GENETIC AND COMMUNITY STRUCTURE OF NATIVE BEES OF THE PALOUSE PRAIRIE

A Dissertation
Presented in Partial Fulfillment of the Requirements for the
Degree of Doctorate of Philosophy
with a
Major in Entomology
in the
College of Graduate Studies
University of Idaho
by
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May 2016

AUTHORIZATION TO SUBMIT DISSERTATION

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ABSTRACT

Many aspects of bee biology and conservation remain unresolved despite the crucial role bees play in both native plant and crop pollination. Bee communities associated with specific habitats are largely uncharacterized, the influence of changing land use, exotic grass invasion, and intensifying agriculture on bee populations and species is often unclear, and ways in which sampling techniques alter the detectability of habitat influence or bee functional classes are unevaluated. This thesis addresses knowledge gaps of bee biology and conservation in the inland Pacific Northwest with emphasis on the Palouse Region. Chapter 1 describes the bee fauna of Palouse Prairie fragments, including six state records and two range expansions, and provides community level information on bee species of unknown conservation status. Chapter 2 examines the presence and abundance of *Bombus occidentals* across the inland Pacific Northwest, where it was once common but now is rare. Chapter 3 compares three common bee collection methods and evaluates the response of bee communities collected using each method to habitat variables known to affect bees. The collection methods are also compared their detection of bees with different functional classes such as bee size or floral specialization. Chapter 4 compares the influence the amount of natural land, exotic grass invasion, and plant species richness and diversity on bee community metrics in the Palouse. Chapter 5 uses molecular markers to assess the genetic structure and permeability of different land cover types including intensive agriculture to dispersal and gene flow of *Bombus bifarius*. Chapter 6 describes an interdisciplinary study focused on conservation of Palouse Prairie plant communities, which are interdependent with bee communities of the region. Economic valuation of the cultural services provided by these communities can help motivate their conservation, but may be inadequate or insensitive to certain cultural perspectives. This final chapter, coauthored with other members of an interdisciplinary team of doctoral students, investigates the social value of culturally significant plants of the Palouse Prairie and evaluates the comfort people have with cash valuation of these plant communities. These results are relevant to bee conservation in fragmented grasslands surrounded by agriculture, a globally common land use pattern.

ACKNOWLEDGEMENTS

First I'd like to thank the members of my graduate committee: Sanford Eigenbrode, Lisette Waits, Nilsa Bosque-Pérez and Steve Sheppard for their continued help throughout this process. Especially Sanford for being always ready to listen to an idea, Lisette for keeping us focused and Nilsa for answering my many questions.

The help of the USDA Bee Biology and Systematics Laboratory was absolutely indispensable throughout this project. Terry Griswold, Harold Ikerd, Michael Orr, and Skyler Burrows all provided excellent taxonomic and bee collection curation assistance. Jamie Strange and Jon Koch provided help with the *Bombus* taxonomy and population genetics.

Thanks to Karen Wright for help with *Melissodes* taxonomy.

Thanks to Jen Adams for her help with PCR and microsatellite analysis

My IGERT team: Cleve Davis, Kevin Decker, and Chris Baugher all deserve thanks.

I appreciate all the effort all the IGERT students and faculty put into the project. It was challenging for everyone but we made something really unique and great.

DEDICATION

This dissertation is dedicated to my lovely fiancée Emily, my wonderfully supportive parents Ray and Palma and all the friends who have helped make this experience not only bearable but wonderful.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: THE NATIVE BEE FAUNA OF THE PALOUSE PRAIRIE

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This chapter is currently under in review for publication in the Journal of Melittology

Abstract

While the range and general composition of North American bee fauna have been mostly described based on random collections, bee communities associated with specific habitats are largely uncharacterized. This report describes the community of native bees currently found in remnant fragments of the Palouse Prairie of northern Idaho and southeastern Washington State. Native bees were collected using standardized collection techniques including blue vane traps, colored pan traps and aerial netting. More than 13,000 individuals were collected representing at least 179 species in 29 genera. These data provide the most thorough characterization of the bee fauna of this vulnerable ecosystem, and community level information on bee species of mostly unknown conservation status. These results are relevant to regional conservation efforts and more broadly are representative of conditions in fragmented grasslands surrounded by intense agriculture, a common global land use pattern of conservation concern.

Introduction

By 2005, cultivated systems covered one quarter of earth’s terrestrial surface (Sarukhan et al., 2005). This habitat loss is responsible for worldwide reductions in both general biodiversity and in the diversity of specific taxa, notably bees (Foley et al., 2005; Brown and Paxton, 2009; Senapathi et al., 2015). Temperate grasslands are particularly vulnerable to anthropogenic land use change with more than half of all temperate grassland, shrubland or savannah converted to agriculture or urban use (White et al., 2000). For bees, habitat loss lowers biodiversity (Vanbergen, 2013) and can exacerbate reductions in biodiversity caused by pesticides (Park et al., 2015). Additionally, fragmentation caused by habitat loss can impact remaining populations through inbreeding of isolated populations (Zayed, 2004; Zayed and Packer, 2005; Darvill et al., 2006; Ellis et al., 2006), inability of small habitat fragments to support populations (Lennartsson, 2002), and through degradation of the remaining habitat, where depauperate bee communities inadequately pollinate necessary forage plants (Fontaine et al., 2005). The Palouse Prairie is a good model system to evaluate the character of bee communities that may still be present in highly fragmented systems dominated by intensive agriculture.

The Palouse Prairie is part of the Pacific Northwest bunchgrass biome which originally occupied an area of about 8 million hectares appearing in discontinuous patches from northeastern Oregon and west-central Idaho northwards through western Montana to the Fraser Valley in central British Columbia (Tisdale, 1982) disjunct from the larger grasslands of the Great Plains. The Palouse Prairie is a discrete component of the Pacific Northwest bunchgrass biome, differentiated by its distinctive soils and topography. It is considered a subsection within Bailey’s ecoregions (Bailey, 1995), a subregion in Omernik’s ecoregions (1987), and unit in Ertter and Moseley’s floristic regions of Idaho (1992). It is situated in northern Idaho and adjacent eastern Washington bounded by the arid channeled scablands of central Washington to the west, the canyon grasslands adjacent to the Snake and Clearwater Rivers to south and southeast, and the forests of the Selkirk and Bitterroot Mountains to the north and east. The Palouse Prairie was continuous habitat across this region until the late

1800s when conversion to agriculture began. Now approximately 1% of the Palouse Prairie remains (Black et al., 1998) and so the ecosystem could be considered ‘Critically Endangered’ using the criteria of Keith et al. (2013). The remaining fragments are small (most less than 2 ha) with high perimeter-to-area ratios and located disproportionately along streams or on land too rocky or steep to farm (Looney and Eigenbrode, 2012). Although fragmented and surrounded by intensive agriculture, the Palouse Prairie still supports a diverse community of vascular plants (Daubenmire, 1942; Lichthardt and Moseley, 1997) with more than 150 species found in a recent thorough survey (Davis, 2015).

Although agricultural production and ecosystem health are dependent on native bee pollinators (Ashman et al., 2004; Klein et al., 2007), it is unclear to what extent habitat loss and fragmentation have affected bee populations throughout North America, and the conservation status of most native bee species remains unknown (NRC, 2007). Nonetheless, reductions in bee species’ range and abundance have been documented throughout the world (Biesmeijer et al., 2006; Fitzpatrick et al., 2007; Potts et al., 2010; Cameron et al., 2011). Some combination of habitat loss, disease, invasive plant spread, and pesticide use is likely responsible for bee declines (Brown et al., 2002; Vanbergen, 2013; Goulson et al., 2015, but see Winfree et al., 2007). The degree of bee species decline can be difficult to resolve because baseline data necessary to identify species of concern is lacking in many cases (Meffe et al., 1998; NRC, 2007; Goulson et al., 2008). While there have been some efforts to determine the extent and magnitude of bee species decline using museum specimens (Bartomeus et al., 2013; Scheper et al., 2014), systematic surveys of bee fauna presence and abundance are lacking in most parts of the world. Although historic collections can document species presence in an area, community level studies are needed to determine population trajectory and evaluate the conservation status of individual species. Moreover, for many rare bees in the western US, basic biological information regarding range or habitat associations is lacking.

There have been two recent, thorough bee community surveys near the Palouse. One occurred using pan traps and aerial nets at flowers at 35 locations in forest meadows of montane areas in the Okanogan National Forest in north central Washington State (Wilson et al., 2010). Each site was visited twice, first in in late June or early July and again in mid-August. Although this survey was performed in forest meadows, not prairie, many common plant are shared between the Palouse Prairie and these forest meadows [e.g. *Achillea millefolium, Geranium viscosissimum, Lupinus sericeus, Potentilla gracilis, Rosa* spp., and *Penstemon* spp., among others (Wilson et al., 2010; Davis, 2015)]. A total of 1,975 bees were collected in the Okanogan National Forest representing 140 species. The other nearby bee community survey occurred in the similar Zumwalt Prairie using only blue vane traps (Kimoto et al., 2012). Bees were sampled in the Zumwalt Prairie at 16 locations over two years. During the first year bees were sampled in late June and again in early July. The second year bees were sampled in early June, mid-July and late August. Over two years, 9,158 bees were collected representing 94 species and 117 morphospecies.

Additionally the *Bombus* community of the Palouse Prairie was characterized using bees collected from pitfall traps in 5 prairie remnants in 2002 and 2003 (Hatten et al., 2013). Bees were collected between June and September in 2002 and between May and August in 2003. In total, 1,192 *Bombus* representing 10 species were collected.

A compilation of historical records for the Palouse suggests at least 257 species of bees can be found there, among the highest of all ecoregions in the Columbia Basin (Tepedino and Griswold, 1995). However, these data were compiled from many different sources using a variety of collection methods, so the relative abundance of species in this assemblage is unknown. The bee community of the Palouse was recently described (Hatten et al. in prep), but the collection method (pitfall traps) was not optimal for bees and collection only occurred on 4 very large prairie fragments. The objectives of this study were to: 1) provide a comprehensive species list of bee fauna of the Palouse Prairie, 2) assess the relative abundance of bee species and 3) identify range expansions or state records for bee species.

Materials and Methods

Bee collection occurred at 32 sites on 29 fragments of Palouse Prairie (Figure 1.1) between May and July in 2012 and 2013. Each site was sampled four times in each year, at sampling intervals of approximately three weeks. Sampling location within the fragment was determined by generating a random point within each prairie fragment at least 10 meters from the fragment edge when possible using the Create Random Points tool in ArcMap 10.0 (ESRI, Redlands CA). If the sampling location fell within a thicket of shrubs or small trees, which would inhibit trap placement, the sampling location was moved 5 meters beyond the nearest edge of the thicket. Multiple methods of bee sampling were employed to maximize detection of the existing fauna: pan traps, blue vane traps, and aerial netting. Recent attempts have been made to standardize bee collection techniques, but the relative performance of netting, pan trapping and blue vane trapping remain unknown, and so all three were used in this study. Pan traps have been extensively used in standardized bee sampling regimes but are known to have bias in bee capture, recovering Halictinae and

Perdita at greater rates than the genera *Anthidium, Colletes,* and *Epeolus,* as compared with netting in the same locations (Wilson et al., 2008). Blue vane traps were used so we could better compare results with the only other thorough bee collection effort in Pacific Northwest bunchgrass prairie performed using only blue vane traps (Kimoto et al., 2012). Blue vane traps filled with soapy water (Springstar Inc., Woodinville, WA) (Stephen and Rao, 2007) were hung about one meter off the ground on a bamboo tripod at the randomly determined sampling location. Three colored pan traps (3.25 oz. soufflé cups, Solo model #p325w-0007) filled with soapy water, one each of fluorescent yellow, fluorescent blue and white, were set three meters apart in a transect leading away from the blue vane trap on a random heading. Pan trap colors were randomized within each transect. Traps were left open for 24 hours. Finally, an aerial net was used to collect bees from flowers within 50 meters of the random point for 5 minutes at the time of trap placement and again at removal for a total of 80 minutes of net collection at each site over the 2 years of sampling.

Sampling was only initiated on mostly sunny days with highs above 16°C but quickly changing weather during the spring and early summer in this region meant some light rain fell during the 24 hours traps were left open. The average high temperature for sampling days was 22.7C in 2012 and 27C in 2013; the average low temperature was 5.4C in 2012 and 6.2C in 2013; 1.16 cm of precipitation fell over four sampling days in 2012 and 0.15 cm fell over two days in 2013; the largest daily rainfall total on a sampling day was 0.71 cm in 2012 and 0.1 cm in 2013.

Netted bees were kept frozen before processing. Bees collected in blue vane traps or pan traps were rinsed in ethanol and then placed in a Whirl-Pak bag (Nasco, Fort Atkinson, WI) and covered with ethanol for temporary storage. Bees stored in ethanol were then washed and dried before further processing (methods adapted from Droege 2009). In the first year of collection all bees were pinned. In the second year very common and easily identifiable species including *Agapostemon angelicus* Cockerell, *A. virescens* (Fabricius), *A. femoratus* Crawford and *Halictus tripartitus* Cockerell were identified without pinning to save time and resources. Additionally, because identification of *Lasioglossum* belonging to the *Hemihalictus* series (Michener 2007) to species was not possible, they were counted then stored without pinning. Other unrevised genera only identified to genus were *Nomada*, *Epeolus*, *Sphecodes,* and *Panurginus*. Voucher specimens reside in the William F. Barr insect museum at the University of Idaho and the U. S. National Pollinating Insects Collection, USDA Bee Biology and Systematics Laboratory housed on the campus of Utah State University.

To determine historical records of bee occurrence in the Palouse region, data from the Global Biodiversity Information Facility (GBIF) was downloaded and used in conjunction with raw data used in a report on the bees of the Upper Columbia River basin (Tepedino and Griswold 1995) obtained from the authors. Only records falling within the Palouse ecoregion (Omernik, 1987) were used.

Bombus taxonomy is relatively stable presently and historically, so good information on bumblebee community composition is readily available where similar information for other genera is not. There are four instances where *Bombus* community data can be compared to the Palouse: 1.) A 2003 *Bombus* survey was performed in the Palouse Prairie reflecting recent community composition (Hatten et al., 2013); 2) A GBIF derived dataset with 1,675 records of *Bombus* occurrence when limited to pre-2000 records (1805- 1999) within the Palouse reflecting historical community composition. The preponderance of post-1999 records in the GBIF database were from the Hatten et al. (2013) study; 3) A recent survey of native bees on the Zumwalt prairie (Kimoto et al., 2012); 4) *Bombus* community data extracted from a bee study of the nearby Okanogan National Forest (Wilson et al., 2010). Bray-Curtis dissimilarity was calculated among these four datasets to evaluate: 1) the similarity of the Palouse *Bombus* community through time, and 2) the similarity of the contemporary Palouse *Bombus* community to nearby habitats. All community data were normalized to account for differing sampling regimes.

Rarefaction was performed using EstimateS (Colwell et al., 2012) to evaluate the number of bee species that remain undetected in the study area using incidence based rarefaction without replacement. Estimated species richness was extrapolated to three times the total number of collected individuals. Rarefaction was performed with and without the *Hemihalictus* series but the total number of estimated species was similar.

Results

Over two years of sampling, 13,241 bees were collected comprising more than 179 species in five families and 29 genera (Table 1.1). Rarefaction analysis indicates the total number of trappable bees was 204 ±20 species so it’s possible our collection is a nearly complete representation of the bee fauna in the area (Figure 1.2).

The Halictidae were the most abundant family, comprising more than 64% of all collected bees (Table 1.1), followed by the Apidae (16%) and Megachilidae (11%). The most abundant species also belonged to Halictidae: *Halictus tripartitus* made up 10.3% of total collected bees, *Agapostemon virescens* (6.6%), *A. angelicus* (4.6%), and *Lasioglossum sisymbrii* (4.0%). The most abundant genus was the halictid *Lasioglossum* comprising 37%

of all collected bees, with the *Hemihalictus* series making up nearly 75% of collected *Lasioglossum*. *Halictus* (15%), *Agapostemon* (11%), the megachilid genus *Osmia* (8%), and the andrenid genus *Andrena* (7%) were also abundant genera (Table 1.1). The most speciose families were Megachilidae (66 species) and Apidae (49 species) followed by Andrenidae (31 species) and Halictidae (27 species) (Table 1.1). (It is important to note that the *Hemihalictus* series with 28% of collected individuals, were not identified to species. A similar study in a nearby Pacific Northwest bunchgrass system (Kimoto et al., 2012), detected as many as 38 morphospecies within the *Hemihalictus* series. If species richness within the *Hemihalictus* series is comparable in the Palouse Prairie it would nearly make the Halictidae the most speciose family. Colletidae were poorly represented (8 species) and Melittidae absent. The most speciose genera include *Andrena* (Andrenidae, 29 species), *Osmia* (Megachilidae, 34 species), *Bombus* (Apidae, 16 species) and *Lasioglossum* (14+ species) (Table 1.2).

Discussion

This is the first thorough examination of the wild bee fauna in the Palouse Prairie. We were able to note a number of state records and range expansions. This data will provide a baseline of presence and abundance of prairie inhabiting bee species which will be useful in evaluating declines or range contractions of wild bees in the western United States. As Kimoto et al. (2012) noted the Pacific Northwest bunchgrass ecosystem supports a rich community of wild bees. By utilizing a more diverse array of trapping methods and by identifying more individuals to species rather than morphospecies we were able to more fully characterize the community of bees inhabiting bunchgrass prairie.

