorp et al. 1983; Williams et al. 2014). Since little genetic structure was observed across

subpopulations of these species, it is likely that they will be able to cope with rapid global change by dispersing to favorable habitats if their present day habitats are not suitable. In contrast to the more broadly distributed species, *B. sylvicola*, is primarily restricted to alpine environments (Williams et al. 2014), with emergence dependent on the timing of snowmelt (Pyke et al. 2011, 2012). As such, their temporal window for a spring queen to find a suitable nesting site, raise a colony, and produce offspring is much shorter than species that are less dependent on the timing of snowmelt in the Pacific Northwest. A recent study by Miller-Struttmann et al. (2015) found that *B. sylvicola* populations in alpine Colorado have evolved shorter proboscis in response to the reduction of their flowering resources associated with rapid climate change over the past 40 years in the region. Given the sensitivity of bumble bees to changes in the angiosperm community, as well as warmer days in the early spring, it is likely that the behavioral changes in alpine species will cascade down to rapid genetic drift and decreased gene flow (Rubidge et al. 2012).

Given that U.S. National Parks experience little to no urbanization and agricultural impact, the primary threats to bumble bee communities in these federally protected areas relate to global climate change. There have been recent proposals by some researchers to transport bumble bees to northern latitudes due to suspected decline of suitable bioclimatic niches at lower latitudes (Kerr et al. 2015). Before such efforts are contemplated I suggest that careful study of bumble bee communities in protected or remote areas be made to determine how different species have responded to continued global change (Miller-Struttmann et al. 2015). Long range transport of bumble bees will likely affect the pathogen dynamics of endemic conspecific or heterospecific populations

(Goka et al. 2006), and may further facilitate population collapse (Cameron et al. 2011). Furthermore, recent studies of bumble bees in alpine communities have found that species with either a long (*i.e., B. balteatus*) or medium (*i.e., B. sylvicola*) in length proboscis appear to have kept pace with changes to the flowering plant community by adjusting their foraging preferences (Miller-Struttmann et al. 2015).

Conclusion

Pollinator species worldwide are undergoing dramatic changes in both genetic diversity and structure, putting them at greater risk to falling below effective population sizes and exposing them to increased inbreeding (Goulson et al. 2008). The decline of suitable bumble bee habitat and increased pathogen and pesticide pressures is associated with rapid decline of bumble bees across the globe (Grixti et al. 2009; Cameron et al. 2011). High-elevation bumble bee communities are in a special position for rapid evolutionary change due to changes in flower phenology and availability due to global climate change (Miller-Struttmann et al. 2015). National Parks in the Pacific Northwest are associated with unique rainforest and alpine ecosystems, and are ideal areas to study the evolution and ecology of bumble bees. My results suggest that bumble bee demes located in the Olympic Mountains, an isolated mountain province of the Pacific Northwest, have reduced genetic diversity relative to conspecific subpopulations located in the Cascade Mountains. Furthermore, the alpine specialist bumble bee, *B. sylvicola* and the relatively uncommon bumble bee *B. mixtus*, were both found to constitute genetically distinct Olympic Mountains populations relative to the populations in the Cascade Mountains and San Juan Islands. Given the projected changes in temperature and precipitation patterns for the Pacific Northwest, in-depth studies of *B. sylvicola*

populations distributed in Olympic National Park may provide insight into the effects of global change on alpine pollinators.

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CHAPTER 7

SUMMARY AND CONCLUSIONS[7]

Pollinator communities worldwide are undergoing dramatic changes in both abundance and composition that may put pollination service at risk in many terrestrial ecosystems (Winfree et al. 2009). These changes may not be unidirectional declines in species abundance, but may manifest as shifts in geographic range or increases in abundance where new habitat is formed. To date, documented changes in pollinator communities have been attributed to many factors, including pathogen outbreaks, pesticides, and land-use change (Williams & Osborne 2009). However, the underlying mechanisms of these changes are not fully understood (Cameron et al. 2011; Bartomeus et al. 2011). Identifying the factors affecting pollinator communities can be difficult as most strategies investigate distinct taxonomic groups (guilds) (Cameron et al. 2011; Miller-Struttmann et al. 2015) or attempt to isolate specific threats, measuring a single species' responses to the threat in question (Bartomeus et al. 2011; Miller-Struttmann et al. 2015). Furthermore, the underlying patterns of genetic diversity and structure of most bumble bees are undocumented, and the species boundaries of some species have yet to be investigated (Cameron et al. 2010).

In this dissertation I gather data, at both the genetic and species level, to examine several important themes relevant to the ecology and evolution of bumble bees in North America. In Chapter 2, I investigate the population genetic structure and diversity of a widespread bumble bee, *Bombus huntii*, in western North America. Populations of *B. huntii* have recently been commercialized to deliver pollination services to glasshouse

[7] This chapter is formatted for *Conservation Biology*.

and open field crops in western North America. Laboratory trials have revealed *B. huntii* to be a promising bumble bee pollinator for commercialization due its widespread distribution, abundance, large colony size, and ability to effectively pollinate glasshouse tomatoes (Strange 2015). However, given the history of collapse among commercial bumble bees, and the threat of pathogen introduction and transport, there is concern by industry producers, growers, and scientist on whether *B. huntii* will be a successful pollinator. My study of *B. huntii* primarily investigated the species' population genetic structure and ecological niche across Canada, USA, and Mexico. I found that populations north of Mexico were largely panmictic with high genetic diversity and low genetic structuring. However, populations in Mexico were highly structured, and were associated with low population genetic diversity. Furthermore, I found that the high instability of *B. huntii* habitat suitability since the Last Glacial Maximum was correlated with high effective allelic diversity. As *B. huntii* diverged from its extant sister taxa, *B. vosnesenskii* by the start of the Pleistocene, it is likely that climate oscillations during this time period greatly influenced the mosaic of contemporary population genetic diversity observed in this current study (Hines 2008; Duennes et al. 2012). Given the results of my study, a degree of caution should be made when transporting bumble bees away from their endemic location into novel locations (Mexico to USA, and vice versa). As *B. huntii* in Mexico represent a distinct and highly structured population, the introduction of *B. huntii* from the USA and Canada may introgress with local populations or spread novel pathogens (Goka et al. 2006; Goulson et al. 2008; Cameron et al. 2011; Sachman-Ruiz et al. 2015). Future work is needed to determine whether outbreeding depression between Mexican and USA/Canada populations of *B. huntii* is possible, and if there is a special

risk for different susceptibilities for economically important bee pathogens (Sachman-Ruiz et al. 2015; Goulson & Hughes 2015).

Species delimitation is of great concern and debate among conservation biologist studying bumble bees (Carolan et al. 2012; Williams et al. 2012b; Duennes et al. 2012). Bumble bees are extremely cryptic as different species may converge on setal coloration patterns (Williams et al. 2012a; Hines & Williams 2012). Identifying bumble bees to species can be challenging when solely dependent on color pattern variability as a diagnostic tool (Stephen 1957; Thorp et al. 1983; Koch et al. 2012; Williams et al. 2014). In Chapter 3 I test the hypothesis that *B. californicus* and *B. fervidu*s are conspecific as suggested by Williams et al. (2014). The combination of wing morphometric characteristics, three mitochondrial loci, and multiple microsatellite loci was able to recover well supported clades composed of two distinct species with convergent phenotypes. These species are largely sympatric throughout the central portion of their distribution Intermountain West. However, where they are allopatric, they appear to show phenotype convergence with other bumble bee species that occur in sympatry. In most of eastern North America, where *B. californicus* is absent, *B. fervidus* converges with *B. pensylvanicus, B. auricomus, B. griseocollis*, and several others in exhibiting black and yellow setal color patterns. Consequently, the mitochondrial and microsatellite genotype data I present show that *B. californicus* and *B. fervidus* are legitimate species. However, given the degree of phenotype convergence exhibited by both species, future work should include an investigation on the genetic architecture associated with color variability across the species. Furthermore, an effort should be made to determine if the species have the capacity to hybridize in lab conditions (Milliron 1973).