Four bee species exotic to North America were recovered in the Palouse Prairie (Table 1.1): *Osmia caerulescens* (L.) is a Palearctic species that was accidentally introduced to the east coast of North America from Eurasia (Rust, 1974) and has been well established in Idaho and the American West since the late 1970s (Parker, 1981). Twenty four individuals of *O. caerulescens* were recovered in 2012 and 2013. *Megachile apicalis* Spinola is native to Europe and has been established in California since the early 1980s at least (Cooper, 1984) and has since been observed in Oregon, Washington, British Columbia, Wyoming, Montana and Utah (GBIF, 2015). The two individuals collected in 2013 are the first record of this species in Idaho. *Megachile rotundata* (Fabricus), another bee native to Europe, was first detected in the United States in the 1940s and is now common throughout the US (Cane, 2003; Pitts-Singer and Cane, 2011). Four individuals of *M. rotundata* were recovered in 2013. *Anthidium manicatum* (L.) has been present in North America since the 1960s (Jaycox, 1967) but was restricted to the eastern US and central Canada until 2001 when it began a rapid range expansion (Gibbs and Sheffield, 2009). It is now naturalized in many parts of the mountain west and has since been recovered from British Columbia, Washington, Oregon, California, Utah, Colorado and southern Idaho (Strange et al., 2011; GBIF, 2015). A single individual of *Anthidium manicatum* was recovered in 2012. Male *A. manicatum* have been known to wound or kill other species of flying insects that enter their territory, a behavior unusual for bees (Wirtz et al., 1988). This behavior can deter foraging by other species of bees, particularly *Bombus* (Pechuman, 1967), thus its presence in the Palouse may pose a threat to native bee species (Cane, 2003). Like most exotic bees in North America, all four exotic bees detected in this survey nest in preexisting cavities and may influence native bee communities through competition for nesting sites with native bees that use the same resource (Barthell et al., 1998; Cane, 2003). *Megachile apicalis*, in particular, is an aggressive nest usurper (Barthell and Thorp, 1995). However, populations of these exotic bees are small in the Palouse and they don’t appear to reduce species richness of other cavity nesting bees at the sites where they were collected. This study indicates further expansion of the invasive range of these species.

Twelve species were recovered that are apparently new records for Washington, Idaho, or both (Table 1.1). *Andrena fuscicauda* Viereck has been found mostly in the Sierra Nevada and Cascade Mountains and coastward, although it has also been found in central Oregon and Nevada (LaBerge, 1973). This is the first record of the species in Idaho. *Andrena semipunctata* Cockerell is common in the arid southwestern US and California, Oregon, and Washington in the Cascade Mountains and coastward. A single individual was previously documented from eastern Washington State (LaBerge, 1973) but this is the first record of the species in Idaho. *Andrena shoshoni* Ribble is a rare species previously only known from South Dakota and Wyoming (Ribble, 1974). Three individuals were recovered in this study in Washington. *Andrena waldmerei* LaBerge & Bouseman is another rare species mostly observed in southern California with one individual collected as far north as Redding, California (LaBerge and Bouseman, 1970). The species was collected in this study in both Washington and Idaho, far from any previous records of that species. *Anthophora affabilis* Cresson is common in the arid southwestern US and has been found in Oregon and Idaho. The single individual collected in this study is the first record of this species in the state of Washington. *Melissodes plumosa* LaBerge is mostly found along the California coast and Sierra Nevada and Cascade Mountains with one individual collected in North Dakota (LaBerge, 1961). This is the first record of this species in Idaho. *Hylaeus granulatus* (Metz) was found to be distributed from the Sierra Nevada and San Gabriel Mountains of California to Colorado in a revision of the genus (Snelling, 1970) and more recently found as far north as central Oregon (GBIF, 2015). This represents the first collection of this species in Idaho and Washington. *Osmia aglaia* Sandhouse is known primarily from California with a few records in Oregon (GBIF, 2015). This is the first specimen recovered in Washington. *Osmia thysanisca* Michener is a rare species previously recovered from Oregon, Wyoming and California (Hurd, 1979). This is the first record of *O. thysanisca* in Washington. *Osmia trifoliama* Sandhouse is a rare species observed previously in Oregon, Washington and California mostly coastward of the Sierra Nevada and Cascade Mountains (GBIF, 2015). We recovered the first record of this species in Idaho. *Stelis interrupta* Cresson is mostly found in the Southwest with the nearest previous recovery in the Cascade Mountains in Oregon (Hurd, 1979). This is the first record in Idaho.

Expansions of the known range for a few additional species should be noted. *Megachile snowi* Michell has been primarily collected in the desert southwest in Arizona and Utah but also in Colorado, New Mexico, California and southern Idaho (Bzdyk, 2012). This is the first record of *M. snowi* in the Palouse region extending the known range several hundred miles to the north. However, *M. snowi* was recently elevated to the rank of species, being previously regarded as a subspecies of *Megachile mendica* Cresson, for which there are records in the Palouse region so it is not clear if this species has been previously observed in the Palouse region or not. *Osmia raritatis* is widespread in the Mountain West appearing throughout California, Arizona, Utah and Colorado. The species has also been recovered in the Cascade Mountains and in southern Idaho but this is the first instance of *O. raritatis* this far into the inland Pacific Northwest.

Historic records from GBIF and Tepedino and Griswold (1995) indicate at least 275 species of native bees have been found in the Palouse region. Of the 179 species observed in this study, 117 were documented by Tepedino and Griswold (1995) and between 54 and 62 are new records for the Palouse. Species previously unknown to the area are primarily in *Osmia* (11 species), *Andrena* (8 species), *Megachile* (5 species), *Anthophora* (5 species), *Hylaeus* (4 species), and *Eucera* (4 species).

Although historic data for non-*Bombus* bee species are sparse, there is one species that was once relatively common in the Palouse that was not recovered in this collection: *Osmia lignaria* Say is a common mason bee throughout North America and was consistently found in the Palouse region by various collectors between 1905 and 1991 (GBIF, 2015). We did not recover this species in 2012 and 2013. However, *O. lignaria* is active very early in the spring and the preponderance (60%) of records in GBIF were collected in March or April, before sampling commenced in the present study. So our failure to detect this species may be because we began sampling too late in the year.

Bombus occidentalis Green was once reported to be common in the inland Northwest but is now rare in the region (Stephen, 1957; Rao and Stephen, 2007; Rao et al., 2011; Rhoades et al. 2016). GBIF data for the Palouse region shows 292 *B. occidentalis* collected between 1888 and 1997, forming about 16% of all pre-2000 *Bombus* occurrence recorded in GBIF for the Palouse region. *Bombus occidentalis* was present in our study but at lower rates than is evident in the historical data (2.9% of total *Bombus*) mirroring trends found throughout its range (Cameron et al. 2011).

The biogeography of bees in the Pacific Northwest remains little known. There are only two other recent surveys of bee communities near the Palouse Prairie. One occurred in forest meadows of montane areas in the Okanogan National Forest in north central Washington (Wilson et al., 2010), the other occurred in the similar Zumwalt Prairie (Kimoto et al., 2012). At the family level the three assemblages are similar: Halictidae are abundant and Megachilidae and Apidae are speciose. Bees in the Colletidae were rare in all three areas but more abundant and speciose in the Okanogan National Forest than in either grassland area. At the generic level, *Andrena* composes 16% of the species recovered from the Palouse, significantly more than in either the Zumwalt (6.4%) or the Okanogan National Forest (11.2%). *Bombus* and *Melissodes* are much more abundant in the Zumwalt (composing 30% and 12% of total individuals collected respectively) and Okanogan National Forest (20% and 2.4% of total individuals) than in the Palouse (5.8% and 1.7% of total individuals collected). In contrast, *Ceratina* (Apidae) is quite abundant on the Palouse, composing 5.6% of collected individuals, compared to 0.02% of individuals captured on the Zumwalt Prairie and undetected from the Okanogan National Forest. *Agapostemon* and *Halictus* are extremely abundant in the Palouse (11.3% and 13.9% of total individuals collected, respectively) but mostly lacking in the Okanogan (0.7% and 2.2% of total individuals) and the Zumwalt (1.4% and 7.5% of total individuals). Although species richness in the Megachilidae is similar in all three studies, Megachilidae, mostly *Osmia*, were far more abundant in the Okanogan National Forest than in the other two areas.

Differences between these assemblages could be attributed to differences in collection techniques as well as differences in the season of collection. Net collecting was primarily used in the Okanogan National Forest (pan traps were only used at 6 of 35 sites). Compared to net collecting, pan traps often collect large numbers of gregarious or semisocial halictid bees. This could explain the low numbers of *Agapostemon* and *Halictus* in the Okanogan National Forest. Bees in the Zumwalt Prairie were collected using only blue vane traps which have poorly characterized biases but may underrepresent Andreninae and Halictini in capture (Joshi et al., 2015), possibly explaining the paucity of these taxa in the Zumwalt.

Differences in bee assemblages among the three areas could also be ascribed to differences in the season of collection. Bee sampling in the Zumwalt and Okanogan continued well into August when *Melissodes* is most abundant. *Melissodes* abundance on the Palouse increases toward late summer (Table 1.1); extending sampling into August might reveal similar *Melissodes* abundance among the three areas.

Despite differing trapping methods Bray-Curtis dissimilarity analysis shows the three *Bombus* datasets from the Palouse Prairie (historic net collections, 2002 and 2003 pitfall traps, contemporary mixed methods) to be more similar to one another than to the Zumwalt Prairie or the Okanogan National Forest suggesting the Palouse Prairie has a distinctive *Bombus* community not shared by similar, nearby systems (Table 1.2).

Remarkably, bee richness in the Palouse is greater than recorded for most studies in the extensive tallgrass prairie (Table 1.3). This may be due in part to sampling effort since a larger number of collected individuals will yield more detected species. Additionally, a wider variety of collection methods could have increased the bee fauna sampled as trap type can affect the taxa detected (Geroff et al., 2014). Finally the Palouse and Zumwalt regions both have a more diverse array of habitats nearby. The Palouse Prairie is surrounded by forest, sagebrush steppe and arid grasslands. Many detected taxa could be adapted to more mesic or arid environments, only marginally present on the Palouse.

The Palouse Prairie is a unique region that has been heavily impacted through fragmentation and habitat loss caused by conversion to agriculture (Donovan et al. 2009). Despite this, remaining fragments of prairie contain large amounts of biodiversity. Palouse Prairie fragments support over 160 species of vascular plants including rare and threatened plant species like *Silene spaldingii*, *Symphyotrichum jessicae*, *Astragalus arrectus*, and *Calochortus nitidus* (Lichthardt and Moseley, 1997; Hanson et al., 2008; Davis, 2015). The native earthworm *Driloleirus americanus* still persists in the Palouse (Sánchez-de León and Johnson-Maynard, 2008). Weevils (20 species), darkling beetles (5 species) and scarab beetles (6 species) present in Palouse Prairie fragments and adjacent agricultural fields have been characterized (Hatten et al., 2004, 2007) and all eight regional species of carrion beetles are found in Palouse Prairie fragments (Looney et al., 2004). This study adds bees to this list of distinctive and diverse Palouse fauna and contributes to our limited but growing knowledge of the bees of the inland Northwest.

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Table 1.1 List of all collected bee species and their abundance in each month in which collection occurred.

	May 2012	June 2012	July 2012	May 2013	June 2013	July 2013	Total
Andrenidae	**322**	**233**	**47**	**190**	**204**	**26**	**1022**
Andrena angustitarsata Viereck	13						13
Andrena astragali Viereck & Cockerell	13	2		18	20		53
Andrena candida Smith	2						2
Andrena chlorogaster Viereck	4	1					5
Andrena cressonii infasciata Lanham		5	2	7	3		17
Andrena fuscicauda Viereck[1]	6	3					9
Andrena hemileuca Viereck	11			3			14
Andrena hippotes Robertson	1						1
Andrena melanochroa Cockerell	4						4
Andrena merriami Cockerell	10	20	1	2	15	1	49
Andrena microchlora Cockerell	2			4	3		9
Andrena nigrihirta (Ashmead)	39	13		16	5		73
Andrena nigrihirta aff (Ashmead)	2			3			5
Andrena nigrocaerulea Cockerell				8	25		33
Andrena nivalis Smith	112	62	3	69	45		291
Andrena pallidifovea Viereck	13	6		12	13		44
Andrena piperi Viereck	3	22	2		18		45
Andrena prunorum Cockerell				1	1		2
Andrena semipunctata Cockerell[1]	11	42	11	26	18	15	123
Andrena shoshoni Ribble[2]		3	1		3		7
Andrena sola Viereck				3			3
Andrena spp.	6	2	1	2	7		18
Andrena subtilis Smith	3	8	2	2	2		17
Andrena thaspii Graenicher		1	1	2	3	2	9
Andrena topazana Cockerell		2	2				4
Andrena trevoris Cockerell			2			5	7
Andrena vierecki Cockerell	1	1	1			1	4
Andrena aff *waldmerei* LaBerge & Bouseman[1,2]	1	1		2			4
Panurginus atriceps (Cresson)	60	35	16	10	23	2	146
Panurginus spp.	5	3					8
Perdita sp.		1					1
Perdita wyomingensis Cockerell			2				2
Apidae	**446**	**351**	**190**	**301**	**377**	**592**	**2256**
Anthophora affabilis Cresson[2?]					1		1
Anthophora bomboides Kirby	1		5		5	1	12
Anthophora edwardsii Cresson	1			1			2
Anthophora occidentalis Cresson						1	1
Anthophora pacifica Cresson				8			8
Anthophora porterae Cockerell				1			1
Anthophora terminalis Cresson					2	1	3
Anthophora urbana Cresson			1		2	17	20

Anthophora ursina Cresson	2	1	1	3	4		11
Apis mellifera Linnaeus	18	36	23	3	13	9	102
Bombus appositus Cresson	13	4	3	4	13	12	49
Bombus bifarius Cresson	69	18	4	2	1	1	95
Bombus californicus Smith	1	2	2	2	3	2	12
Bombus centralis Cresson	25	4	4	17	7	9	66
Bombus fernaldae (Franklin)				1			1
Bombus fervidus (Fabricius)	27	9	5	48	17	16	122
Bombus flavifrons Cresson	2					2	4
Bombus griseocollis (DeGeer)		1				7	8
Bombus huntii Green	7	2		3	1	1	14
Bombus insularis (Smith)	12	5	1	1			19
Bombus melanopygus Nylander	1						1
Bombus mixtus Cresson	12		3	1		2	18
Bombus nevadensis Cresson	71	6	8	28	22	25	160
Bombus occidentalis Green	9		1	1	1		12
Bombus rufocinctus Cresson	41	37	26	18	27	21	170
Bombus vagans Smith	1	1	1				3
Ceratina acantha Provancher	18	45	20	30	22	17	152
Ceratina nanula Cockerell	71	118	14	78	62	92	435
Ceratina pacifica H.S. Smith	3	4	34	8	18	82	149
Ceratina spp.	4						4
Diadasia enavata (Cressson)						6	6
Diadasia nigrifrons (Cressson)		1	2				3
Epeolus sp.			1				1
Eucera delphinii (Timberlake)	1						1
Eucera edwardsii (Cresson)		9	4	4	21		38
Eucera frater (Cresson)	8	26	14	12	102	49	211
Eucera hurdi (Timberlake)	2			1	2		5
Eucera spp.	2	1	3	1	9	9	25
Habropoda cineraria (Smith)	1						1
Melecta pacifica Cresson	2	1		1	1		5
Melissodes agilis Cresson			1				1
Melissodes communis Cresson					1	3	4
Melissodes lupina Cresson					1	45	46
Melissodes spp.			4		1	90	95
Melissodes menuachus Cresson			1				1
Melissodes metenua Cockerell						20	20
Melissodes microsticta Cockerell			1		3	19	23
Melissodes plumosa LaBerge[1]						24	24
Melissodes rivalis Cresson			2			4	6
Melissodes sp.2						2	2
Nomada spp.	21	20		24	15	1	81
Triepeolus heterurus (Cockerell & Sandhouse)						2	2
Colletidae		**13**	**6**	**1**	**16**	**3**	**39**
Colletes spp.		1			2		3
Hylaeus affinis (Smith)		2	1		1	1	5