In Chapter 4 of my dissertation, I determine the capacity of color pattern variation to predict the structure of bumble bee communities in Yosemite National Park in California. Müllerian mimicry appears to have played a central role in the ecology and evolution of bumble bees across the globe (Owen et al. 1980; Plowright & Owen 1980; Williams 2007; Hines 2008; Duennes et al. 2012; Hines & Williams 2012). However, most studies on Müllerian mimicry examine regional patterns of color variability across species participating in a mimicry ring. I tested the hypothesis that the functional trait of phenotype (phenotype convergence) was a better predictor than proboscis length or species diversity in structuring bumble bee communities across an elevation gradient. I found no relationship between elevation and species richness across studies sites in Yosemite, nor did I observe a trend in proboscis length. Rather, I found that species richness and proboscis length were relatively homogenous (neutral, no positive or negative relationship) across communities, which supports the hypothesis of resource partitioning within bumble bees based on proboscis length (Inouye 1980). However, I did detect a distinct trend in setal color pattern variation, with low-elevation communities composed of black and yellow bumble bees (*i.e., B. vosnesenskii, B. californicus, B. vandykei*) and high-elevation communities composed of bumble bees with red setae (i.e., *B. sylvicola*, *B. balteatus*, *B. mixtus*). Sympatric convergence of phenotypes across unrelated bumble bee species (different subgenera) suggest that there is strong selection for bumble bees with similar setal patterns and colors. Selection for Müllerian mimicry across prey is dependent on spatial and temporal co-occurrence of predators. My study identified two distinct Müllerian mimicry rings distributed at either high or low-elevation sites, thereby supporting the hypothesis of mimetic evolution in bumble bees to avoid

predation. Future work could test the differential effect of bumble bees with or without red setae in Yosemite on predator avoidance behavior (Brower et al. 1960).

In Chapter 5, I expand the known range of *B. sylvicola* and *B. vandykei* into Olympic Mountains of Olympic National Park. Bumble bees are generally thought to be well studied, however my study reports that *B. sylvicola*, an alpine specialist south of the US-Canadian border, is quite abundant in this mountain province. Furthermore, a population of *B. vandykei* with an uncommon phenotype was detected in a relatively high-elevation habitat in Olympic Mountains. In this chapter I discussed the importance of surveying bees in wilderness areas, away for transportation corridors, as there is likely a great deal to discover about bumble bees in North America. Finally, in Chapter 6 of my dissertation I examine the population genetic structure and diversity of four bumble bee species in the Pacific Northwest: *B. sylvicola*, *B. flavifrons*, *B. melanopygus*, and *B. mixtus*. I specifically compare the effect of geographic distance on genetic fixation across conspecific subpopulations of each species in the Cascade Mountains, Olympic Mountains, and the San Juan Islands (when the species was detected, *i.e.*, *B. mixtus* and *B. flavifrons*). I found that recently discovered *B. sylvicola* populations in the Olympic population composes a unique lineage of the species, distinct from the population found in the Cascades. However, across subpopulations of *B. melanopygus* and *B. flavifrons*, I found both species to be in panmixia. I also found a degree of genetic fixation across *B. mixtus* subpopulations, with the San Juan Island lineage to be distinct from populations in the Olympic and Cascade Mountains. While I observed different patterns of genetic fixation and structure across all four species, I did find that populations of each species showed significantly less genetic diversity, primarily effective allelic diversity, in the

Olympic Mountains relative to the Cascades. Given the unique Pleistocene history of the Pacific Northwest and geographic isolation of the Olympic Mountains, future work could address the phylogeography of multiple bumble bees in the area. As bumble bees have responded to global change behaviorally through early emergence and shorter proboscis length relative to historic populations (Bartomeus et al. 2011; Miller-Struttmann et al. 2015), the study of bumble bees in federally protected areas like Olympic National Park may provide insight into how bumble bees respond to global change.

In conclusion, my dissertation reports on the patterns of genetic diversity in economically important and wild bumble bees throughout North America. My body of work also illuminated the cryptic phenotypes associated with two monophyletic bumble bee species. Finally, I explored the role of Müllerian mimicry in structuring bumble bee communities at narrow spatial scales as an explanation for co-located phenotypes across different species. I expect that the results of my dissertation will enhance our knowledge on the natural history of bumble bees, which will help biologists, growers, and policy makers make informed decisions on managing and conserving North America's most iconic pollinator.

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APPENDICES

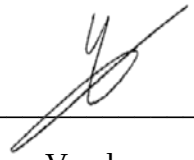
APPENDIX A

Co-author release forms

September 30, 2015

Jonathan B. Koch has my permission to include the following paper, which will be submitted for publication, of which I was a co-author, in his doctoral dissertation in the Biology Department at Utah State University.

Koch, J.B., Vandame, R. Pineda, E., Philippe, S., Strange, J.P. “Range-wide assessment of *Bombus huntii* genetic diversity and niche suggests population divergence during the Pleistocene”.



Dr. Rémy Vandame

September 30, 2015

Jonathan B. Koch has my permission to include the following paper, which will be submitted for publication, of which I was a co-author in his doctoral dissertation in the Biology Department at Utah State University.

Koch, J.B., Vandame, R. Pineda, E., Philippe, S., Strange, J.P. “Range-wide assessment of *Bombus huntii* genetic diversity and niche suggests population divergence during the Pleistocene”.

Mr. Philippe Sagot

September 30, 2015

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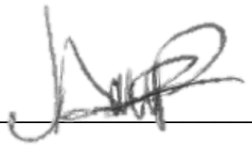


Mr. Jorge Mérida-Rivas

September 12, 2015

Jonathan B. Koch has my permission to include the following paper, which will be submitted for publication, of which I was a co-author, in his doctoral dissertation in the Biology Department at Utah State University.

Koch, J.B., Rodriguez, J. R., Pitts, J. P., Strange, J. P. “Multigene phylogeny and microsatellite genotypes illuminates an enigmatic bumble bee species complex”.

Dr. Juanita R. Rodriguez

September 12, 2015

Jonathan B. Koch has my permission to include the following paper, which will be submitted for publication, of which I was a co-author in his doctoral dissertation in the Biology Department at Utah State University.

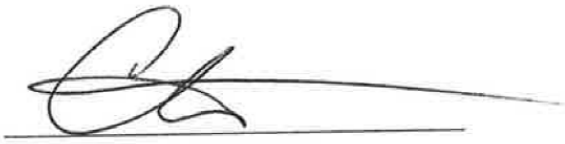
Koch, J.B., Florez, J., Griswold, T., Pitts, J. P., Strange, J. P. “Müllerian mimicry structures local bumble bee communities across an elevation gradient.”

Mr. Jaime A. Florez

October 1, 2015

Jonathan B. Koch has my permission to include the following paper, of which I was a co-author, in his doctoral dissertation in the Biology Department at Utah State University. He has submitted the paper to *Northwest Science*.

Koch, J. B., Looney, C., Sheppard, W. S., Strange, J. P. “Range extension of two bumble bee species (Hymenoptera: Apidae) into Olympic National Park”.