Hylaeus conspicuus (Metz)					1		1
*Hylaeus granulatus (*Metz)		10	2	1	8	1	22
Hylaeus spp.			2		1		3
Hylaeus modestus Say					1	1	2
Hylaeus verticalis (Cresson)			1				1
Hylaeus wootoni (Cockerell)					2		2
Halictidae	**1635**	**1722**	**1030**	**1346**	**1708**	**966**	**8426**
Agapostemon angelicus Cockerell	50	145	88	24	192	98	597
Agapostemon coloradinus (Vachal)	3				1	3	7
Agapostemon femoratus Crawford				7	11	4	22
Agapostemon virescens (Fabricius)	35	136	219	25	219	225	859
Halictus confusus Smith	4	16					20
Halictus farinosus Smith	11	9	11	19	17	3	70
Halictus spp.	11	1					12
Halictus ligatus Smith	46	21	6	13	29	10	125
Halictus rubicundus (Christ)	45	51	22	170	78	7	373
Halictus tripartitus Cockerell	259	213	113	324	276	172	1357
Lasioglossum anhypops McGinley	1	2					3
Lasioglossum athabascense (Sandhouse)	4		2		1	1	8
Lasioglossum colatum (Vachal)	3	16	14	5	14	1	47
Lasioglossum Hemihalictus series	778	643	365	660	637	323	3406
Lasioglossum egregium (Vachal)	16	91	45	3	51	41	246
Lasioglossum (*Evylaeus*) *sp.*	32	18	1	34	15	5	105
Lasioglossum mellipes (Crawford)				2	7		9
Lasioglossum olympiae (Cockerell)	16	29	4	2	5		56
Lasioglossum ovaliceps (Cockerell)		1					1
Lasioglossum pacificum (Cockerell)		4	7	8	6	5	30
Lasioglossum paraforbesii McGinley						2	2
Lasioglossum sisymbrii (Cockerell)	64	238	81	27	91	24	525
Lasioglossum spp.	172	4	1				177
Lasioglossum tegulare (Robertson)	16						16
Lasioglossum titusi (Crawford)	50	54	27	13	18	40	202
Lasioglossum trizonatum (Cresson)	3	3	1	4	30	2	43
Lasioglossum zonulum (Smith)		5	12	8	4		29
Sphecodes spp.	16	23	11	6	19	4	79
Megachilidae	**214**	**385**	**198**	**180**	**287**	**234**	**1498**
*Anthidium manicatum (*Linnaeus)[3]						1	1
Anthidium utahense Swenk			1		1	1	3
Atoposmia copelandica (Cockerell)					1		1
Dianthidium curvatum (Smith)						3	3
Dianthidium subparvum Swenk			2		2	4	8
Heriades carinata Cresson			1				1
Heriades carinatus Cresson						5	5
Hoplitis albifrons argentifrons (Cresson)		3	3		1	4	11
Hoplitis fulgida (Cresson)		8	4	3	13		28

Hoplitis grinnelli (Cockerell)	1	6	2		3	1	13
Hoplitis hypocrita (Cockerell)	3	3	1	7	6		20
Hoplitis producta (Cresson)	1	29	37	5	26	15	113
Hoplitis sambuci Titus			1		1	4	6
Megachile apicalis Spinola[3]						2	2
Megachile brevis Say				1	2	4	7
Megachile gemula Cresson		1					1
Megachile gentilis Cresson					2	6	8
Megachile lippiae Cockerell						1	1
Megachile sp.						1	1
Megachile melanophaea Smith						2	2
Megachile mellitarsis Cresson	1						1
Megachile montivaga Cresson		12	12		9	24	57
Megachile parallela Smith						2	2
Megachile perihirta Cockerell		3	9		13	65	90
Megachile relativa Cresson						1	1
Megachile rotundata (Fabricius)[3]						4	4
Megachile snowi Mitchell						1	1
Osmia spp.	23		1				24
Osmia aglaia Sandhouse	1						1
Osmia albolateralis Cockerell	3	2	2	5	26	15	53
Osmia atrocyanea Cockerell	20	19	3	15	22	4	83
Osmia bakeri Sandhouse	1			1			2
Osmia brevis Cresson	2	10					12
Osmia bruneri Cockerell	8	5			7	2	22
Osmia caerulescens (Linnaeus)[3]		8	12		8	1	29
Osmia californica Cresson	3		1	1	1		6
Osmia calla Cockerell			4	1	5	4	14
Osmia cobaltina Cresson	1	1					2
Osmia coloradensis Cresson	2	3		8		1	14
Osmia densa Cresson	6	5		25	9	1	46
Osmia dolerosa Sandhouse					2		2
Osmia giliarum Cockerell	6	10	2	1		1	20
Osmia grindeliae Cockerell					1		1
Osmia integra Cresson					1		1
Osmia iridis Cockerell & Titus	1	3		5			9
Osmia kincaidii Cockerell	10	16	5	7	7	3	48
Osmia marginipennis Cresson			1				1
Osmia nemoris Sandhouse			1	4	2		7
Osmia nigrifrons Cresson		1		1			2
Osmia paradisica Sandhouse			1		2	3	6
Osmia proxima Cresson	4	11	7	3	3		28
Osmia pusilla Cresson	30	44	12	2	10	14	112
Osmia raritatis Michener	1			1	1		3
Osmia simillima Smith	5	6	1	1	2	2	17
Osmia texana Cresson					1		1
Osmia thysanisca Michener	1						1
Osmia trevoris Cockerell	72	167	63	77	81	29	489
Osmia trifoliama Sandhouse		4		4	3	1	12

Osmia tristella Cockerell			2		2		4
Osmia unca Michener	2						2
Osmia vandykei Sandhouse	1			2			3
Stelis holocyanea (Cockerell)		1			5		6
Stelis interrupta Cresson			3				3
Stelis montana Cresson	5	3	1				9
Stelis monticola Cresson			1		1	1	3
Stelis sp.					1		1
Stelis (*Stelis*) sp. 1		1	1				2
Stelis sp. 2			1		1		2
Stelis sp. B					1	1	2
Stelis subemarginata Cresson					2		2

1. New state record for Idaho
2. New state record for Washington
3. Exotic

Table 1.2 Bray-Curtis dissimilarity matrix for *Bombus* community data from the Palouse Prairie (this report), Okanogan National Forest (Wilson et al., 2010), the Zumwalt Prairie (Kimoto et al., 2012), the Palouse Prairie from 2002 and 2003 (Hatten et al., 2013), and Palouse Prairie data collected prior to 2001 (GBIF, 2015).

	Current Palouse	Okanogan	Zumwalt	Palouse 2002,2003
Okanogan	72.8	-		
Zumwalt	63.5	43.3	-	
Palouse 2002,2003	55.5	87.6	78.4	-
Historic Palouse	36.6	62.0	60.7	68.1

Table 1.3 Studies of prairie or grassland inhabiting bees.

Habitat	Years Collecting	Specimens Collected	Species Detected	Sampling Method	Sampling Period	Citation
Iowa tallgrass prairie	2	3,566	86	pan trap	May-August	Davis et al., 2008
grasslands near Boulder CO	5	5,207	104	pan trap and net	May-August	Kearns and Oliveras, 2009
Minnesota tallgrass prairie	3	3,702	127	net	May-September	Reed, 1995
Illinois tallgrass prairie	1	4,622	111	malaise trap, pan trap, vane trap	June to October	Geroff et al., 2014
Iowa tallgrass prairie	1	1,149	73	pan trap and net	June-August	Hendrix et al., 2010
Iowa tallgrass prairie and ruderal areas	1	582	56	pan traps and nets	June-August	Kwaiser and Hendrix, 2008
Wyoming shortgrass prairie	2	-	200	net	May-August	Tepedino and Stanton, 1981
Zumwalt bunchgrass prairie	2	9,158	~211	blue vane trap	June-August	Kimoto et al., 2012
Palouse bunchgrass prairie	2	13,241	179	vane trap, pan trap, net	May to July	this report

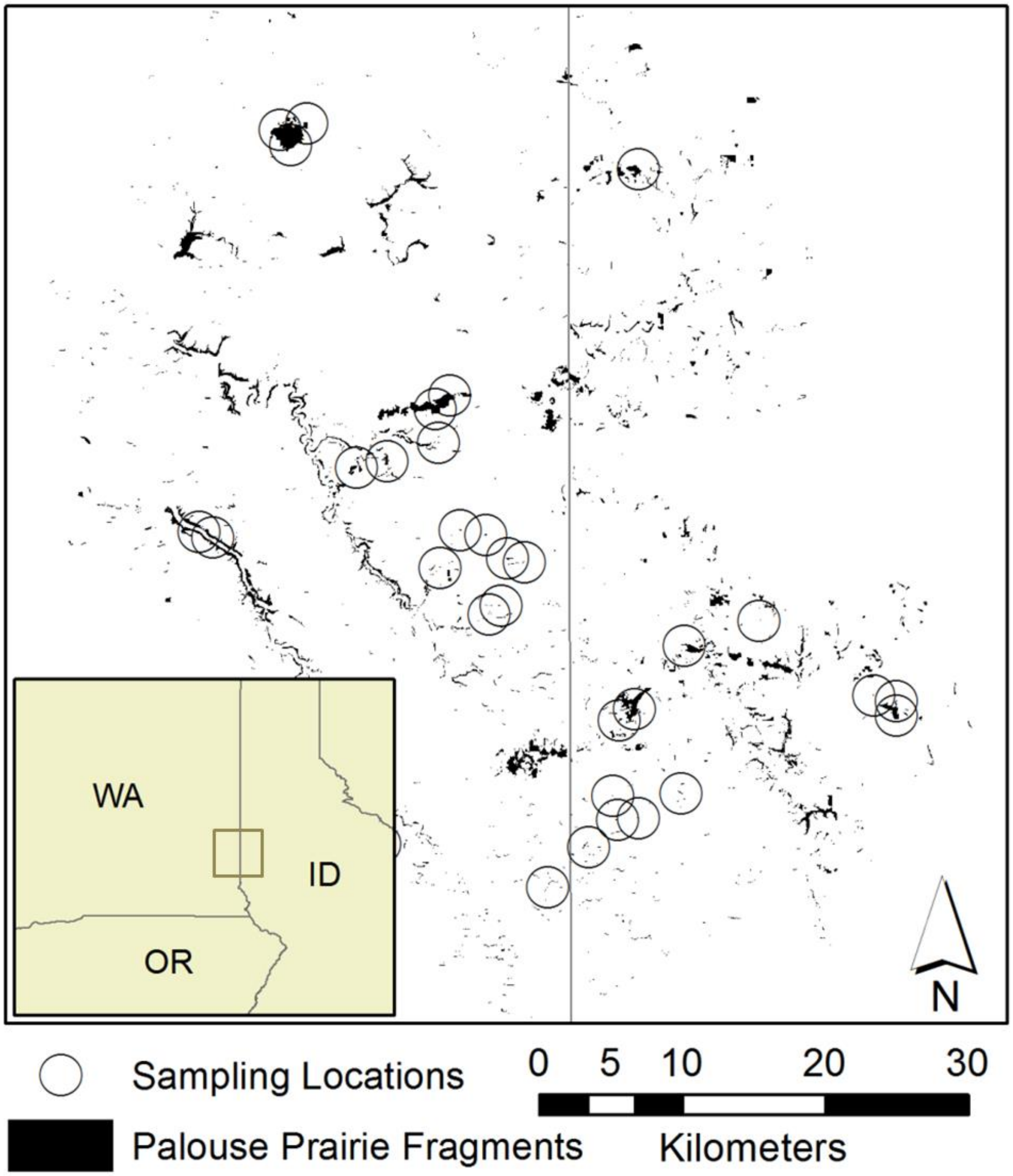

Figure 1.1 Map of sampling locations and Palouse Prairie fragments. Circles denote collection sites.

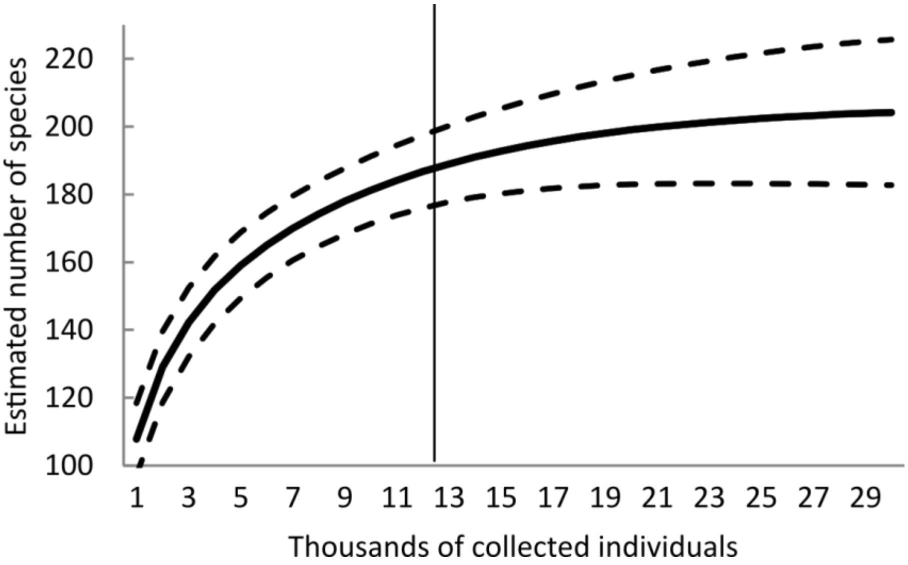

Figure 1.2 Extrapolated rarefaction curve with 95% confidence intervals based on all collected bees. Vertical line indicates the actual number of collected bees.

CHAPTER 2: EVIDENCE FOR *BOMBUS OCCIDENTALIS* (HYMENOPTERA: APIDAE) POPULATIONS IN THE OLYMPIC PENINSULA, THE PALOUSE PRAIRIE, AND FORESTS OF NORTHERN IDAHO

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Abstract

Since the mid-1990's *Bombus occidentalis* (Green) has declined from being one of the most common to one of the rarest bumble bee species in the Pacific Northwest of the USA. Although its conservation status is unresolved, a petition to list this species as endangered or threatened was recently submitted to the U.S. Fish and Wildlife Service (USFWS). To shed light on the conservation situation and inform the USFWS decision we report on the detection and abundance of *B. occidentalis* following bumble bee collection between 2012 and 2014 across the Pacific Northwest. Collection occurred from the San Juan Islands and Olympic peninsula east to northern Idaho and northeastern Oregon, excluding the arid region in central Washington. *Bombus occidentalis* was observed at 23 collection sites out of a total of 234. With the exception of three sites on the Olympic peninsula all of these were in the southeastern portion of the collection range.

Introduction

Bee species decline is an issue of global concern due to the vital ecosystem service and function provided by pollinators (Biesmeijer et al. 2006, Potts et al. 2010, Lebuhn et al. 2013, Kleijn et al. 2015). Bumble bees (Hymenoptera: Apidae, *Bombus*) are a conspicuous and charismatic group, and declines in abundance and range contraction have been observed in Europe and North America (Williams 1982, Rasmont et al. 2005, Colla and Packer 2008, Grixti et al. 2009, Cameron et al. 2011).While comprehensive analyses of bee species abundance and conservation status at continental scales continue to accumulate (Cameron et al. 2011, Kleijn et al. 2015), intensive local and regional assessments are sorely lacking (Tripodi and Szalanski 2015). Systematic surveys of bumble bees at local scales have the capacity to discover rare and declining bumble bee species (Colla and Packer 2008, Tripodi and Szalanski 2015) and therefore greatly contribute to a thorough assessment of conservation status.

In North America, *Bombus occidentalis,* along with several other *Bombus* species, has undergone recent dramatic range contraction and reduction in relative abundance possibly due to disease (NRC 2007, Rao and Stephen 2007, Cameron et al. 2011, Colla et al. 2012, Koch and Strange 2012). *Bombus occidentalis* was once a very common bee in North America, evidenced by the abundance of pinned specimens associated with natural history collections throughout the United States (Cameron et al. 2011). Although there are no explicit baseline records of historic relative abundance (*i.e.*, community-level study, *e.g.*, Colla and Packer 2008), Stephen (1957 p.71) described the species as "…abundant throughout the Pacific Northwest and Northern California becoming less numerous in the southern Rocky Mountain area." Now the species is rare across its historic range having substantially declined in relative abundance and in geographic range by 28% (Cameron et al. 2011). The historic range of *B. occidentalis* extended from Arizona to Alaska and from the western parts of the Great Plains to the Pacific (Stephen 1957), but it is currently rare west of the Sierra-Cascade Crest (Rao and Stephen 2007, Cameron et al. 2011). The species is listed as “Vulnerable” by the IUCN (IUCN 2015) and has an active petition for federal listing under the endangered species act. Decline of *B. occidentalis* may be caused by spread of *Nosema bombi* into wild populations from commercially reared colonies used for greenhouse pollination (Colla et al. 2006, Winter et al. 2006). However, in Alaska, *B. occidentalis* along with *B. cryptarum* (=*B. moderatus*) is abundant, despite high disease prevalence (Koch and Strange 2012). Localized decline in abundance has been observed in the Fraser Valley of British Columbia between 1981 and 2004 (Colla and Ratti 2010). Localized decline in Washington State may have happened in the early 1990s (Figure 2). The species was not detected in Washington state between 2002 to 2009 despite intensive surveying (Cameron et al. 2011, Hatten et al. 2013; but see Doughton 2013). A survey in the Zumwalt Prairie in northeastern Oregon did detect *B. occidentalis* in both years of a 2 year study, albeit at fairly low levels (1.83% of total collected *Bombus*) (Rao et al. 2011). The current range, conservation status, and habitat associations of *B. occidentalis* is not fully known in the Pacific Northwest. Monitoring for further decline or recovery is important to inform conservation efforts. Herein we present data collected between 2012 and 2014 at 234 sites across Washington, Oregon and Idaho and provide evidence for the persistence of *B. occidentalis* populations on the Olympic peninsula as well as in the Palouse Prairie and highland areas of Northern Idaho and Eastern Washington.

Materials and Methods

Data were collected in three phases:

Phase one – Palouse Prairie collection sites.

The Core Palouse Prairie is located in Northern Idaho and adjacent Eastern Washington (Donovan et al. 2009) (Figure 2.1). Historically this region was dominated by extensive bunchgrass prairie but the fertility of the area led to near complete conversion to agriculture, leaving a mosaic of fragments mostly on land too steep or rocky to farm (Looney and Eigenbrode 2012). Collection sites were densely located in this region but never less than 1 km apart (Figure 2.2). Blue vane traps (SpringStar LLC, Woodinville, WA, USA) and colored pan traps (bee bowls, Wilson et al. 2008) were placed for 24 hours at 32 locations in Palouse Prairie fragments four times each year in the summers of 2012 and 2013 (Figure 2.2). Aerial netting was performed during trap placement and removal. All bumble bees collected in this manner were pinned and identified to species**.**

Phase two – Inland Northwest collection sites.