Dr. Chris Looney

October 1, 2015

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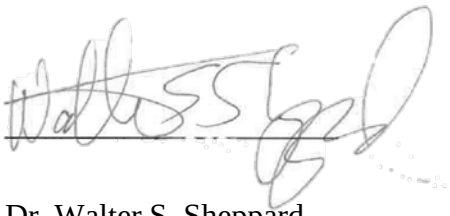
Koch, J. B., Looney, C., Sheppard, W. S., Strange, J. P. “Range extension of two bumble bee species (Hymenoptera: Apidae) into Olympic National Park”.



Dr. Walter S. Sheppard

October 1, 2015

Jonathan B. Koch has my permission to include the following paper, which will be submitted for publication, of which I was a co-author, in his doctoral dissertation in the Biology Department at Utah State University.

Koch, J. B., Looney, C., Sheppard, W. S., Strange, J. P. “Patterns of population genetic differentiation across bumble bee communities in the Pacific Northwest”.

Dr. Chris Looney

October 1, 2015

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Koch, J. B., Looney, C., Sheppard, W. S., Strange, J. P. “Patterns of population genetic differentiation across bumble bee communities in the Pacific Northwest”.

Dr. Walter S. Sheppard

APPENDIX B

Chapter 2 Supplementary Tables and Figures

Appendix B1, Table B-1. Data providers of the georeferenced occurrence records used to construct habitat suitability models and characterize bioclimatic niche of *Bombus huntii*. Data were accessed on 28 June 2015.

Institution
iNaturalist.org: iNaturalist research-grade observations doi:10.15468/ab3s5x
USDA-ARS Bee Biology and Systematics Laboratory: Bee Biology and Systematics Laboratory doi:10.15468/anyror
Bombus of Canada doi:10.15468/ip9oon
Museum of Biological Diversity, The Ohio State University: C.A. Triplehorn Insect Collection (OSUC), Ohio State University doi:10.15468/efb17f
Luis Martínez, M. A. 2001. Computarización de la colección de abejas (Hymenoptera: Apoidea) del Museo de Zoología Alfonso L. Herrera, de la Facultad de Ciencias de la UNAM. Universidad Nacional Autónoma de México. Facultad de Ciencias. Bases de datos SNIB2010-CONABIO. Proyecto No. Q035. México, D.F. doi:10.15468/wzfnf2
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Biodiversity Institute of Ontario (UBCZ) from University of Guelph. http://dx.doi.org/10.5886/qzxxd2pa (accessed on [date]), doi:10.5886/qzxxd2pa doi:10.5886/qzxxd2pa
Yale University Peabody Museum: Entomology Division, Yale Peabody Museum doi:10.15468/95waq3
USDA-ARS Bee Biology and Systematics Laboratory: Patterns of widespread decline in North American bumble bees doi:10.15468/kjpwz1
iNaturalist.org: iNaturalist research-grade observations doi:10.15468/ab3s5x
Illinois Natural History Survey: Illinois Natural History Survey doi:10.15468/eol0pe
University of Kansas Biodiversity Institute: Snow Entomological Museum Collection doi:10.15468/fhntpy

APPENDIX C

Chapter 3 Supplementary Tables and Figures

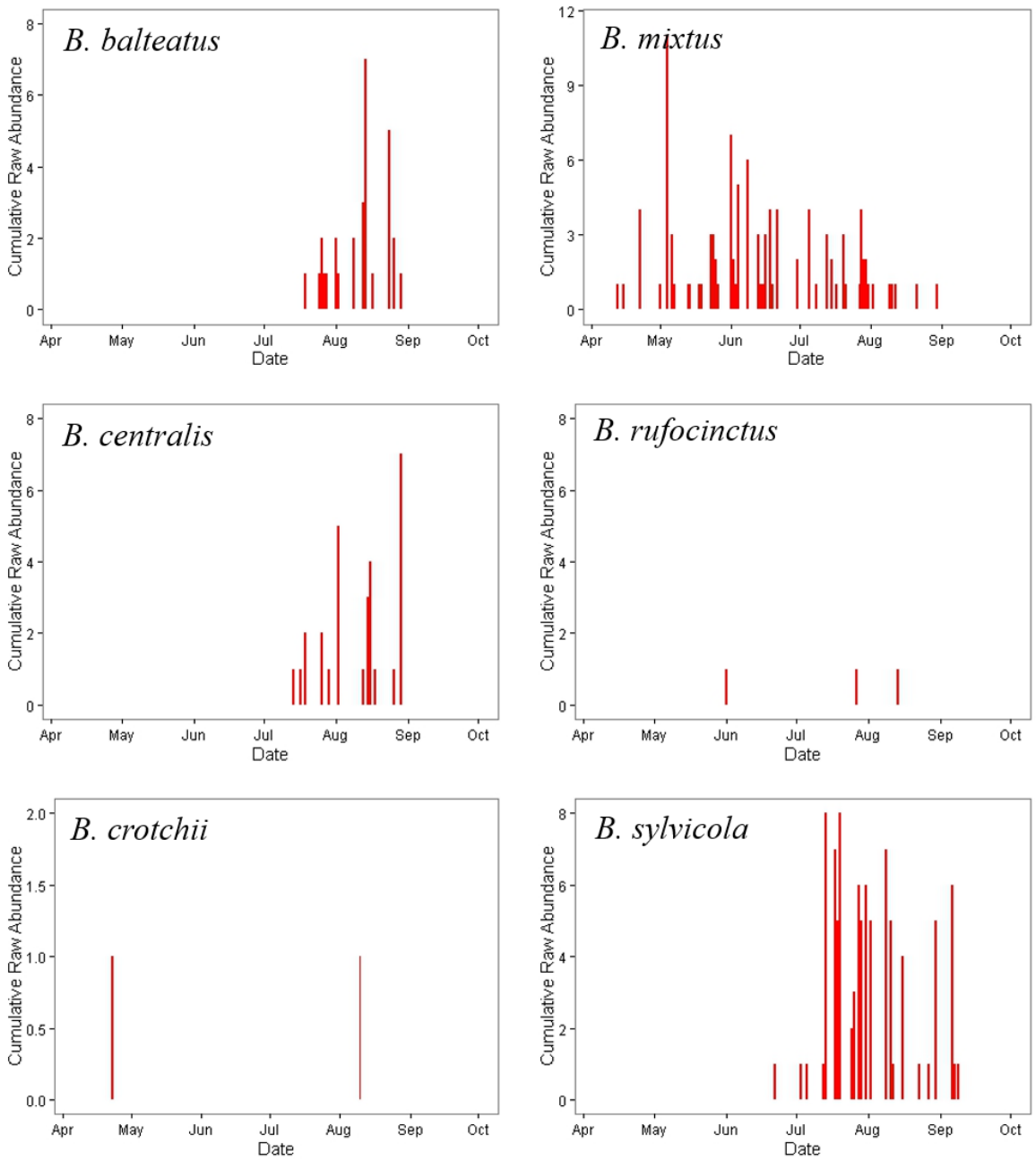
Appendix C1, Table C-1. Data providers of the georeferenced occurrence records used to characterize bioclimatic niche. Data were accessed on 2 September 2014.

Species	Institution
B. californicus	Naturalis Biodiversity Center: Naturalis Biodiversity Center (NL) - Hymenoptera
B. californicus	USDA-ARS Bee Biology and Systematics Laboratory: Bee Biology and Systematics Laboratory
B. californicus	Essig Museum Online Database. University of California, Berkeley., http://essigdb.berkeley.edu
B. californicus	ARC
B. californicus	University of Alaska Museum Observation data (non-marine arthropods)
B. californicus	York University: Morandin PhD Thesis / La Crete, Alberta
B. californicus	Yale Peabody Museum, (c) 2009. Specimen data records available through distributed digital resources.
B. californicus	Field Museum: Field Museum of Natural History (Zoology) Insect, Arachnid and Myriapod Collection
B. californicus	York University: Ratti Master Thesis / Fraser Valley, British Columbia
B. californicus	iNaturalist.org: iNaturalist research-grade observations
B. californicus	University of Kansas Biodiversity Institute: Snow Entomological Museum Collection
B. californicus	National Museum of Natural History, Smithsonian Institution: NMNH occurrence DwC-A
B. fervidus	Naturalis Biodiversity Center: Naturalis Biodiversity Center (NL) - Hymenoptera
B. fervidus	University of Alberta Museums: University of Alberta Entomology Collection (UASM)
B. fervidus	Iziko
B. fervidus	Luis Martínez, M. A. 2001. Computarización de la colección de abejas (Hymenoptera: Apoidea) del Museo de Zoología Alfonso L. Herrera, de la Facultad de Ciencias de la UNAM. Universidad Nacional Autónoma de México. Facultad de Ciencias. Bases de datos SNIB2010-CONABIO. Proyecto No. Q035. México, D.F.
B. fervidus	Biodiversity Institute of Ontario (UBCZ) from University of Guelph. http://dx.doi.org/10.5886/qzxxd2pa, doi:10.5886/qzxxd2pa
B. fervidus	Yale Peabody Museum, (c) 2009. Specimen data records available through distributed digital resources.

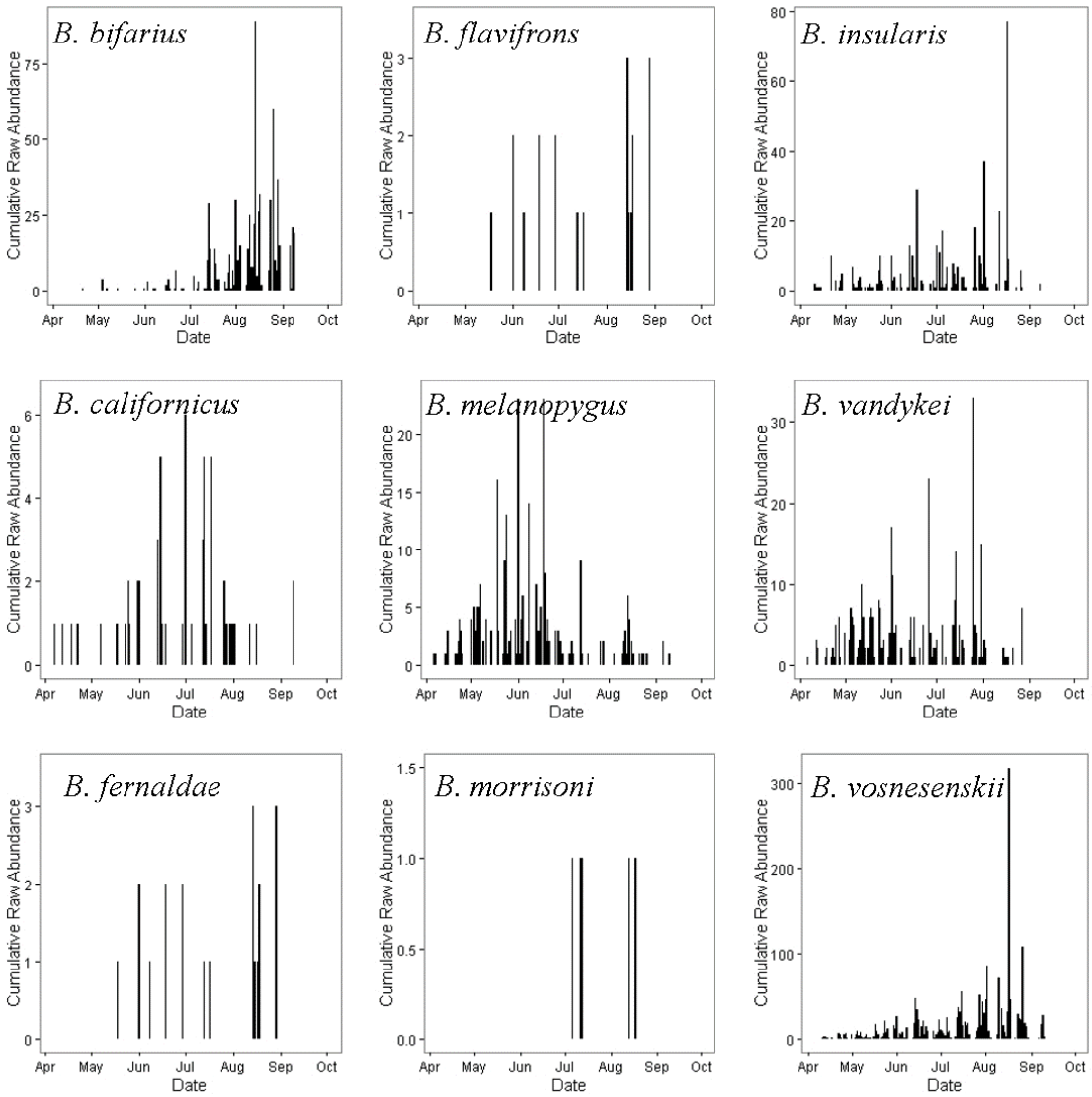
Species	Institution
B. fervidus	Field Museum: Field Museum of Natural History (Zoology) Insect, Arachnid and Myriapod Collection
B. fervidus	iNaturalist.org: iNaturalist research-grade observations
B. fervidus	University of Kansas Biodiversity Institute: Snow Entomological Museum Collection
B. fervidus	York University: Knerer collection / Gschwendtner property
B. fervidus	USDA-ARS Bee Biology and Systematics Laboratory: Bee Biology and Systematics Laboratory
B. fervidus	Bombus of Canada
B. fervidus	Museum of Biological Diversity, The Ohio State University: C.A. Triplehorn Insect Collection (OSUC), Ohio State University
B. fervidus	ARC
B. fervidus	Museum of Comparative Zoology, Harvard University: Museum of Comparative Zoology, Harvard University
B. fervidus	Dr. Ricardo Ayala Barajas, CNIN/Abejas de México/Apoidea, Estación de Biología Chamela, Instituto de Biología, UNAM consultada el dd/mm/yy.
B. fervidus	University of Alaska Museum Observation data (non-marine arthropods)
B. fervidus	USDA-ARS Bee Biology and Systematics Laboratory: Patterns of widespread decline in North American bumble bees
B. fervidus	Illinois Natural History Survey: Illinois Natural History Survey
B. fervidus	National Museum of Natural History, Smithsonian Institution: NMNH occurrence DwC-A
B. fervidus	Borror Laboratory of Bioacoustics audio recording record.
B. fervidus	Museum of Biological Diversity, The Ohio State University: Borror Lab of Bioacoustics (BLB), Ohio State University

APPENDIX D

Chapter 4 Supplementary Tables and Figures



Appendix D1, Figure D-1. Cumulative raw abundance of adult bumble bees with red setae on the dorsal metasoma detected from April to October in Yosemite National Park.



Appendix D1, Figure D-2. Cumulative raw abundance of adult bumble bees with black setae detected from April to October in Yosemite National Park.