Collection also occurred throughout the Inland Northwest. These sites were mostly restricted to wooded upland areas ringing the arid lowlands of central Washington but included additional Palouse Prairie sites (Figures 2.1 and 2.2). Blue vane traps were placed for 24 hours at 167 locations in July and August 2014. Traps were more widely spaced than in Phase one, generally about 20 km apart, except for those immediately north or south of the Palouse prairie, which were more closely spaced, about 2 km apart (Figure 2.2). The purpose of this phase of collection was to obtain genetic material from *B. bifarius* for population genetics analysis but the abundance of certain other bumble bee species were noted. To limit impact on bumble bee populations in sampled areas, most bees were released unharmed. Bees were chilled with ice and only bees that could be rapidly and reliably identified from hair color patterns were noted. The brief period in which bees were incapacitated following chilling prevented a comprehensive analysis of the *Bombus* community during this collection phase.

Phase three – National Park collection sites.

Finally, collection occurred in 2013 and 2014 at 35 locations in seven National Parks in Washington State (Figure 2.1). These collection sites were clumped spatially. Twenty-three of 35 total sites were in one of three national parks (North Cascades, 5 sites; Mt. Rainier 7 sites; or Olympic, 11 sites). Bees were netted for 0.5 to 6 collector-hours (mean 1.5 collector-hours). Collected *Bombus* were identified in the field. Voucher specimens were retained and the remainder was released. Overall, these collections yielded two types of data: relative abundance data from Phase one and Phase three and presence-absence data from Phase two.

Collections during all three phases took place in 10 ecological sections (Cleland et al. 1997): Oregon and Washington Coast Ranges (M242A), Willamette Valley (242B), Puget Trough (242A), Northern, (M242D), Western (M242B) and Eastern (M242C) Cascades, Okanogan Highland (M333A), Bitterroot Mountains (M333D), Blue Mountains (M223G), Palouse Prairie (331A). Ecological sections are a fine-scale ecological classification and mapping unit used by the National Framework of Ecological Units.

To assess historic relative abundance of *Bombus occidentalis* and to determine when the species decline began in Washington State we extracted information from the Global Biodiversity Information Facility (GBIF; http://gbif.org). GBIF is a major repository of geo-referenced bumble bee records in North America, and has been used in a variety of contemporary studies (e.g., Kerr et al. 2015). After eliminating records that lacked location or year of collection we were left with 12,047 records of bumble bee occurrence in the state of Washington from 1888 to 2014. To determine collection effort of *B. occidentalis* we combined data available on GBIF with our collection efforts from 2012 to 2014 in Washington (*n* = 1918). Our contemporary survey data has not been deposited onto GBIF. We estimated the proportion of *B. occidentalis* captured over time from 1888 to 2014 by simply dividing the number of *B. occidentalis* detected by the total bumble bees that have been recorded on GBIF.

Results and Discussion

Historic relative abundance of Bombus occidentalis

Prior to 1990, the abundance of *B. occidentalis* reported in Natural History Collections relative to all bumble bees surveyed, digitized, and deposited on GBIF is estimated to be 27% (Figure 2). However, after 1990, the abundance of *B. occidentalis* in combined survey efforts across the state is estimated to be < 0.1%. We chose 1990 as a useful cut-off of decline because the prevailing hypothesis associated with the decline of *B. occidentalis* is tied to the spread of pathogens into wild populations from commercial colonies (Colla et al. 2006). It was in the early 1990s that commercial bumble bees were introduced into the North American continent to deliver pollination services in greenhouse and open field crops (Velthuis and Van Doorn 2006). Although the date of decline in Washington State is slightly earlier than suggested by data from southern Oregon and California (Evans et al. 2008) our survey of natural history collection records supports the hypothesis that the general decline of wild *B. occidentalis* occurred in the 1990s.

Phase one – Palouse Prairie collection sites.

In 2012, 439 bumble bees were collected at Palouse Prairie sites representing 16 species. Ten *B. occidentalis* were collected at six sites making up about 2.3% of total collected *Bombus*. In 2013, 316 bumble bees were collected representing 14 species. Two *B. occidentalis* were collected at two sites making up 0.63% of total collected *Bombus*. Over two years, *B. occidentalis* was observed at 7 sites in the Palouse Prairie (Figure 2.2).

Phase two – Inland Northwest collection sites.

Out of 167 collection sites, 25 *B. occidentalis* were observed at 14 sites, all in the southeastern portion of the sampling area (Figures 2.1 & 2.2). Because the occurrence of only *B. occidentalis*, *B. nevadensis* and, *B. bifarius* were noted, relative abundance data are not available.

Phase three – National Park collection sites

In 2013, 774 bumble bees representing 15 species were collected at 28 sites (Figure 2.1). Six *B. occidentalis* were collected at two sites both within Olympic National Park comprising about 3.5% of total *Bombus* collected in Olympic National Park in 2013 and. In 2014, 531 bumble bees representing 12 species were collected at 12 sites. Four *B. occidentalis* were collected at one site in Olympic National Park comprising 1% of total *Bombus* collected within the park in 2014. Of the seven national parks from which collection occurred, *B. occidentalis* was only observed in Olympic National Park.

Throughout all three phases of this study, *B. occidentalis* was recovered on four of the ten ecological sections on which collection took place including Oregon and Washington

Coast Ranges (M242A), Blue Mountains (M223G), Palouse Prairie (331A) and adjacent locations in Okanogan Highlands (M333A).

Observance of *B. occidentalis* was mostly restricted to the southeastern portion of the sampling area with one occurrence in central Oregon and 10 individuals observed at three sites on the Olympic Peninsula. However, the area in southeastern Washington and adjacent Idaho had far more closely spaced collection sites (Figure 2.2); about half of all collection sites were situated in this relatively small area. Moreover, some of these sites were sampled over a longer period of time. Failure to recover *B. occidentalis* in other parts of the northwest may be due to lower sampling effort, rather than the absence of the species in those areas.

Despite fairly intense sampling effort, *B. occidentalis* was not detected in the Cascade Range. Natural history data and species distribution models suggest the area is suitable habitat for the species (Stephen 1957, Koch and Strange 2009, Koch et al. 2012), and occurrence in the Cascade Range has been noted in contemporary surveys (Cameron et al. 2011). Our failure to detect it suggests any remaining populations may be isolated and small.

Bombus occidentalis was not observed in bees collected in the Palouse Prairie in 2002-2003 but sampling effort was less intense and the sampling technique (pitfall trapping) may be less suitable for bee collection (Hatten et al. 2013). While the presence of *B. occidentalis* in the Palouse is encouraging, it has declined in relative abundance from one of the more common to one of the more rare *Bombus* species in the Pacific Northwest (Cameron et al. 2011).

Similar rates of relative abundance of *B. occidentalis* were detected in both the Palouse Prairie and the Zumwalt Prairie in Northeastern Oregon. (Rao et al. 2011). Higher rates of relative abundance of *B. occidentalis* in both the core Palouse and in the Zumwalt Prairie suggests that it may be particularly suited to less disturbed prairie habitats, underscoring the need for conservation both of the Zumwalt and of the remaining fragments of Palouse Prairie.

While we have established populations of *B. occidentalis* exist in the Palouse Prairie and in Olympic National Park we are unable to comment on the sustainability of these populations. Sampling bumble bees at the same sites in future years can help determine population trajectories, which are still unknown. Additionally, examining patterns of genetic diversity to evaluate levels of geneflow and the severity of any genetic bottlenecks the species has endured will help assess the long term persistence of detected populations.

Acknowledgments

We would like to thank the landowners of the Palouse Prairie who gave us access to prairie sites for insect collection, Dr. Sujaya Rao for her review of this manuscript, and Dr. Tim Hatten for general advice. Surveys in Pacific Northwest U.S. National Parks would not have been possible without the assistance of Dr. Brandon Hopkins, Craig Huntzinger, Houston Judd, Jason Long, Dr. Elinor Lichtenberg, Dr. Walter S. Sheppard, Dr. Chris Looney, and Joyce Knoblett. This project was funded through the NSF-IGERT program to P. Rhodes and S. Eigenbrode and a research grant from the North Coast and Cascades Science Learning Network (#NPS P13PG00149/FSN) to J. Strange.

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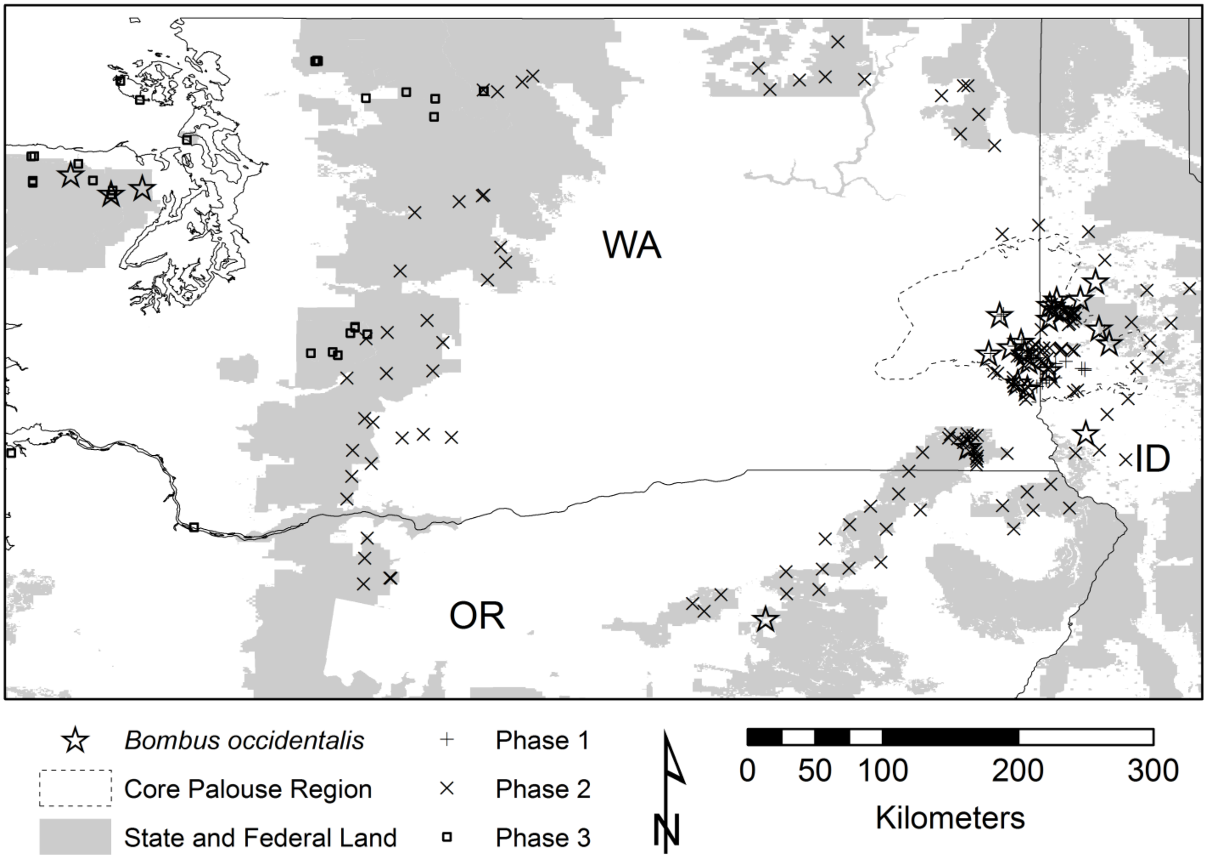

Figure 2.1 Locations of bumble bee sampling sites across the Pacific Northwest. The dashed line denotes the Palouse Prairie where sampling site density was high. Phase one, two, and three denote different types of sampling regimes: Sampling at phase-one sites includes netting, blue vane traps and colored pan traps; sampling at phase two sites used only blue vane traps; sampling at phase three sites used only an aerial net. Stars denote sites where *B. occidentalis* was collected.

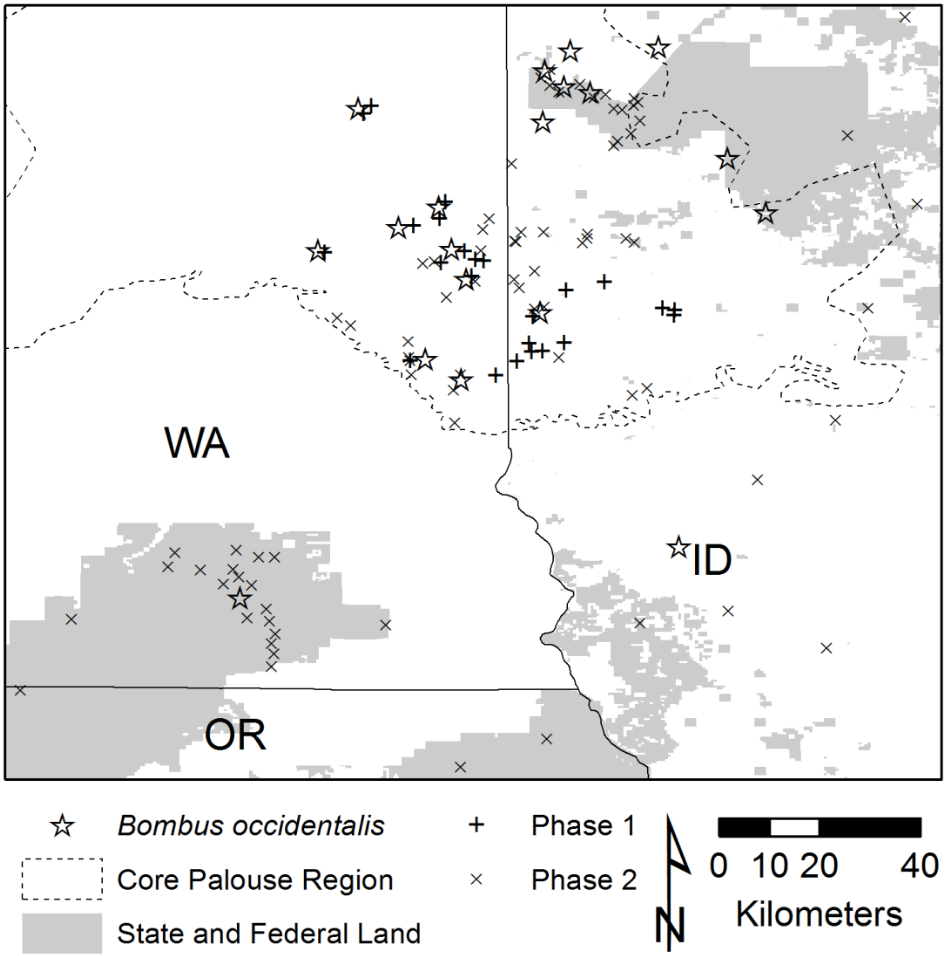

Figure 2.2 Locations bumble bee sampling sites in and around the Palouse Prairie. The dashed line denotes the Palouse Prairie where sampling site density was high. Phase one, two, and three denote different types of sampling regimes: Sampling at phase-one sites includes netting, blue vane traps and colored pan traps; sampling at phase two sites used only blue vane traps; sampling at phase three sites used only an aerial net. Stars denote sites where *B. occidentalis* was collected.

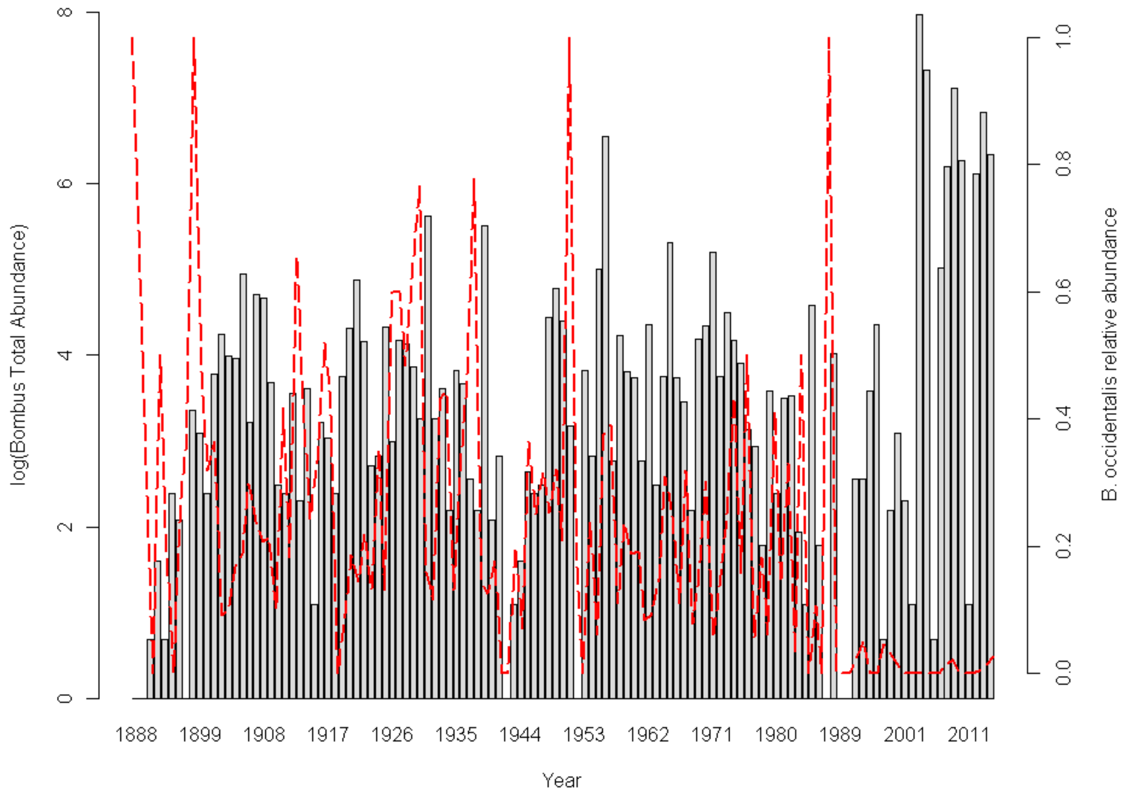

Figure 2.3 Survey of bumble bee collection effort in Washington from 1888 – 2014. The log of total bumble bee abundance (all species) is represented by a gray bar and relative abundance of *B. occidentalis* relative to total bumble bee abundance is represented by dashed red line.