APPENDIX E

Chapter 6 Supplementary Tables and Figures

Appendix E1, Table E-1. Table of four probabilities of model fit implemented with the Evanno Method associated with different values of *K* (i.e., clusters) based on 8 microsatellites implemented in STRUCTURE Harvester in four species (A) *B. flavifrons*, (B) *B. mixtus*, (C) *B. melanopygus*, and (D) *B. sylvicola*. Bold text represents the indices that suggests the value of *K* that best predicts the microsatellite genotypes assigned in the STRUCTURE analysis. High values of Delta K suggest better fit of the genotype data to the number of proposed K.

(A) *B. flavifrons*

# K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-5123.71	0.2807	-	-	-
2	**10**	**-5139.39**	**11.7357**	**-15.68**	**258.12**	**21.99435**
3	10	-5413.19	63.9267	-273.8	371.38	5.809466
4	10	-6058.37	413.3857	-645.18	443.79	1.07355
5	10	-6259.76	493.1711	-201.39	216.97	0.439949
6	10	-6244.18	987.9128	15.58	460.37	0.466003
7	10	-5768.23	113.5797	475.95	923.48	8.130676
8	10	-6215.76	960.9537	-447.53	221.7	0.230708
9	10	-6441.59	1705.53	-225.83	362.75	0.212691
10	10	-6304.67	1545.959	136.92	-	-

(B) *B. mixtus*

# K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-2331.6	0.3887	-	-	-
2	10	-2270.09	11.8345	61.51	28.3	2.391304
3	**10**	**-2236.88**	**4.8508**	**33.21**	**100.89**	**20.79845**
4	10	-2304.56	14.6658	-67.68	19.38	1.321437
5	10	-2391.62	38.3121	-87.06	35.68	0.931298
6	10	-2443	42.8971	-51.38	17.29	0.403058
7	10	-2511.67	23.9753	-68.67	60.07	2.505499
8	10	-2520.27	69.3742	-8.6	4.02	0.057947
9	10	-2524.85	33.6123	-4.58	46.07	1.370628
10	10	-2575.5	54.7021	-50.65	-	-

Appendix E1, continued.

(C) *B. melanopygus*

# K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln"(K)|	Delta K
1	10	-1649.11	0.4067	-	-	-
2	**10**	**-1685.32**	**23.2054**	**-36.21**	**36.88**	**1.589288**
3	10	-1684.65	20.7593	0.67	13.01	0.626706
4	10	-1696.99	37.6312	-12.34	12.55	0.3335
5	10	-1696.78	42.7173	0.21	16.77	0.392581
6	10	-1713.34	38.7628	-16.56	5.33	0.137503
7	10	-1724.57	71.1293	-11.23	2.85	0.040068
8	10	-1738.65	92.1577	-14.08	18.38	0.199441
9	10	-1734.35	71.8521	4.3	49.47	0.688498
10	10	-1779.52	102.3651	-45.17	-	-

(D) *B. sylvicola*

# K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln"(K)|	Delta K
1	10	-4674.69	0.247	-	-	-
2	10	-4618.48	6.8256	56.21	166.52	24.39649
3	10	-4728.79	30.1367	-110.31	112.85	3.744603
4	10	-4951.95	45.2308	-223.16	50.17	1.109199
5	10	-5225.28	269.0882	-273.33	305.52	1.13539
6	10	-5193.09	77.7482	32.19	122.13	1.57084
7	10	-5283.03	127.2144	-89.94	0.23	0.001808
8	10	-5372.74	157.7693	-89.71	5.5	0.034861
9	10	-5456.95	101.797	-84.21	113.56	1.115554
10	10	-5427.6	254.8693	29.35	-	-

Jonathan B. Koch

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PROFESSIONAL PREPARATION

Ph.D. Ecology, Utah State University, expected November 2015
Dissertation: Biogeography, population genetics, and community structure of North American Bumble Bees. Advisers: James Strange and James Pitts

M.S. Biology, Utah State University, 2011
Thesis: The decline and conservation status of North American bumble bees.
Advisers: James Strange and James Pitts
Recognized as the *Graduate Student Researcher of the Year in College of Science* (2011)

B.S. Environmental Science, **B.A. Geography**, University of Hawai'i at Hilo, 2008
Thesis: Plant-pollinator interactions across an elevation gradient on Mauna Loa Volcano.
Research Advisers: Heather F. Sahli and Jonathan Price; Academic Adviser: James Juvik
Recognized as a *University of Hawai'i at Hilo Chancellor Scholar* (2003 – 2008)
Recognized as the *Environmental Science Student of the Year* (2008)
Recognized as the *Student Employee of the Year* (2007)

PROFESSIONAL TRAINING

2008 – 15 **Research Assistant**, Department of Biology, Utah State University
Investigated conservation status of North American bumble bees with molecular data (microsatellites and sequence data) and specimens form natural history collections. Conducted intensive field work throughout the American West and Alaska. Curated bumble bee specimens in 13 natural history collections. Mentored underrepresented students in STEM.

2008 – 15 **Biological Science Aid**, USDA-ARS Pollinating Insects Research Unit
Assisted in the insect husbandry of stable and declining bumble bees in a federal lab. Supervised undergraduate researchers and technicians. Performed microscopy for pathogens for thousands of bumble bee specimens in field conditions.

2007 - 08 **Research Assistant**, Department of Biology, University of Hawai'i at Hilo
Investigated plant-pollinator interactions in Hawaii for 1 year across rugged lava landscape in Hawaii. Performed field surveys and assessed pollen species associated with different insect visitors. Conducted analyses to determine the insect and bird visitor dynamics to native Hawaiian plants.

PUBLICATIONS

Refereed Articles
Published (12), Submitted (3), In Preparation (5)
Books (1)
Technical Reports & Published Outreach (5)

Published Refereed Articles

1. **Koch, J.B.**, Love, B., Klinger, E., Strange, J.P. 2014. The effect of sun exposure on bee (Hymenoptera: Anthophila) setal color and its implications for studying aging and behavior. ***Journal of Melittology***, 38: 1-9.

2. Orr, M., **Koch, J.B.**, Griswold, T., Pitts, J.P. 2014. The Re-Evaluation of the Specific Status of a Solitary Bee, *Anthophora* (*Heliophila*) *curta* (Hymenoptera: Apidae). ***Zootaxa***, 3846 (3): 411-429.

3. Holden, A.R., **Koch, J.B.**, Erwin, D.M., Griswold, T., Hall, J. 2014. Leafcutter Bee Nests and Pupae from the Rancho La Brea Tar Pits of southern California: Implications for understanding the paleoenvironment of the Late Pleistocene. ***PLoS ONE,*** 9(4): e94724. doi:10.1371/journal.pone.0094724. Media coverage in > 30 news media outlets include Nature News & Views, Science News, Science Daily, Aljazeera, and EurekAlert.

4. Strange, J., Baur, A., **Koch, J**. 2013. A scientific note on *Bombus (Psithyrus) insularis* invasions of bumble bee nests and honey bee hives in the western United States. ***Apidologie***, 45: 554 – 556.

5. Lozier, J., Strange, J., **Koch, J.** 2013. Landscape heterogeneity predicts gene flow in a widespread polymorphic bumble bee, *Bombus bifarius* (Hymenoptera: Apidae). ***Conservation Genetics***, 14(5): 1099-1110.

6. **Koch, J.B.**, Sahli, H.F. 2012. Patterns of flower visitation across elevation and succession gradients in Hawai'i. ***Pacific Science***, 67(2): 253 – 266.