CHAPTER 3: COLLECTION TECHNIQUE AFFECTS DETECTION OF HABITAT FACTORS INFLUENCING WILD BEE COMMUNITIES

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Abstract

Effective and consistent monitoring is an important part of bee conservation. Correctly interpreting the influence of habitat characteristics on native bee communities is necessary to create strategies for bee conservation as well as the provision of pollination services to agricultural crops or natural plant communities. The method used to monitor bee populations may affect the ability to identify these habitat characteristics. We used three sampling methods (blue vane traps, colored pan traps, and aerial net collection) to assess bee communities in fragments of Palouse Prairie. Traps were placed at 32 sites in 28 fragments of natural land four times each year in 2012 and 2013. Differences in abundance, species richness, generic proportions and functional trait characteristics among the three sampling techniques were noted. We also evaluated differences in relationships between bee community characteristics and two habitat variables known to mediate bee communities: local plant species richness and the extent of suitable bee habitat in the surrounding landscape. Under our sampling regime blue vane traps collect the largest number of individuals and detect the most species. Net collected assemblages have a higher proportion of oligolectic bees. Colored pan traps and blue vane traps collected significantly more parasitic bees per collection event. Community metrics for bees collected using blue vane traps were correlated with the amount of suitable habitat in the surrounding landscape but not local plant species richness. Conversely, community metrics for bees collected using an

aerial net were correlated with the local plant species richness but not amount of suitable habitat in the landscape.

Introduction

Animal pollination, mediated in large part by wild bees, is crucial for natural ecosystem function and crop production (Fontaine et al. 2005; Klein et al. 2007). Declines in bee populations can lead to a cascade of extinctions as codependent plant and pollinator communities decline in parallel (Biesmeijer et al. 2006). Seed production is, at least in some years, pollen limited (Burd 1994), plant community diversity is linked to the functional diversity of flowering plants (Fontaine et al. 2005), and reduction in pollinator abundance can influence plant gene flow (Bawa 1990). Moreover, animal pollination is necessary for 35% of global food production (Klein et al. 2007) and diverse communities of native bees can enhance seed production in crop plants (Klein et al. 2003; Hoehn et al. 2008; Vergara and Badano 2009).

Bee populations worldwide are currently under pressure from invasive organisms, disease and habitat loss and fragmentation (Winfree et al. 2009; Vanbergen 2013). Indirect evidence suggesting a decline in the abundance and range of many bee species has created concern as to the status of remaining bee populations (Biesmeijer et al. 2006; NRC 2007; Burkle et al. 2013; Bartomeus et al. 2013). Bee conservation requires effective monitoring using efficient methods that are comparable across different sampling areas. Monitoring techniques often involve use of standardized sampling protocols based around passive insect traps sometimes supplemented with active net collection. Passive traps can increase the proportion of the bee community sampled and may eliminate bias associated with net capture where a collector's awareness and dexterity may influence the assemblage of insects collected (Nielsen et al. 2011) (but see Cane et al. 2013 who found little difference among trained collectors). Colored pan traps ("Bee Bowls") have been commonly used in native bee surveys for well over a decade and have low bias and high efficiency compared to other bee collection methods (LeBuhn et al. 2007; Westphal et al. 2008; Droege et al. 2010). Colored pan traps are typically deployed using equal numbers of fluorescent blue, fluorescent yellow and white traps (LeBuhn et al. 2007). Blue vane traps were recently introduced as an effective method for collecting bees (Stephen and Rao 2005) and are now commonly used. They consist of two 24x13 cm cross vanes inserted into a funnel that is threaded to screw into a collecting jar. The cross vanes and funnel are UV reflective.

While differences in the assemblage of collected bees among different trap types have been noted (Cane et al. 2000; Giles and Ascher 2006; Roulston et al. 2007; Wilson et al. 2008; Wood et al. 2015), influence of habitat characteristics on trap performance is not fully understood. Characteristics of the collection site can influence colored pan trap and net collection. Presence of abundant floral resources can reduce the abundance and species richness of colored pan trapped samples relative to net collected samples (Cane et al. 2000; Roulston et al. 2007; Wilson et al. 2008). Experimental removal of floral resources increased species richness of bees detected with colored pan traps relative to control sites with the strength of the effect varying across the flowering season (Baum and Wallen 2011). The observable bee community at a location is influenced by both such local factors and more distant factors (Potts et al. 2003; Steffan-Dewenter 2003; Kremen et al. 2004; Hines and Hendrix 2005). For example, in fragments of Palouse Prairie we have found plant species richness within 50m of the bee collection site, as well as amount of useful habitat for bees within 1500 m of the collection site, to be correlated with bee species richness and diversity (Rhoades et al. in prep). However, it is unclear if we would have arrived at these conclusions if different sampling methods had been employed.

Relative biases inherent to pan trapping, net collection and blue vane traps have not been fully characterized. The purpose of this paper is to evaluate these relative biases and determine the utility of each method in assessing the influence of habitat characteristics known to be important in mediating bee communities. A study of the bee communities of the Palouse Prairie utilizing multiple collecting methods provides the opportunity for such an assessment. The objectives are to: 1. Assess potential biases of each method with respect to bee size, floral specialization, and taxonomic composition; and 2. Compare the ability of the three methods to elucidate the impact of local plant community characteristics and landscape context on bee community metrics.

Materials and Methods

Sample collection

Sampling occurred in remnants of Palouse Prairie, a Pacific Northwest bunchgrass prairie in eastern Washington and adjacent Idaho. Study locations exhibited a range of site and landscape characteristics. Like many grassland and prairie systems worldwide, conversion to agriculture has significantly reduced its distribution in the last century. Approximately 1% of the Palouse Prairie remains (Black et al. 1998), and could be classified as ‘Critically Endangered’ (Keith et al. 2013). Most of the remaining fragments are less than 2 ha in size with high perimeter-to-area ratios, but a few larger remnants exist mostly on rocky buttes (Looney and Eigenbrode 2012). Plant communities on Palouse Prairie fragments range from patches highly invaded by exotic grass with low native plant diversity to sites with robust communities of native grasses, shrubs and forbs (Davis 2015). In a recent thorough survey, more than 150 species of vascular plants were observed at the sites in which bee sampling occurred (Davis 2015).

Bee collection occurred in 2012 and 2013 at 32 sites in 29 fragments of natural land. Each site was sampled four times each year at sampling intervals of approximately three weeks. Sampling location within the fragment was determined by generating a random point within each prairie fragment at least 10 meters from the fragment edge (where possible) using the Create Random Points tool in ArcMap10.0 (ESRI, Redlands CA). If the sampling point fell within a thicket of shrubs or small trees, prohibiting trap placement, the sampling location was moved 5 meters beyond the nearest edge of the thicket. These sampling points were used for all 8 sampling events. At each visit, pan traps, blue vane traps, and aerial net collection were used for bee sampling. Three colored pan traps (3.25 oz. soufflé cups, Solo model #p325w-0007) filled with soapy water were placed on the ground in areas without dense plant growth that would decrease visibility. Pan colors (one yellow, one blue and one white) were randomized and were placed in a transect leading away from the sampling point about three meters apart. Blue vane traps were filled with 500 ml of soapy water and hung from a bamboo tripod with the bottom of the trap about 1 meter off the ground. Traps were left open for approximately 24 hours. Net collection occurred at flowers within 50 meters of the sampling point for 5 minutes at the time of trap placement and removal for a total of 10 minutes of net collection for each site at each sampling period.

Sampling occurred on mostly sunny days with highs above 16°C. The average high temperature for sampling days was 22.7°C in 2012 and 27°C in 2013; the average low temperature was 5.4°C in 2012 and 6.2°C in 2013; 1.16 cm of precipitation fell over four sampling days in 2012 and 0.15 cm fell over two days in 2013.

Net collected bees were kept frozen before pinning and identification. Bees collected in blue vane traps or pan traps were rinsed in ethanol and then placed in a Whirl-Pak bag (Nasco, Fort Atkinson, WI), covered with ethanol and eventually washed and dried before pinning and identification (methods adapted from (Droege 2009). In the first year of collection all bees were pinned and identified. In the second year a few very common and easily identifiable species including *Agapostemon angelicus* Cockerell, *A. virescens* (Fabricius), *A. femoratus* Crawford and *Halictus tripartitus* Cockerell were identified without pinning. Additionally, because we were not able to resolve species identities in *Lasioglossum* of the *Hemihalictus* series we recorded them as such and stored without pinning. All other bees were identified to species except for *Nomada, Epeolus, Sphecodes,* and the *Hemihalictus* series, which were only identified to genus. Voucher specimens reside in the William F. Barr Insect Museum at the University of Idaho and the U. S. National Pollinating Insects Collection, USDA Bee Biology and Systematics Laboratory housed on the campus of Utah State University.

Site and landscape characterization

The plant community was characterized by identifying all entomophilous plants in flower within 50m of the sampling point at the time of trap placement. Plants were either identified in the field or in consultation with a botanist (Cleve Davis) using photos.

Landscape composition was characterized using the Cropscape (USDA-NASS) remotely sensed land cover classification supplemented with National Agricultural Imagery Program

(NAIP) imagery and high resolution Google Maps data. Landscape features were heads-up digitized into polygons using ArcView 10.0 and classified into 16 categories: dense forest, open forest, highly developed land, lightly developed land, hay/pasture/CRP, natural land, spring wheat, winter wheat, canola, garbanzos, dry peas, lentils, barley, grass seed, and alfalfa. Lightly developed land included most rural homesteads. Hay fields, pasture, land enrolled in the conservation reserve program (CRP) and Palouse Prairie fragments were all classified similarly in the Cropscape data layer. Deciding whether or not to include a particular piece of land in the natural land category was done by evaluating the heterogeneity of the plant cover using high resolution aerial photographs. Land with high plant heterogeneity was judged to have large amounts of floral resources to support bee populations and considered natural land. These polygons were then reclassified in ArcView using the Reclass tool into useful habitat for bees (open forest, natural land, and lightly developed land) and land not useful for bees (everything else). Concentric rings were generated around the sampling point at ten distances (50m, 100m, 150m, 250m, 400m, 600m, 800m, 1000m, 1250m, and 1500m) using the Multiple Ring Buffer tool. The Iterate Feature Selection tool in model builder in ArcView was used to automate the process of quantifying the area of land cover type at each sampling point/radius combination by using the Tabulate Area tool and the Append tool at each radius for each sampling point. The area of each land cover type for each radius was determined by subtracting that of the next smallest radius to produce a measure of area of each land cover type within each ring (e.g. between 1000m and 1250m). These data were then log transformed.

Data analysis

Spatial variograms were constructed using the geoR package in R (Ribeiro and Diggle 2001; R Core development team 2015) to evaluate any spatial autocorrelation among any variables of interest including bee species richness, diversity, plant species richness, and amount of habitat suitable for bees at each radius. Linear regression was then used to evaluate the correlation between the amount of useful habitat for bees and bee species richness or Shannon-Wiener diversity calculated for each collection method individually and for all collection methods combined. These metrics were correlated to the amount of useful habitat for bees at each ring from 100m to 1500m.

Ordination was performed using non-metric multidimensional scaling on bee communities captured using each collection method at each site for a total of 96 site-method combinations. The Vegan package in R (Oksanen et al. 2015; Team 2015) was used for this procedure. All 8 collection periods for each site-method were combined. Species with fewer than 5 collected individuals were dropped. Three sites with net collection totals of fewer than five were dropped. To visualize relationships between community partitioning in NMDS space and generic composition or functional trait composition (bee size and oligolecty) of the 96 represented communities the following procedure was employed: Proportions of the nine most abundant genera (*Agapostemon, Andrena, Bombus, Ceratina, Halictus, Lasioglossum, Megachlie, Melissodes,* and *Osmia*) were calculated for each site-method combination. Bee size was estimated by measuring the intertegular span using a reticle on a dissecting scope (Cane 1987). The intertegular span was averaged over all collected individuals for each species or, if more than 15 individuals were collected, average span was calculated using 15 individuals chosen at random. Average intertegular span for each site-method combination was calculated. Bee species were scored as oligolectic or not using published information or expert opinion and the proportion of oligolectic bees was calculated for each site-method combination. These values (average size, proportion of oligolectic bees, and proportion of each genus) were then fit to the ordination using environmental vector fitting (Oksanen et al. 2015).

Differences in trap performance during different sampling periods were evaluated with a two factor ANOVA using either abundance or species richness as a response variable and collection method, sampling period, and the interaction between collection method and sampling period as predictor variables. A generalized linear model with a Poisson link function was used in R (Team 2015). Means separation was performed using Tukey's HSD test in the Multcomp package in R. Differences among collection methods in the abundance and proportion of oligolectic or parasitic bees was evaluated using a Tukey's HSD test. Bray-Curtis analysis was performed on bee communities of all sampling sites pooled to quantify the similarity of communities collected using differing trapping methods.

To evaluate species richness among different trap types, rarefaction curves for each collection method were created in EstimateS using 100 permutations of the species by sample matrix (Colwell et al. 2012; Colwell 2013). These curves estimate species accumulation, produce confidence intervals, and allow interpolation of species richness at different numbers of collected individuals. The Chao 1 estimator generated by EstimateS was used to estimate asymptotic species richness (Chao 1984).

Results

In total, 13,241 bees were collected comprising at least 179 species in five families and 29 genera. Blue vane traps captured 7,404 individuals (58% of total) and 154 species (85% of total). Colored pan traps captured 4,351 individuals (34% of total) and 130 species (72% of total). Net collection yielded 1,086 individuals (8% of total) and 105 species (58% of total). Forty species were recovered only from blue vane traps, 20 species were recovered only from pan traps, and 10 species were recovered only through net collection (Figure 3.1).

Net collections yielded a mean of 6.1 bees per collecting event (two five-minute sessions of net collection at each site), colored pan traps yielded a mean of 18.3 bees per collecting event (24 hours of open trap time; all three pan traps combined) and blue vane traps yielded a mean of 30.1 bees per collection event (24 hours of open trap time). Mean bee abundance was highly variable among collection periods and collection methods ranging from 2.2 to 8.1 for net collection, 9.6 to 29.8 for colored pan traps and 19.3 to 40.8 for blue vane traps (Figure 3.2). Blue vane traps collected significantly more individual bees than colored pan traps or net collection except for collection period five early in the spring of 2012 where colored pan traps and blue vane traps collected similar numbers of bees. Net collection yielded fewer bees per collection period than either colored pan traps or blue vane traps (Figure 3.2).

Mean bee richness per collecting event varied more than fourfold among collection methods (Figure 3.3): net collection, 2.1 species (range 1.2 to 3.9); colored pan traps 5.2, (3.4 to 7.8); and blue vane traps, 9.0 (5.9 to 11.7). In 2012 blue vane traps and colored pan traps collected similar numbers of species in the first three collection periods, in the fourth collection period blue vane traps collected significantly more species than colored pan traps. Net collection yielded fewer species in all collection periods that year (Figure 3.3). In 2013, blue vane traps collected the most species in all collection periods (Figure 3.3). Net collection detected the fewest species in all collection periods except period seven when it yielded similar numbers of species to colored pan traps (Figure 3.3).

Species richness estimates and accumulation rates varied among collection methods. Extrapolated estimations of asymptotic species richness suggest blue vane traps will ultimately collect the highest number of species, significantly more than net collections (Table 3.1). Yet, net collection produces the steepest accumulation curve and net collection has the highest interpolated species richness standardized to 1,000 collected individuals, although differences in interpolated species richness were not significant (Table 3.1).

Bray-Curtis dissimilarity analysis shows the assemblages of bees collected using blue vane traps and net collection were most dissimilar from one another, with colored pan traps of intermediate dissimilarity to both. This is corroborated by a community ordination showing clear delineation among collection methods with pan trapped samples intermediate between those collected using blue vane traps and net collections along axis 1 (Figure 3.4). Additionally, blue vane traps and net collected assemblages are somewhat separated from pan trap-collected assemblages on axis 2. Bees in the genera *Lasioglossum*, *Agapostemon* and *Melissodes* composed a larger proportion of blue vane traps assemblages compared to colored pan traps or net collection, bees in the genera *Andrena*, *Ceratina*, and *Megachile* composed a larger proportion of bees collected using a net than the other two collection methods; and bees in genera *Halictus* and *Osmia* compose a larger proportion of colored pan trap collected samples compared to the other 2 methods (Figure 3.4, Table 3.5).

Additionally, pan trap assemblages are smaller in average size compared to the other two methods (Figure 3.4, Table 3.3). Net collected assemblages have a higher proportion of oligolectic bees than the other two methods, but abundance of oligolectic bees per collection event does not differ between the two methods (Figure 3.4, Table 3.3). Finally, colored pan traps and blue vane traps collected significantly more parasitic bees per collection event than net collection but the proportion of collected parasitic bees was similar among all three methods (Table 3.3).