7. **Koch, J.B.,** Strange, J.P. 2012. The status of *Bombus occidentalis* and *B. moderatus* in Alaska with special focus on *Nosema bombi* incidence. ***Northwest Science***, 86(3): 212 – 220.

8. Strange, J.P., **Koch, J.B.**, Gonzalez, V.H., Nemelka, L., Griswold, T. 2011. Global invasion by *Anthidium manicatum* (Linnaeus) (Hymenoptera: Megachilidae): assessing potential distribution in North America and beyond. ***Biological Invasions***, 13: 2115 – 2133.

9. Cameron, S., Lozier, J., Strange, J., **Koch, J.**, Cordes, N., Solter, L., Griswold, T. 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. Media coverage in > 20 news media outlets include Nature News & Views, Science News, Science Daily, Huffington Post, MSNBC News, Aljazeera, and EurekAlert. Study is reviewed by Brown, M.J F. 2011. The trouble with bumblebees. ***Nature*** 469:169-170.

10. Gonzalez, V.H., **Koch, J.B.**, Griswold, T.L. 2010. *Anthidium vigintiduopunctatum* Friese (Hymenoptera: Megachilidae): The elusive "dwarf bee" of the Galapagos Archipelago? ***Biological Invasions***, 12: 2381-2383.

11. **Koch, J.B.**, Strange, J.P. 2009. Constructing a species database and historic range maps for North American bumble bees (*Bombus* sensu stricto Latreille) to inform conservation decisions. ***Uludag Bee Journal***, 9(3): 97-108.

12. Blue, J.D., Armstrong, M., Brym, Z., Chandler, J., Cheluitte, C., Cooley, C., Farinas, S., **Koch, J.B.** 2009. Synthesis and the Future of Ecology. ***Bulletin of the Ecological Society***, 90:283–285.

Technical Reports and Published Outreach (Not Peer-Reviewed)

13. Strange, J.P., **Koch, J.B.**, Sheppard, W., Looney, C., Lichtenberg, E., Long, J., Hopkins, B. 2014. Bumble bee community composition and population genetic diversity in the North Cascades and Coast Network. 30 p.

14. **Koch, J.B**., Sadler, E., Weglarz, K. 2014. Utah State University Entomology Club and Insect Tours Webpage. http://usuinsecttours.wix.com/usuentomologyclub#!about-usu-insect-tours/c1zn5.

15. Burkle, L., Griswold, T., Hernandez, J., Inouye, D., **Koch, J.** 2011. Climate Change and Range Shifts Working Group In *North American Bumble Bee Species Conservation Planning Workshop Final Report* (Cameron, S., S. Jepsen, E. Spevak, J. Strange, M. Vaughan, J. Engler, and O. Byers, Editors). IUCN Species Survival Commission, Conservation Breeding Specialist Group: Apple Valley MN, USA.

16. Adams, L.D., **Koch, J.**, Strange, J. 2011. Bumble Bees of the Western United States Poster. Pollinator Partnership, San Francisco CA, USA. (Poster)

17. **Koch, J.**, Colla, S., Inouye, D., Adams, L.D. 2009. Bumble bees are essential: Helping pollinators thrive. Pollinator Partnership, San Francisco CA, USA. (Pamphlet)

Books

18. **Koch, J.B.**, Strange, J.P., Williams, P.H. 2012. Bumble bees of the western United States. USDA Forest Service and the Pollinator Partnership. 144 p. Recognized by

the American Library Association as "Notable Government Document of the Year 2012". Reviewed by *Native Plants Journal* 14(2): 116 – 117.

Submitted

19. Koch, J.B., Looney, C., Sheppard, W.S. Strange, J.P. Range extensions of two bumble bees (Hymenoptera: Apidae) in Olympic National Park. *Northwest Science*.

20. Rhoades, P.R., **Koch, J.B.,** Waits, L., Strange, J.P., Eigenbrode, S.D. Evidence for *Bombus occidentalis* (Hymenoptera: Apidae) populations in the Olympic Peninsula, the Palouse Prairie, and Forests of Northern Idaho. *Journal of Insect Science.*

21. Koch, J.B., Lozier, J., Strange, J., Griswold, T., Cordes, N., Solter, L., Stewart, I., Cameron, S. *USBombus*, contemporary survey data of North American bumble bees (Hymenoptera, Apidae, *Bombus*) distributed in the United States. *Biodiversity Data Journal.*

In Preparation

22. Koch, J.B., Florez, J., Strange, J.P., Pitts, J., Griswold, T. Community structure predicted by convergent Müllerian mimicry in bumble bees.

23. Hatfield, R., Strange, J.P., **Koch, J.B**., Jepsen, S. Neonicitinoid pesticides drive population dynamics in localized bumble bee (*Bombus* spp., Hymenoptera: Apidae) populations: A case study from Wilsonville, OR.

24. Koch, J.B., Pitts, J., Strange, J.P. Microsatellites and species distribution models reveal cryptic diversity of North American bumble bees.

25. Koch, J.B., Looney, C., Sheppard, S., Strange J.P. Landscape genetics of Pacific Northwest bumble bees reveals restricted gene flow in high-elevation distributed species.

26. Baur, A., **Koch, J.B.**, Strange J.P. Foraging ecology of *Bombus huntii*, a viable bumble bee pollinator for commercialization.

TEACHING EXPERIENCE

2015 **Lecturer,** Bumble Bee Molecular Ecology Workshop, USDA-ARS Pollinating Insect Research Unit and Project Integrated Crop Pollination. *Developed curriculum and materials for three 1-hour lectures for workshop participants that included senior faculty, technicians, and graduate students (15 attendees). Lecture 1: Specimen Handling and DNA Extraction; Lecture 2: Heterozygosity and Genetic Diversity; Lecture 3: Introduction to Population Genetics.* Supervisor: James P. Strange

2014 **Teaching Assistant,** Insect Systematics & Evolution, Utah State University. *Prepared lectures and lab curriculum for insect taxonomy and systematics aimed at graduate and undergraduate students. Curated teaching collection spanning major orders of Hexapoda. Developed and graded lab quizzes and practical. Held office hours. (1 section). Evaluations available upon request.* Supervisors: Carol von Dohlen and James Pitts

2013 **Teaching Assistant**, Introduction to Plant Pathology, Utah State University.
Prepared lectures on plant pathology and sterile technique. Organized lab materials, supplies, reagents, and cultures for 30 undergraduate students. Graded lab assignments, research paper, and exams. Held office hours. (1 section). Evaluations available upon request. Supervisor: Bradly Kropp

2012 **Teaching Assistant**, General Biology I, Utah State University
Prepared short lectures for biology labs (for Biology majors). Led labs of ~60 students and graded assignments. Held office Hours. (2-3 sections per semester). Evaluations available upon request. Supervisor: James Pitts

Lecturer, The Bumble Bee Workshop, USDA-ARS Pollinating Insects Research Unit. *Developed curriculum and materials for three 30-minute lectures directed to the general public and stakeholders invested in bumble bee management for crop pollination (20 attendees). Lecture 1: Bumble Bee Biology and Natural History; Lecture 2: Bumble Bee Health and Conservation; Lecture 3: Gardening for Local Bumble Bees.* Supervisor: James Strange

2011 **Lecturer**, All About Bees Workshop, Utah State University Extension. *Developed curriculum and materials for one 1-hour lecture directed to the stakeholders interested in using bees for pollination services in agricultural and horticultural settings (35 attendees). Lecture 1: Bumble bees and Honey bees. Received Honorarium ($50.00).*

Teaching Assistant, General Microbiology, Utah State University
Prepared lectures on general biology and sterile technique. Organized lab materials, supplies, and cultures for up ~60 students. Graded assignments and lab exams. (2 sections). Evaluations available upon request. Supervisor: Bradly Kropp

INVITED PRESENTATIONS AND SEMINARS

1. **Koch, J.B.** 2016. Digitizing natural history collections to investigate the future of species conservation. International Congress of Entomology. Orlando FL, USA. (Upcoming *September*).