Bee diversity and species richness calculated using data from all collection methods combined were not correlated with prairie remnant size (data not shown) but were correlated with plant species richness at the collection site and amount of habitat useful for bees at all distances 1500m from the collection site and closer (Table 3.4). Correlation statistics were similar for all measured distances so only results from 1000m and 100m are used in subsequent analyses and displayed in Table 3.4. Relationships between plant species richness, landscape configuration, and bee community metrics differ depending on the collection method employed. Community metrics of bees collected using blue vane traps, but not colored pan trap or net collected bees, were correlated with the amount of useful habitat for bees at 100m and 1000m (Table 3.4). Conversely, species richness and diversity of net collected bees was correlated with plant species richness at the collection site, while community metrics of bees collected using blue vane traps or colored pan traps were not (Table 3.4).

Discussion

Consistent bee community characterization and accurate evaluation of the influence of habitat characteristics on these communities are both important in monitoring bee populations and crafting conservation strategies. Our results indicate alterations to the bee community caused by landscape and site variables were not detected by all sampling methods and so care must be taken when evaluating the influence of these factors.

Differences in relative abundance

Differences in abundance among sampling methods are difficult to compare with other studies. Predictably, the effectiveness of net collection compared to passive trapping methods depends on the relative effort collectors dedicate to each method. Roulston et al. (2007) found prolonged net collection (13.5 collector hours) captured far more bee species than 30 pan traps open for 1 day; in another study, more modest amounts of net collection (2 collector hours) collected fewer individuals and species compared to 30 pan traps open for 1 day (Wilson et al. 2008). Our periods of net collection were much shorter (10 minutes per collection event), and the number of colored pan traps deployed (3 total per collection event) was an order of magnitude fewer than many similar studies (Roulston et al. 2007). This led to consistently fewer individuals collected and fewer species detected through net collection or colored pan traps compared to blue vane traps. The only exception to this occurred in collection period 5 when mean abundance was similar between colored pan traps and blue vane traps (Figure 3.3). However, this was driven by large numbers of *Halictus* and *Ceratina* representing few species in colored pan traps, so mean species detection was still higher in blue vane traps during this period (Figure 3.3).

While less relative effort was dedicated to net collection and the use of colored pan traps compared to similar works, a substantial number of individuals and species were collected with these methods. Pan traps were highly effective in this study collecting over 4,000 individuals and 130 species, which is similar or higher than work in other prairie systems (Davis et al. 2008; Kearns and Oliveras 2009). Similarly, although relatively little net collection occurred in this study, 105 species were collected in this manner, again similar or higher than other prairie systems (Reed 1995; Kwaiser and Hendrix 2008; Hendrix et al. 2010).

It is not clear why blue vane traps and colored pan traps collected similar numbers of species only in the first 3 collection periods of 2012, after which, in the final collection period of 2012 and in all of 2013, blue vane traps collected significantly more species (Figures 3.2 and 3.3). Bees may fly farther later in the year as floral resources diminish with the drying of the landscape, but this could not explain performance of colored pan traps in 2013. Alternately, colored pan traps could capture (trap out) most of the bees near the sampling area in the first few weeks of collection. In subsequent sampling periods, immigrants to the now somewhat depopulated sampling location may be typically trapped in the more visible blue vane trap.

Differences in proportions of different taxa

Each collection method yielded a distinct community of bees. Bray-Curtis analysis found between 45-77% dissimilarity among the three trap types (Table 3.2). Apparent predilections for certain trap types among bee taxa and functional groups have been noted in other studies; our data both corroborates and conflicts with these previous works. We found oligolectic bees made up a higher proportion of bees in net collected samples compared with other methods (Table 3.3) supporting the findings of Cane et al. (2000). Others have found *Andrena* (Giles and Ascher 2006; Joshi et al. 2015) and *Lasioglossum* (Giles and Ascher 2006; Wilson et al. 2008; Joshi et al. 2015) to be over-represented in pan traps, whereas we found *Andrena* to make up a disproportionately large part of net collected samples (many are oligolectic) and *Lasioglossum* is equally common in colored pan traps and blue vane traps (Table 3.5). However, Giles and Ascher (2006) and Wilson et al. (2006) did not use blue vane traps and it is possible that Lasioglossum is generally more common in passive traps. Like Joshi (2015) and Geroff (2014) we found *Melissodes* and *Bombus* to be relatively more abundant in blue vane trap samples than in colored pan traps. On the other hand, unlike Joshi (2015) whose redundancy analysis (a form of constrained ordination) found *Osmia* and *Ceratina* to compose a larger proportion of assemblages collected with blue vane traps, we found *Osmia* to be a large part of both colored pan trap and blue vane trap assemblages and *Ceratina* composed a large part of assemblages collected using all three methods (Table 3.5).

Interactions between habitat characteristics and sampling method

Although it is increasingly evident that different collection methods will yield different assemblages of bees, the effect of specific methods on characterizations of important habitat characteristics and bee community metrics of assemblages have not been previously evaluated. We found that the method of sampling altered detectable relationships between bee community metrics and important habitat variables. Specifically, community metrics of bees captured using net collection are correlated with plant species richness at the collection site and not with the amount of useful habitat for bees at a distance. While increasing the total amount of habitat in an area does increase bee species richness and abundance (Steffan-Dewenter 2003), net collection will not detect many of these species in areas without abundant and diverse floral resources. On the other hand, community metrics of bees captured using blue vane traps are correlated with the amount of natural land within 1,000 meters, but not plant species richness at the collection site suggesting the community of bees captured using blue vane traps may be originating from areas outside the immediate sampling area (Table 3.4). Additionally this indicates that passive trapping may miss a lot of the bees currently foraging in the area, as shown by Cane et al. (2000). Alternately, passive trapping may draw bees from a larger geographic area and so the influence of factors at the collection site is overwhelmed by the influence of distant landscape features. This hypothesis is supported by the fact that models which incorporate all trapping methods correlate most closely with plant species richness (Table 3.4). Regardless, differences in community composition between blue vane trap-collected and net collected bee communities strongly suggest that many individuals collected with blue vane traps were not foraging in the area where sampling occurred but were just passing through.

Community metrics of bees collected in colored pan traps were not correlated with either local plant species richness or amount of natural land in the landscape. Nonetheless, models evaluating the relationship between local plant species richness and bee community metrics that incorporated data from colored pan traps, blue vane traps and net collection have higher r^2 values than models that used data from net collection alone (Table 3.4). Conversely, models evaluating the relationship between bee community metrics and the amount of useful habitat for bees at 1000 m or 100 m had the best fit when only data from blue vane traps were used (Table 3.4). This implies the assemblage of bees detected by colored pan traps set on the ground respond to local, rather than large scale habitat characteristics. This may not be true for colored pan traps in all contexts. The placement of pan traps on the ground may have made them less conspicuous to immigrant bees and so these visitors may be collected by the blue vane trap before become aware of the pan traps. Geroff et al. (2014) found that similarity between communities of bees captured using blue vane traps and pan traps increased when pan traps were elevated to 1 meter compared to their standard deployment at ground level. Alternatively, foraging altitude and visual cues for finding forage may be different in bees that are larger and presumably stronger fliers (*e.g. Anthophora, Bombus, Apis, Eucera*) thus targeting vane traps.

Recommendations for conservation of the bee community in various habitats are predicated on relationships between bee community characteristics and landscape and site variables. We have shown that collection method alters the detected influence of habitat variables at different scales and suggests at least a mixture of passive traps and netting, if not all three methods, is essential to evaluate these factors. If full community characterization is desired, our data and data from other studies (Cane et al. 2000; Geroff et al. 2014) strongly indicate that a variety of collection methods should be employed.

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Table 3.1 Detected, extrapolated and interpolated species richness estimates by collection method. Letters denote significant differences in estimates as determined by non-overlapping confidence intervals.

	Detected	Extrapolated (Chao 1)	Interpolated (n=1000)
Blue vane trap	154	187.0 ±18.9[a]	95.6 ±10.4
Colored pan trap	130	157.6 ±16.0[ab]	94.1 ±9.1
Net collecting	105	125.7 ±12.2[b]	108.8 ±8.8

Table 3.2 Bray-Curtis dissimilarity matrix for bee species assemblages collected using blue vane traps, colored pan traps, and aerial nets.

	Colored pan trap	Blue vane trap
Blue vane trap	0.45	-
Net collecting	0.60	0.77

Table 3.3 Differences in functional trait make-up of bees collected using different sampling methods. Size is measured by the intertegular distance (IDT). Different letters denote significantly different means ($p < 0.05$) within each functional category as determined by Tukey's HSD test.

	Blue vane trap	Colored pan trap	Net collecting
Mean ITD	2.62 mm	1.84 mm	2.19 mm
Mean number of parasitic bees per collection event	0.4[a]	0.4[a]	0.2[b]
Mean proportion of parasitic bees per collection event	2.4%[a]	3.7%[a]	4.5%[a]
Mean number of oligolectic bees per collection event	0.3[a]	0.5[a]	0.4[a]
Mean proportion of oligolectic bees per collection event	1.9%[a]	3.2%[a]	6.0%[b]

Table 3.4 Regression output of the relationships between bee community metrics based on different collection methods and plant species richness (≤50m of sampling point) and extent of suitable surrounding habitat . Correlations significant at p=0.05 are in bold.

		Plant species richness	Amount of habitat 1000m	Amount of habitat 100m
All trap types combined	richness	**p<0.01** **R^2=0.30**	**p<0.01** **R^2=0.24**	**p<0.01** **R^2=0.31**
	diversity	**p<0.01** **R^2=0.25**	**p=0.01** **R^2=0.19**	**p<0.01** **R^2=0.29**
blue vane trap	richness	p=0.09 R^2=0.09	**p<0.01** **R^2=0.24**	**p<0.01** **R^2=0.34**
	diversity	p=0.12 R^2=0.07	p=0.11 R^2=0.08	**p=0.01** **R^2=0.19**
colored pan trap	richness	p=0.09 R^2=0.09	p=0.54 R^2=0.01	p=0.37 R^2=0.03
	diversity	p=0.12 R^2=0.07	p=0.36 R^2=0.03	p=0.18 R^2=0.06
net collecting	richness	**p=0.01** R^2**=0.21**	p=0.27 R^2=0.04	p=0.33 R^2=0.03
	diversity	**P=0.01** R^2**=0.21**	p=0.12 R^2=0.07	p=0.23 R^2=0.04

Table 3.5 On left, the number of individuals of each genus collected using colored pan traps, blue vane traps, and net collection. On right, the percentage of the total number of individuals collected by that trap type represented by each genus.

	Number of individuals collected			Percent of total trap capture represented by each genus		
	Colored pan trap	Blue vane trap	Net collection	Colored pan trap	Blue vane trap	Net collection
Agapostemon	165	1313	7	3.64%	17.11%	0.67%
Andrena	275	340	257	6.06%	4.43%	24.45%
Anthidium	0	4	0	-	0.05%	-
Anthophora	3	55	1	0.07%	0.72%	0.10%
Apis	7	52	46	0.15%	0.68%	4.38%
Atoposmia	1	0	0	0.02%	-	-
Bombus	59	641	55	1.30%	8.35%	5.23%
Ceratina	391	213	137	8.61%	2.78%	13.04%
Colletes	1	0	2	0.02%	-	0.19%
Diadasia	1	8	0	0.02%	0.10%	-
Dianthidium	1	9	1	0.02%	0.12%	0.10%
Epeolus	1	0	0	0.02%	-	-
Eucera	65	191	28	1.43%	2.49%	2.66%
Habropoda	0	0	1	-	-	0.10%
Halictus	1048	795	121	23.09%	10.36%	11.51%
Heriades	1	2	3	0.02%	0.03%	0.29%
Hoplitis	72	109	10	1.59%	1.42%	0.95%
Hylaeus	9	13	14	0.20%	0.17%	1.33%
Lasioglossum	1727	2976	202	38.05%	38.78%	19.22%
Megachile	31	126	21	0.68%	1.64%	2.00%
Melecta	1	3	1	0.02%	0.04%	0.10%
Melissodes	27	183	12	0.59%	2.38%	1.14%
Nomada	37	6	38	0.82%	0.08%	3.62%
Osmia	489	531	57	10.77%	6.92%	5.42%
Panurginus	56	74	24	1.23%	0.96%	2.28%
Perdita	1	0	2	0.02%	-	0.19%
Sphecodes	52	17	10	1.15%	0.22%	0.95%
Stelis	17	12	1	0.37%	0.16%	0.10%
Triepeolus	1	1	0	0.02%	0.01%	-

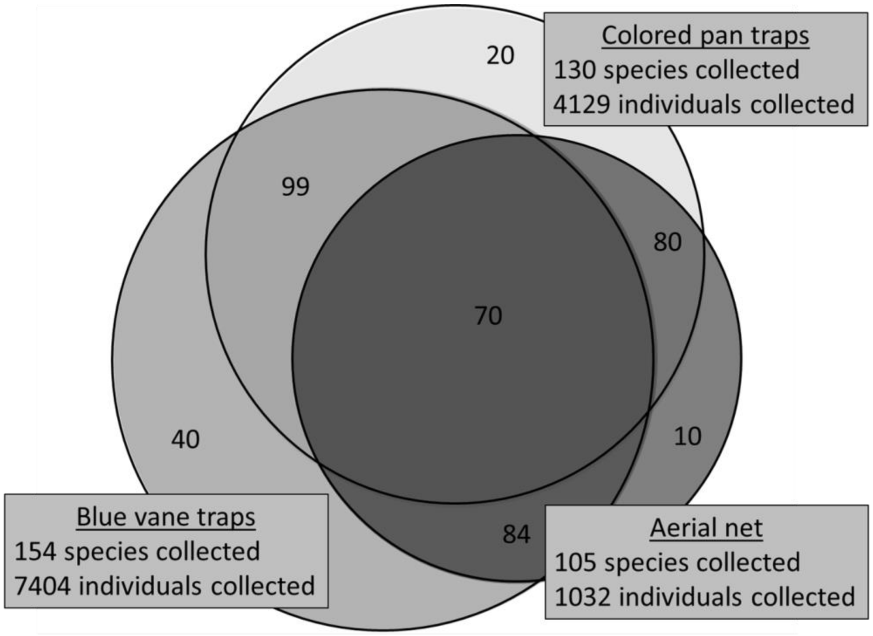

Figure 3.1 Venn diagram indicating the numbers of shared species, out of 179 total, captured by blue vane traps, colored pan traps, and aerial netting

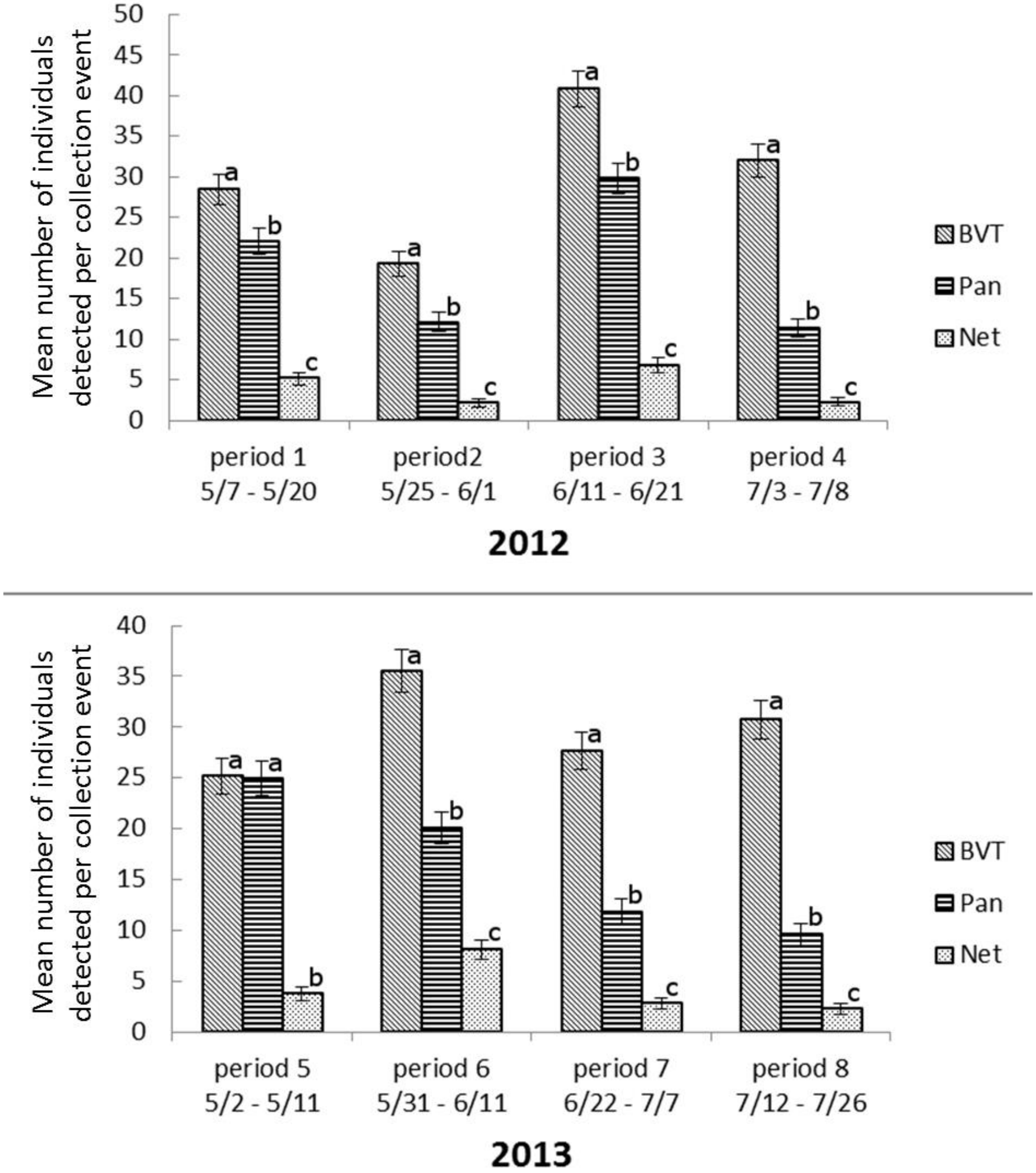

Figure 3.2 Mean abundance of wild bees from 3 collection methods per sampling period per site in 2012 and 2013. Colored pan traps and blue vane traps (BVT) were open for 24 hours and netting occurred for 10 minutes for each sampling period. All 32 sites were sampled once each period. Letters above bars denote significantly different means within each collection period. Bars are 95% confidence intervals

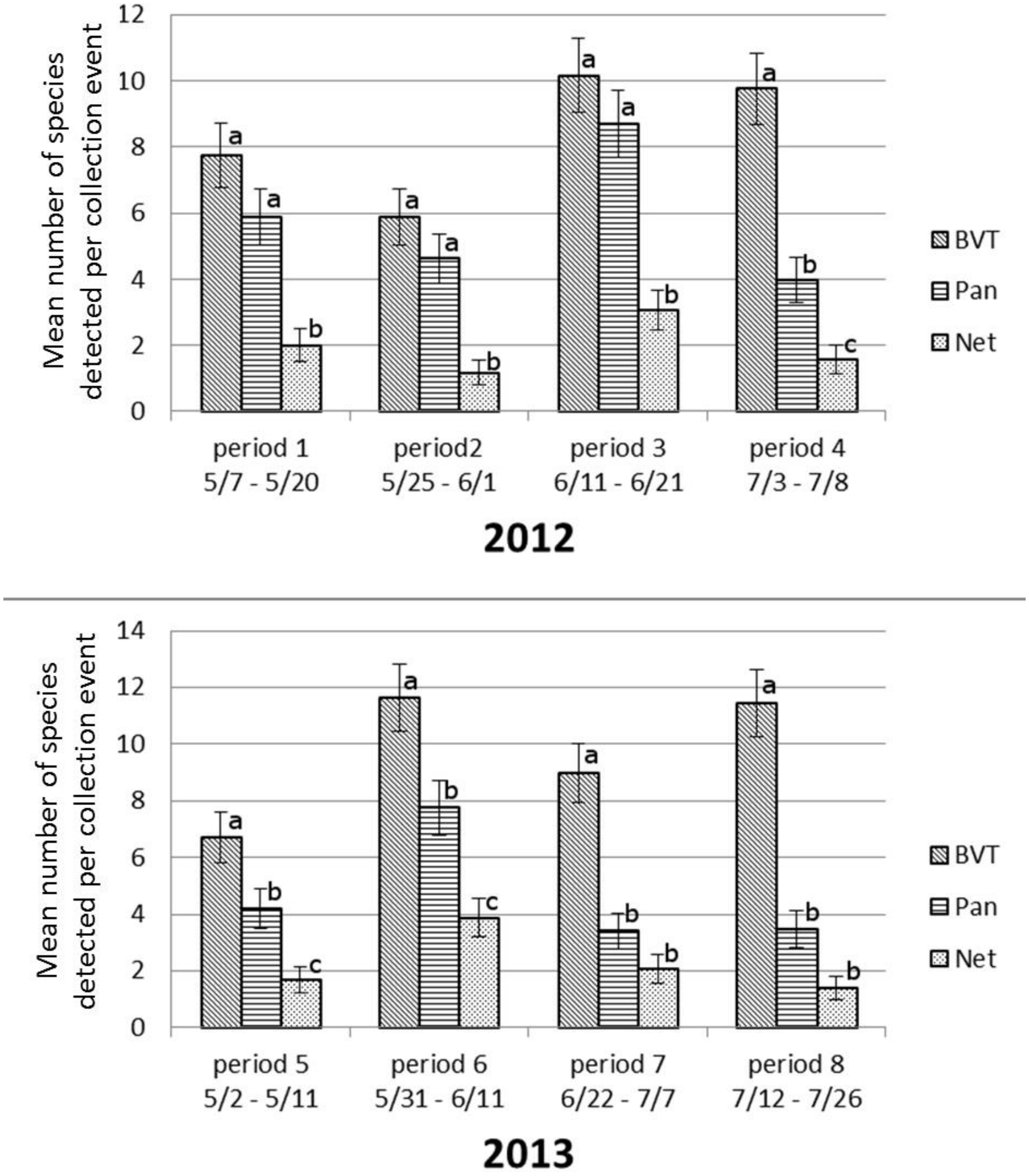

Figure 3.3 Mean bee species richness per collection event in 2012 and 2013. Colored pan traps and blue vane traps (BVT) were open for 24 hours and net collection occurred for 10 minutes for each sampling period. All 32 sites were sampled once each period. Bars are standard error. Different letters indicate significant differences in means within a collection period using Tukey's Honestly Significant Difference

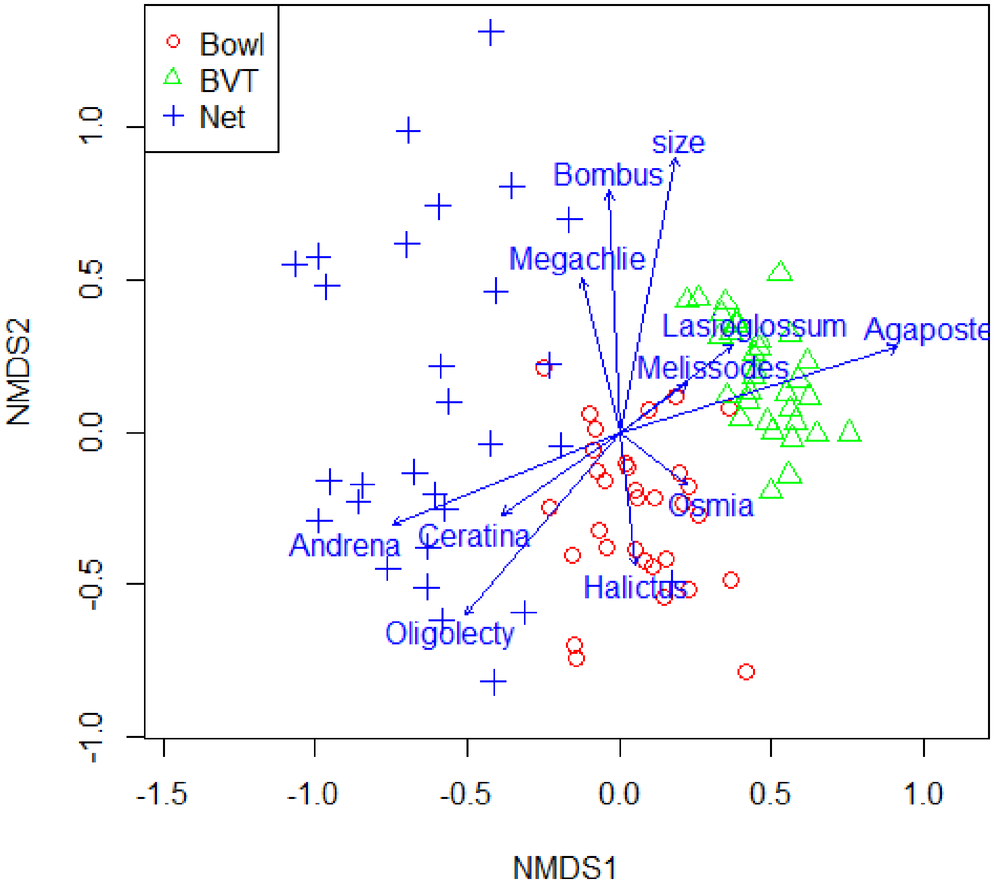

Figure 3.4 Ordination plot of wild bee communities associated with each collection method. Each point represents a single site and collection method. Direction of the vector indicates increasing generic abundances, average bee size, and proportion of oligolectic bees associated with each site/collection method combination. The length of the vector indicates the strength of the correlation.

CHAPTER 4: LANDSCAPE GENETICS OF *BOMBUS BIFARIUS* AT COARSE AND FINE SCALES IN THE INLAND PACIFIC NORTHWEST

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Abstract

Declines in abundance and range contractions of native bee species have been observed in North America and Europe. Changing land cover, along with pesticides and disease, is an important contributor to declines in *Bombus* species. Land cover influences native bee species by altering availability of forage and nesting resources. However, the influence of land cover on gene flow of native bee species is poorly studied, particularly at fine spatial scales. To examine the effects of land use and environmental features on gene flow of *Bombus bifarius* we used microsatellites to genotype individuals at 2 spatial scales. Coarse scale sampling occurred across the inland Pacific Northwest and fine scale sampling was centered on the Palouse agricultural region of northern Idaho and southeastern Washington State. We show human altered land use, primarily conversion of prairie habitats to agricultural land in the Palouse region, can reduce genetic diversity and gene flow of *B. bifarius*.

Introduction

Bee populations are crucial for native plant reproduction and agriculture worldwide (Klein *et al.* 2007; Ollerton, Winfree & Tarrant 2011). Many bee species are declining due to pressure from pesticides, pathogens and land use change (Potts *et al.* 2010; Goulson *et al.* 2015). Reductions in range and abundance for bee species in the genus *Bombus* are among the most well documented and prominent in the Apoidea (Colla & Packer 2008; Grixti *et al.*

2009; Winfree *et al.* 2009; Cameron *et al.* 2011). While possible causes of decline in *Bombus* are diverse including disease and reductions in available forage and nesting resources, genetic isolation, loss of diversity and reduced gene flow (Williams & Osborne 2009; Cameron *et al.* 2011) are likely contributing factors. Indeed, *Bombus* species decline and reduced genetic diversity are often linked (Cameron *et al.* 2011), and deleterious effects of reduced genetic diversity, including sterile male production, increased susceptibility to parasites and reduced colony size, have been observed in some cases (Baer & Schmid-Hempel 1999; Zayed, Roubik & Packer 2004; Herrmann *et al.* 2007). As part of conservation efforts, it is therefore important to document genetic diversity within and gene flow among populations of *Bombus* species and to maintain historical patterns and processes.

Characterizing patterns of genetic differentiation among populations can shed light on the patterns and processes of gene flow that mediate genetic diversity in populations. Traditional approaches examine correlations between pairwise genetic distance (e.g., F_{ST}) and pairwise geographic distance (Rousset 1997). A correlation between genetic and geographic distance (isolation by distance, IBD) suggests limited dispersal across the sampled area. A lack of isolation by distance could mean distant populations are well connected through dispersing individuals or intervening landscape features could be altering dispersal patterns. The field of landscape genetics uses characteristics of the landscape (elevation, precipitation, land cover, etc.) along with measures of genetic differentiation to infer the influence of these landscape variables on dispersal and gene flow (Manel *et al.* 2003). Isolation by resistance (IBR) models use characteristics of the landscape to produce spatially explicit hypotheses of resistance to dispersal (McRae 2006; Spear *et al.* 2010).

IBR models can describe patterns of genetic differentiation better than IBD models by predicting actual routes of dispersal among surveyed populations (Spear *et al.* 2010). Elucidating factors contributing to connectivity among populations can improve species conservation by providing a better understanding of habitat suitability and corridors of dispersal (Kool, Moilanen & Treml 2013; Boff *et al.* 2014; López-Uribe *et al.* 2015). Evaluating genetic structure at local and regional scales may shed light on patterns of bee movement and reveal barriers to bumble bee dispersal, such as environmentally inhospitable regions or intensive agriculture, as well as regions that facilitate gene flow such as areas of continuous habitat.

Bombus species can exhibit high gene flow even at continental scales (>1,000 km) (a range of D_{est} 0.020 – 0.118; G_{st} 0.003 – 0.035 for 6 North American *Bombus* species), even among species experiencing population decline (Cameron *et al.* 2011; Lozier *et al.* 2011). Nonetheless, gene flow at both large and small spatial extents can be restricted by sufficiently inhospitable landscape features, such as ocean or urban land (Darvill *et al.* 2010; Charman *et al.* 2010; Jha & Kremen 2013a; Boff *et al.* 2014; Jha 2015; López-Uribe *et al.* 2015). Landscapes with extensive cereal agriculture are relatively inhospitable to bees, with overall bee abundance and species richness negatively correlated with the amount of farmed land (Steffan-Dewenter 2003), so these landscapes may restrict dispersal and gene flow for some bee species. In an analysis of the genetic structure of five *Bombus* species in an extensive cereal-legume agricultural landscape, slight but significant isolation by distance was observed in two species at a distance of less than 10 km (Dreier *et al.* 2014). Yet, this distance is smaller than the documented foraging distance of some bumble bee workers, which can be as long as 11 km (Rao & Strange 2012). and may be smaller than the dispersal distance of a bumble bee queen, which could be as long as 200 km in certain cases (Lepais *et al.* 2010; Schmid-Hempel *et al.* 2014). Therefore, although the genetic structure observed by Dreier *et al.* (2014) was weak, it may be greater across longer distances in extensively farmed landscapes.

The widespread North American bumble bee *Bombus bifarius* (Cresson) is a good model organism to evaluate genetic structure because it exhibits regional genetic substructure and is sufficiently common to make capturing enough individuals for analysis possible (Lozier, Strange & Koch 2013). This bee is widespread in western North America, ranging from northern Mexico to Alaska and from the montane areas of Colorado to the Pacific Ocean (Koch, Strange & Williams 2012; Lozier *et al.* 2013). This species prefers wetter, upland habitat and is mostly restricted to forested areas within the study area (Figures 4.1 and 4.2), but the core Palouse Prairie, which is not forested, is an exception as it is wet enough to support this species (Lozier *et al.* 2013). Unlike many North American *Bombus* species studied to date, *B. bifarius* exhibits regional genetic structure (Cameron *et al.* 2011; Lozier *et al.* 2011). Cameron *et al.* (2011) and Lozier *et al.* (2011) examined genetic structure of *B. bifarius* across the western U.S., from Colorado west to the Sierra Nevada Mountains of California and north to the Canadian border using 8-11 nuclear DNA microsatellite loci. Both found evidence for three distinct genetic clusters. In a more recent examination of the coarse scale genetic structure of *B. bifarius*, Lozier *et al.* (2013) found evidence for an additional fourth genetic cluster and placed these four into a geographic context: one cluster in southern Wyoming, Colorado and Utah, one cluster on the islands of the Salish Sea off the coast of Washington State, one cluster stretching from the Sierra Nevada Mountains of California north to the Cascade Range in Oregon, and one centrally located, somewhat admixed cluster stretching from the Cascade Mountains of Washington east through northeastern Oregon, Idaho, Montana, northern Utah and into Wyoming. This study examines genetic diversity and structure in a portion of this centrally located cluster.

The purpose of this paper is to evaluate the genetic structure of *B. bifarius* at two spatial scales in the Pacific Northwest of the United States: 1. a local scale analysis in the Palouse, which is an intensely agricultural region characterized by high fragmentation and isolated patches of natural prairie land (Looney & Eigenbrode 2012) and 2. a regional scale analysis across an area where gradients of precipitation and habitat quality may influence dispersal and gene flow. We tested three hypotheses. 1. Genetic diversity is lower at sites with a lower proportion of suitable habitat in the surrounding area because the effective population size is reduced and isolation of populations in these regions is likely to be higher. 2. Gene flow is restricted across areas with a high proportion of human altered land use because dispersing bees may be reluctant to enter these areas, survivability may be low in these areas and low population size could reduce mating success. 3. Gene flow is restricted across areas of low precipitation (<35 cm annually) because these areas are poor *B. bifarius* habitat and individuals may have the same challenges dispersing, surviving and mating as in human altered habitat. We test these hypotheses by genotyping 571 individuals of *B. bifarius* at eight nuclear DNA microsatellite loci. Samples were gathered across the inland northwest with a focus on the Palouse region. Genetic diversity, quantified as homozygosity by locus and internal relatedness, was regressed with the amount of suitable habitat in the surrounding area to evaluate influence of habitat isolation on genetic diversity. Mantel tests were used to evaluate correlations between genetic distance among individuals of *B. bifarius* and geographic or resistance distance using either precipitation or land cover to parameterize resistance surfaces.

Methods

Sample collection and genotyping

We used an individual-based, rather than population-based, collection scheme in which the number of sampled individuals per location is decreased as the number of sample locations is increased (Prunier *et al.* 2013). We collected a mean of 4.2 individuals per location at 130 locations. This approach is better suited to the intensely agricultural study region than population based collecting because the small, degraded habitat patches that predominate usually do not support populations large enough for a population-based analysis. Additionally, individual-based sampling allows for more spatially continuous coverage with fewer unsampled populations between sampled sites and performs similarly to population-based methods when resistance models are used (Prunier *et al.* 2013).