2. **Koch, J.B.** 2015. Maximizing natural history collections for biodiversity conservation. Ecological Society of America IGNITE Series. Baltimore MD, USA. Audio/Visual Recording available at: https://vimeo.com/136524119. (50 attendees)

3. **Koch, J.B.** 2015. Where the wild bees are. University of Florida, Florida Bee Symposium. Gainesville, FL, USA. **Keynote Speaker**.

4. **Koch, J.B.** 2015. The plight of the pollinator. Utah State University IGNITE Series. Logan UT, USA. (178 attendees) http://ignite.usu.edu/jonathan-koch/; YouTube: https://youtu.be/SQ4lS937TY8. (**537 views,** 18 August 2015).

5. **Koch, J.B**. 2015. Teaching entomology in Cache Valley. Utah State University Entomology Club. Logan, UT, USA. (15 attendees)

6. **Koch, J.B.** 2014. The plight of the pollinator. USGS Pacific Island Ecosystems Research Center. Hawai'i Volcanoes National Park, Volcano HI, USA. (15 attendees)

7. **Koch, J.B.** 2014. Pacific Northwest bumble bees and Climate Change. Utah State University Entomology Club. Logan UT, USA. (10 attendees)

8. **Koch, J.B.** 2013. The would-bee Biologist: Taking advantage of your undergraduate education. Southwestern Oklahoma State University. Weatherford OK, USA. (50 attendees)

9. **Koch, J.B.** 2013. Pollinator decline and conservation. Society for the Advancement of Chicano/Hispanics and Native American Scientists- Utah State University. Logan UT, USA. (30 attendees)

10. **Koch, J.B.** 2013. Gardening for Utah bees. Utah State University, Horticultural Entomology. Kayesville UT, USA. (30 attendees)

11. **Koch, J.B.** and Strange J.P. 2013. Bumble bees of the Pacific Northwest. Olympic National Park. Port Angeles, WA. (10 attendees)

12. **Koch, J.B.** and Strange J.P. 2013. Bumble bees of the Pacific Northwest. North Cascades National Park. Sedro Wooley WA, USA. (10 attendees)

13. **Koch, J.B**. and Strange, J.P. Mt. 2013 Bumble bees of the Pacific Northwest. Rainier National Park. Ashford, WA. (10 attendees)

14. **Koch, J.B.** 2011. First Annual Bee-A-Thon. The Plight of the bumble bee. Global Online Event (> 500 attendees)

15. **Koch, J.B.** 2011. Weber Public Library. North American pollinator decline. Huntsville UT, USA. (20 attendees)

CONTRIBUTED PRESENTATIONS (FIRST AUTHOR)*

*titles of 14 additional non-first author presentations are available upon request.

1. **Koch, J.B.**, Vandame, R. Strange, J.P. 2015. Population genetic diversity of wild *Bombus huntii* in North America, a viable pollinator for commercialization. Florida Bee Research Symposium, Gainesville, Florida, USA.

2. **Koch, J.B.**, Pitts, J., Strange, J.P. 2015. Genetic diversity of a cryptic bumble bee species, *Bombus californicus* in western North America. Entomological Society of America, Pacific Branch. Coeur d'Alene ID, USA. (Invited Symposium Presentation)

3. **Koch, J.B.** Orr, M., Sadler, E. 2015. Is this Real? Exploring Insect Biodiversity with local communities. Entomological Society of America, Pacific Branch. Coeur d'Alene ID, USA. (Invited Symposium Presentation)

4. **Koch, J.B.**, Strange, J.P.* 2015. Population genetic diversity of Alpine bumble bees in western North America. Entomological Society of America, Pacific Branch. Coeur d'Alene ID, USA. *Presenting Author

5. **Koch, J.B.,** Strange J.P. 2014. Population genetic diversity patterns of bumble bee (*Bombus*) communities in the wild lands of the Pacific Northwest. Entomological Society of America. Portland OR, USA.

6. **Koch, J.B.**, Strange J.P. 2014. Bumble bee population genetic structure and diversity in the context of global climate change. North American Congress for Conservation Biology. Missoula MT, USA.

7. **Koch, J.B.**, Orr, M. 2013. The Buzz on Bee Biodiversity in Utah, Maps on the Hill. Salt Lake City UT, USA. (Poster)

8. **Koch, J.B.**, Strange J.P. 2013. Conservation-minded approaches to surveying bumble bees. North American Pollinator Protection Campaign. Washington D.C., USA.

9. **Koch, J.B.**, Strange, J.P. 2013. Environmental diversity and genetic structure of North American Bumble Bees. Intermountain Graduate Research Symposium. Logan UT, USA. **Awarded 1st place for Graduate Student Presentation.**

10. **Koch, J.B.**, Strange, J.P. 2013. Environmental diversity and genetic diversity of North American *Pyrobombus*. Entomological Society of America. Stateline NV, USA. **Awarded 1st place for Graduate Student Presentation.**

11. **Koch, J.B.**, Love, B., Klinger, E., Strange, J.P. 2013. Revisiting the utility of morphometric measurements in assessing senescence across Apoidea. Entomological Society of America. Stateline NV, USA.

12. **Koch, J.B.**, Strange, J.P. 2012. Investigating the species concept of North American *Fervidobombus*. Intermountain Graduate Research Symposium. Logan UT, USA.

13. **Koch, J.B.**, Strange, J.P. 2012. Investigating the species concept of North American *Fervidobombus*. Entomological Society of America. Portland OR, USA. **Awarded 1st place for Graduate Student Presentation.**

14. **Koch, J.B.**, Strange, J.P. 2011. The conservation status of *Bombus moderatus* and *B. occidentalis* in Alaska, USA. Entomological Society of America. Reno NV, USA. **Awarded 1st place for Graduate Student Presentation.**

15. **Koch, J.B.,** Strange, J.P. 2011. The conservation status of North American bumble bees. Intermountain Graduate Research Symposium. Logan UT, USA. **Awarded 2nd place for Graduate Student Presentation.**

16. **Koch, J.B.**, Strange, J.P. 2010. Revisiting the subspecies conundrum of the bumble bee *Bombus bifarius* Cresson (Hymenoptera: Apidae) in North America. Entomological Society of America. San Diego CA, USA.

17. **Koch, J.B.**, Strange, J.P., Lozier, J.D., Griswold, T.L., Cameron, S.A., Thorp, R.W. 2010. The conservation status of nine bumble bee species in North America. Entomological Society of America. San Diego CA, USA.

18. **Koch, J.B.**, Strange, J.P., Ikerd, H., Griswold, T.L. 2010. The importance of entomological collections in assessing the conservation status of the western bumble bee, *Bombus occidentalis* Greene. Entomological Society of America, Pacific Branch. Boise ID, USA. (Invited Symposia talk)

19. **Koch, J.B.**, Sahli, H.F. 2008. Pollination webs across elevation and succession in Hawai'i. Ecological Society of America. Milwaukee WI, USA.

20. **Koch, J.B.,** Sahli, H.F. 2008. Pollination webs across elevation and succession in Hawai'i. EPSCoR Hawai'i Conference, Waikoloa HI, USA.

RESEARCH GRANTS

2015 **National Science Foundation Postdoctoral Fellowship** ($138,000) Awarded Jan. 2016. PI: **Jonathan B. Koch**, Mentors: Donald Price (University of Hawaii at Hilo), Peter Follett (USDA-ARS Daniel K. Inouye Pacific Basin Research Center). Title: "Invasion biology, population genomics, and adaptive evolution of *Drosophila suzukii*"

2014 **Utah State University Ecology Center Graduate Research Grant** ($4000) Awarded May 2014. PI: **Jonathan B. Koch.** Mentors: James Strange (USDA-ARS Pollinating Insects Research Unit) and James Pitts (Utah State University). Title: "Landscape genetics of Pacific Northwest Bumble Bees"

SIGNIFICANT ACCOMPLISHMENTS AND AWARDS

1. 2015, Ecological Society of America SEEDS Grad. Student Travel Award ($800)
2. 2015, Graduate Student Travel Award, Utah State University ($300)
3. 2014**,** Graduate Enhancement Award, Utah State University ($4000)
4. 2014, MacMahon Endowed Ecology Graduate Student Research Award ($1000)
5. 2014, Graduate Student Travel Award, Utah State University ($300)
6. 2013, 1st place Paper, Entomological Society of America, PBESA ($100)
7. 2013, 1st place Paper, Intermountain Grad. Stu. Research Symposium ($300)
8. 2012, 1st place Paper, Entomological Society of America, PBESA ($150)
9. 2012, 1st place Paper, Entomological Society of America Ann. Meeting ($150)
8. 2012. 2nd place Paper, Intermountain Grad. Stu. Research Symposium ($50)
9. 2012, Notable Government Document of the Year, American Library Association GODORT Award for Bumble bees of the western United States
10. 2011, Graduate Researcher of the Year, Utah State University CoS ($100)
11. 2009, 2010, 2011, USDA-ARS PIRU, On-the-Spot Award ($50 each)
12. 2007, Student Employee of the Year, University of Hawai'i at Hilo ($2000)
13. 2008, Environmental Science Student of the Year, Univ. of Hawaii Hilo ($100)
14. 2007, NSF REU Intern, University of Hawai'i Hilo ($4000)
15. 2008, Ecological Society of America SEEDS Travel Award ($3000)
16. 2008, Nominee, Who's who in American Colleges and University
17. 2003 – 2008, Dean's List, University of Hawai'i Hilo
18. 2003 – 2008, University of Hawai'i at Hilo Chancellor Scholar (4-yr tuition waiver)

PROFESSIONAL SERVICE AND LEADERSHIP

Ad hoc reviewer for: Caldasia, Checklist Journal, Environmental Entomology, Journal of Economic Entomology, Journal of Melittology, Oecologia

1. 2016, *Symposium Co-organizer*, PBESA- Entomological Soc. of America, "Celebrating North America's Iconic Native Pollinator - Contemporary advances in the study of bumble bee ecology and evolution".
2. 2016, *Symposium Co-organizer*, PBESA - Entomological Soc. of America, "Celebrating Diversity in the Pacific Branch, and the next 100 years".
3. 2015, *Co-Chair*, Celebrating National Pollinator Week with the USDA-ARS at the Cache Valley Gardener's Market.
4. 2015, *SEEDS Alumni Mentor*, Ecological Society of America.
5. 2015, *Reviewer*, Ecological Soc. of America SEEDS Travel Award Committee.

6. 2015, *Symposium Co-organizer***,** PBESA- Entomological Soc. of America, "Discovering Patterns and Process of Insect Biodiversity in the American West".
7. 2013 – 15, *Petition Contact*, Proclamation for "Utah Pollinator Week" (mid-June).
8. 2013 – 15, *Regional Administrator*, Bumble Bee Watch, Xerces Society.
9. 2013, *Co-Chair*, NAPPC Bumble Bee Task Force.
10. 2008 – 15, *Member*, NAPPC Bumble Bee Task Force.
11. 2013 – 14, *Science Outreach Coordinator*, USU Insect Tours & Entomology Club.
12. 2010 – 15, *Member*, IUCN Species Survival Commission, Bumble Bee Group.
13. 2008 – 15, *Entomology Club Tour Guide*, USU Entomology Club.
14. 2012, *Organization Committee*, USDA-ARS Bumble Bee Workshop.
15. 2010 – 13, *Biology Seminar Committee*, Utah State University.
16. 2008 – 15, *Member*, USDA-ARS National Pollinator Week Community Event.
17. 2008 – 15, *GLBTQA student services*, Allies on Campus Program, Utah State University.
18. 2011, *GLBTQA office volunteer*, Utah State University.
19. 2009 – 10, *Co-chair*, Ecology Center Steering Committee, Utah State University.
20. 2010, *Participant*, North American Bumble Bee Species Conservation Strategy Workshop.
21. 2008, *Vice President*, Biology Graduate Student Association, Utah State University.
22. 2008 – 2009, *Member*, Ecology Center Seminar Committee, Utah State University.
23. 2008, *Entomology Leader*, Antelope Island Bioblitz, Utah State Parks.
24. 2008, *Member*, Smithsonian Institute Bumble Bee Task Force.
25. 2008, *Attendee*, Ecological Society of America SEEDS Leadership Meeting.

STUDENTS MENTORED

(+ = Underrepresented students in STEM)

2015 Nicole Bittner+, Major: Biology, Brigham Young University

2014 Hattie Cadreact+, Major: Biology, Utah State University
Research Project: "Setal color variability in cryptic bumble bees"
Taylor Peacock, Major: Biology, Utah State University
Cody Darrington, Major: Biology, Utah State University

2013 – 14 Abby Baur+, Major: Wildlife Science, Utah State University
Research Project: "Foraging ecology of bumble bees"

2013 Amir Ghazi+, Major: Biology, Utah State University
Research Project: "*Nosema bombi* incidence in managed bumble bee populations"

MEDIA COVERAGE OF RESEARCH AND OUTREACH

Agricultural Research Magazine, Huffington Post, The Utah Statesman, Deseret News, USA Today, MSNBC, AOL News, Daily Kos, Science News, CBC, National Geographic, EurekAlert, Science Magazine, Smithsonian, Weather.com, Phys.org, Science Newsline, Science Daily, LiveScience, Christian Science Monitor, Headline and Global News, IFLS, University Herald, Science World

Report, Examiner, Scientific American, Delhi Daily News, News Tonight Africa, Austrian Tribune, French Tribune, Saigon, Perfect Integro, News Now UK, Native Bee Research, Beyond Bee, Aljazeera TechKnow

RELEVANT SKILLS AND TECHNIQUES

1. **Laboratory skills**: PCR, PCR Optimization, Microsatellite Analysis, Sequencing Analysis, Microscopy
2. **Programming Languages**: R, Python, SQL, Linux
3. **Software**: Geneious, GeneMapper, ArcGIS, R Studio, STRUCTURE
4. **Insect husbandry.** I have assisted in rearing out colonies of rare (*Bombus occidentalis*) and common bumble bees (*Bombus huntii*) for agricultural studies.
5. **General Insect Identification & Curation**. I taught graduate and undergraduate students basic insect identification (to Family). I am considered an expert in bumble bee identification (Hymenoptera, Apidae, *Bombus*), and have identified and curated thousands of specimens from >15 natural history collections in the United States.
6. **Science Communicator**, I have 6+ years of experience in effectively communicating agriculture needs and science to growers, community members, and master gardeners.