Sampling occurred at two spatial scales: a fine scale centered on the Palouse region of northern Idaho and southeastern Washington State (Figures 4.1 and 4.2), and a coarse scale across the inland Pacific Northwest. The Palouse region was a continuous bunchgrass prairie (Palouse Prairie) until Euro-American settlement in the late 19th century, after which rapid conversion to agriculture occurred (Black *et al.* 1998). Approximately 1% of the original Palouse Prairie remains today (Black *et al.* 1998), confined to small fragments (most less than 2 ha) with high perimeter-to-area ratios (Looney & Eigenbrode 2012). Fine-scale sampling site density was relatively high with an average distance between adjacent sites of 1 km. Sampling occurred in agricultural fields and fragments of prairie in the

Palouse region as well as in the surrounding forested hills (Figure 4.1). Bees were collected from 40 sites within the agricultural region and 61 sites in the surrounding forest in 2011, 2012 and 2013. Bees were collected using blue vane traps (Stephen & Rao 2005), colored pan traps (Kirk 1984), and an aerial net. For each sample, blue vane traps and colored pan traps were left out for 24 hours. An aerial net was used to collect bees off of flowers for five minutes at the time of trap placement and removal. Bees collected using blue vane traps or colored pan traps were placed in Whirl-Pak® bags (Nasco, Fort Atkinson, Wisconsin) and covered with ethanol for storage. Bees collected using an aerial net were placed on ice and then pinned, dried and identified before DNA extraction.

At the course spatial scale, gene flow and genetic diversity were examined across the inland Pacific Northwest extending for 500 km from the northern Cascade region to the forested area southeast of the Palouse Prairie (Figure 4.2). Collection was confined to the forested hills surrounding central Washington State, as the arid region in central Washington State is not preferred habitat for this species (Lozier *et al.* 2013). Bees were collected at 40 sites in 2013. Site density was low with an average distance between adjacent sizes of 25km. Only blue vane traps were used for collection at this scale. Traps were left in place for 24 hours. Collected bees were anesthetized by chilling on ice and the right mid basitarsus was removed and placed in ethanol after which bees were released. At both fine and course scales very few males were observed and only females were collected.

DNA was extracted from either flight muscles in the thorax or the right mid basitarsus of collected bees using a Qiagen DNeasy tissue kit (Qiagen, USA) with one modification to the standard protocol: tissue was allowed to incubate in the ATL buffer for at least 4 hours, often overnight. Eight microsatellite loci were used for genotyping: BL13, BTERN01, B124, BT10, BT30, B96, BL11, BT28 (Estoup *et al.* 1995, 1996; Reber Funk, Schmid-Hempel & Schmid-Hempel 2006). Relative concentrations of primers were varied and optimized to produce a single multiplex reaction using all eight primer pairs. A 7ul PCR reaction consisted of 3.31 ul of dH_20, 3.5ul of Qiagen multiplex PCR master mix, 0.7 ul Q-solution (Qiagen, USA) and final primer concentrations of: B96 – 0.114 uM, B124 – 0.050 uM, BT10 – 0.029 uM, BT28 – 0.039 uM, BT30 – 0.057 uM, BTERN01 – 0.039 uM, BL11 – 0.033 uM, BL13 – 0.057 uM. The PCR conditions began with a primary denaturing step of 95°c for 15m followed by a touch down program consisting of a denaturing step of 94°c for 30s, an annealing step of 60°c for 30s, and an extension step of 72°c for 1m. The annealing step was reduced by 0.5°c each cycle for 10 cycles, arriving at final cycling conditions of 94°c for 30s, 55°c for 30s, and 72°c for 1 min which were repeated for 25 cycles for 35 total cycles of amplification. The protocol finished with a final cooldown of 4°c for 1m. One primer from each pair was labeled with fluorescent dye: 6-FAM, NED, VIC, or PET. Electrophoresis and genotyping were performed using ABI 3730xl capillary DNA sequencers (Applied Biosystems, Carlsbad, CA, USA). Alleles were scored manually using GeneMapper (Applied Biosystems). Fifteen percent of samples were re-run to estimate genotyping error rates.

Analysis

Relatedness

Although we ultimately collected few siblings, a large bumble bee colony can flood an area with many full siblings with an expected relatedness of 0.75. To avoid bias caused by sibship, the program COLONY 2.0 (Jones & Wang 2010) was used to identify siblings. The genotyping error rate was set to 0.001 as determined by replicate genotyping of random individuals. One individual from any group of siblings was retained, the rest were removed from analysis.

Population genetic structure

Although we used an individual-based collection scheme, we grouped bees into 9 putative populations that were generally defined based on natural potential barriers such as arid canyons, the Columbia River, or urban or developed areas that form poor habitat (Figure 4.2). These putative populations were used to evaluate Hardy-Weinberg equilibrium, expected heterozygosity, and to measure genetic differentiation using G'_{ST} and D. The spatial extent of the 9 putative populations varied from 30km (in the Mt Hood region) to 230 km (in the Umatilla National Forest). While it is not clear if the spatial extent from which each population was drawn is consistent with single panmictic populations in other studies, very low genetic differentiation is seen between populations separated by a much larger distance with greater intervening environmental heterogeneity (Lozier *et al.* 2011, 2013). Hardy-Weinberg equilibrium analyses and Nei's expected heterozygosity were calculated within these populations and pairwise values for G'_{ST} among these populations were calculated using GenAlEx (Peakall & Smouse 2006). Linkage disequilibrium was calculated using Genepop version 4.2 (Raymond & Rousset 1995; Rousset 2008). HP-RARE (Kalinowski 2005) was used to estimate allelic richness and private allelic richness rarefied to the smallest population.

Bayesian cluster analysis

We used Bayesian clustering programs GENELAND and STRUCTURE (Pritchard, Stephens & Donnelly 2000; Guillot, Mortier & Estoup 2005) to search for potential discrete populations. Preliminary analysis using the program STRUCTURE showed 21 individuals strongly separated from the rest. Subsequent analysis using the program GENELAND 4.0.0, implemented in R (R core development team 2014) showed no spatial clustering of these individuals. It was determined that these 21 individuals were not *B. bifarius* and were misidentified in the field, thus these were removed from all analyses. Misidentified specimens are likely to be *B. huntii*, which has similar coloration and habitat preference, but this was not confirmed genetically. Following removal, structure was run with admixture and 100,000 burn-in steps with 100,000 iterations and 10 replications for each k, 1-9. Individuals were arranged based on geographic proximity to aid in detecting subtle clustering this admixed region. GENELAND differs from STRUCTURE by using spatial location as a prior when estimating the optimal number of population clusters. We ran 5 replicates of 100,000 iterations (thinning = 100) with a random adjustment of spatial coordinates of 0.001 decimal degrees. The number of possible clusters ranged from one to nine.

Resistance modeling

We used resistance surfaces with the program Circuitscape (McRae 2006) to evaluate the influence of landscape features on genetic structure (Spear *et al.* 2010). Our general hypothesis is that suitable habitat facilitates dispersal and gene flow. We approached spatial quantification of *B. bifarius* habitat in two ways: by using precipitation as a proxy for suitable habitat and by using land cover classification data to delineate suitable habitat. Both methods of quantifying resistance to geneflow were applied to local and regional scale analysis.

Resistance surfaces based on precipitation

Precipitation is a convenient and appropriate way to quantify potential landscape resistance to gene flow for *B. bifarius* because precipitation maps are available throughout its range (Hijmans *et al.* 2005) and because precipitation was the most important component of a Maxent analysis of habitat suitability for *B. bifarius* (Lozier *et al.* 2013). It is unlikely habitat suitability scales linearly with precipitation, thus we reclassified a map of annual precipitation data into areas of suitable habitat (>50 cm of annual precipitation), moderately suitable habitat (35-50cm annual precipitation) and unsuitable habitat (<35cm annual precipitation). These classifications were made by observing the habitat quality and *B. bifarius* abundance on the ground during collection as well as using habitat suitability maps developed by Lozier *et al.* (2013). Worldclim precipitation data (Hijmans *et al.* 2005) was downloaded and reclassified with the above criteria. Cell size for this layer is 10 arc seconds (~300m). Values for resistance to gene flow were set to 0.1 for suitable habitat, 0.25 for moderately suitable habitat and 0.9 for unsuitable habitat. These data were used to evaluate gene flow at both a local and regional scale.

Resistance surfaces based on proportion of suitable habitat

Defining suitable habitat based on observed land cover characteristics may be superior to precipitation as measure of resistance to gene flow because precipitation is an imprecise proxy for bee habitat. For example, dense, mature conifer forest predominates under higher precipitation, but generally supports lower bee abundance and species richness compared to more open habitat or earlier successional stages (Taki, Kevan & Ascher 2007; Sugiura *et al.* 2008). Additionally areas with suitable precipitation may be converted to agriculture or urban use. Therefore, National Land Cover Dataset classifications representing percent canopy cover and land cover change between 2001 and 2011 (Coulston *et al.* 2013; Jin *et al.* 2013) were used to produce alternate resistance surfaces representing suitable habitat in the inland northwest region. First, percent tree canopy cover was used to identify regions of suitable *B. bifarius* habitat, defined as between 15% and 65% canopy cover. This excluded areas too arid or shaded to support abundant forage for *B. bifarius*. Second, early successional stages, also suitable *B. bifarius* habitat, were identified as any areas that changed from evergreen or mixed forest to shrubland, barren land, or grassland between 2001 and 2011, indicating recent logging. Definitions for suitable habitat were based on *B. bifarius* abundance during collection. This produced a map of *B. bifarius* habitat in the region with a 30m cell size. Useful habitat was given a value of 1 and all other land was given a value of 0.1. This layer was coarsened to a 300m pixel size by finding the mean of all cells falling within the new larger cell. These values were used to represent resistance to gene flow in Circuitscape.

Resistance surfaces representing *B. bifarius* habitat in the Palouse region were made by combining the layer for suitable habitat based on canopy cover used for regional scale analysis with Palouse Prairie fragment maps because Palouse Prairie serves as suitable that would not be listed as such using canopy cover data alone. High resolution habitat maps exist for the Palouse Prairie from previous work (Looney & Eigenbrode 2012) as well as unpublished data produced by the Palouse Conservation District. These maps delineate patches of natural land set into the agricultural matrix of the Palouse region. The resulting combined map is a comprehensive representation of potential *B. bifarius* habitat in the Palouse region. Suitable *B. bifarius* habitat was given a value of 1 and unsuitable habitat a value of 0.1. This layer was coarsened to a 150m pixel size by finding the mean of all cells falling within the new larger cell and then reclassified into ten categories representing the percentage of habitat within each pixel. The resulting layer is shown in Figure 4.1. These values were used to represent resistance to gene flow in Circuitscape with pixels with less habitat being more resistant to gene flow.

Pairwise ecological distances were calculated using the program Circuitscape (McRae 2006) using maps of landscape resistance for both the Palouse region for the inland Pacific Northwest(McRae 2006). Individual genetic distance was calculated using GenAlEx (Smouse & Peakall 1999; Peakall & Smouse 2006). A pairwise geographic distance matrix was generated using the Geographic Distance Matrix Generator with the WGS84 spheroid (Ersts 2011). Mantel and partial mantel tests were used in the R package Vegan (Oksanen *et al.* 2015) to evaluate associations between genetic distance and geographic or resistance distance.

Influence of landscape context on genetic diversity

Landscape context for each collection point was quantified using the map of suitable habitat for the Palouse region generated as described previously. The proportion of suitable habitat around each collection point was determined by recalculating each raster value to be the average of all raster values within 4 distances (1 km, 2 km, 5 km, and 10 km) using the Focal Statistics tool. Raster values were then extracted to collection points. Individual heterozygosity was quantified as internal relatedness (IR) (Amos *et al.* 2001) and homozygosity by locus (HL) (Aparicio, Ortego & Cordero 2006) using Excel macros provided by the authors. The relationship between the proportion of usable habitat at each distance and the 2 individual heterozygosity metrics was analyzed for each individual bee using linear regression.

Results

In total, 571 individuals were collected at 130 sites across the entire sampling area. Of these, 21 were determined to be an unknown alternate species, 7 were discarded due to sibship, and 9 failed to amplify. This leaves reliable genotypes for 534 individuals for the entire sampling area; or 435 individuals at 101 sites in the Palouse region and 99 individuals at 29 sites throughout the rest of the sampling area. Most populations deviated from Hardy-Weinberg equilibrium at one or two loci, however the Palouse population taken as a whole deviated at five loci (Table 4.1a). When divided into three populations based on year of collection, the Palouse deviated at four loci in one year and two loci each of the other two years (Table 4.1b). No loci were out of Hardy-Weinberg equilibrium in >38% of tests and no pair of loci were in linkage disequilibrium across more than 2 populations, so all loci were retained.

Population genetic structure

Genetic diversity was high in all sampled populations. Average unbiased expected heterozygosity ranged from 0.78 to 0.81 with a mean of 0.78 (Table 4.2). Rarefied allelic richness averaged for all loci ranged from 6.99 to 7.88 among different putative populations with an overall mean of 7.17 (Table 4.2). The rarefied number of private alleles averaged over all loci ranged from 0.29 to 0.48. Using 2 indices, genetic differentiation among the 9 putative populations was extremely low, (G'_{ST} = -0.001 and D_{est} = -0.003), and pairwise values were not significantly different from zero among the 9 putative populations (Tables 4.3a and 4.3b). Likewise, Bayesian cluster analysis found no structure across the study area. Both GENELAND and STRUCTURE suggest a single population (Figure 4.3).

Mantel tests of genetic distance and resistance distance or geographic distance

Mantel tests detected no association between genetic distance and either geographic or resistance distance for the coarse scale inland Pacific Northwest region (Table 4.5). At a finer spatial scale, in the Palouse region, there was also no detectable association between genetic distance and geographic distance. There was, however, a significant association between resistance distance based on suitable habitat and genetic distance in the Palouse region (Table 4.5, Figure 4.1).

Landscape influence on genetic diversity

Regression analysis shows a weak but significant relationship between homozygosity by locus or internal relatedness and the amount of usable habitat at all evaluated distances from the collection site. Although differences in r^2 values among spatial scales between 1 km and 20 km are slight, the proportion of natural land within 10 km of the collection site has the highest correlation with genetic diversity using HL and IR (Table 4.4).

Discussion

Bee populations worldwide are under pressure from a variety of stressors, one of which is genetic isolation and inbreeding. Constraints to geneflow are still unclear. The influence of agriculture, which is increasing in intensity throughout the world, has not been fully evaluated. This study examined genetic structure of *B. bifarius* at a fine spatial scale in the predominantly agricultural Palouse region, and at a courser spatial scale across the inland Pacific Northwest. We find evidence that intensive agriculture inhibits dispersal and gene flow at the finer spatial scale in the Palouse region. Both individual genetic differentiation and measures of genetic diversity were influenced by agriculture in that region. No genetic structure was detected at the courser spatial scale despite gradients of precipitation, making portions of the study area less suitable habitat. This suggests that human land use and not precipitation gradients are the important dispersal barriers to *B. bifarius* gene flow and human land use can induce genetic structure at fine spatial scales.

High gene flow throughout the inland northwest

Low levels of genetic structure and high connectivity is the norm for North American *Bombus* species with stable populations (Cameron *et al.* 2011; Lozier *et al.* 2011). Our STRUCTURE and GENELAND results are similar to other bumble bee population genetic studies where low genetic differentiation and high levels of co-ancestry are typical (Cameron *et al.* 2011; Lozier *et al.* 2011; Jha 2015). However, our values of genetic differentiation were low even by these standards and far lower than other results for *B. bifarius*, which showed higher levels of differentiation than other *Bombus* species with stable populations examined over a similar spatial scale (Cameron et al. 2011, Lozier et al. 2011). Values for genetic differentiation over the entire range for *B. bifarius* in the contiguous U.S. excluding parts of California are $F_{ST} = 0.026$, $D_{est} = 0.14$ (Cameron *et al.* 2011) and $D_{est} = 0.114$, $G_{ST} = 0.022$ (Lozier *et al.* 2011) when examined at eight or nine microsatellite loci respectively. *Bombus bifarius* and *B. occidentalis* are the only *Bombus* species in North America studied to date exhibiting even weak population subdivisions (Lozier 2011). However, our analysis fell wholly within a single population cluster and so did not include much of the genetic variation examined by Lozier *et al.* (2011) and Cameron *et al.* (2011). Additionally, our individual-based sampling scheme is poorly suited to population-based analysis. While we tried to separate populations using possible barriers to dispersal (e.g. the Columbia River separating population 7 from population 8 or interstate 90 separating population 8 from population 9 [Figure 4.2]), this wasn't always possible (e.g. between populations 2 and 3 [Figure 4.2]). Also, the bulk of our collecting effort was within the southeastern corner of the sampling area (Figure 4.2), 435 of the 534 total collected individuals fell within the area displayed in Figure 1 and the size of sampled populations outside of this region were often quite small (Table 4.2). Finally, the number of examined loci was fewer than other similar studies (Cameron *et al.* 2011; Lozier *et al.* 2011; Jha 2015).

No correlations were found between individual genetic distance and either geographic or resistance distance when examining the entire inland Pacific Northwest (Table 4.5). The lack of any such associations across the region may indicate the arid agricultural region in central Washington is better *B. bifarius* habitat than we suspected. Certain combinations of sequentially flowering crops can support abundant and diverse bumble bee communities (Rao & Stephen 2010). Although it is possible the more varied agriculture in central Washington provides more useful habitat than other agricultural settings, this conclusion is not concordant with either the habitat suitability model created by (Lozier *et al.* 2013) or with studies of bumble bee gene flow across agricultural regions, including regions of California which also have varied agriculture (Dreier *et al.* 2014; Jha 2015). It may be more likely that lack of detected genetic structure using individual based analysis across the Pacific Northwest is caused by some of the same issues contributing to extremely low population based genetic differentiation, including relatively few individuals in the west and north sides of the study area and few examined loci as well as very low actual genetic differentiation. When using individual based sampling schemes, detectability of genetic structure varies by the number of individuals sampled per location, the number of locations and the dispersal distance of the organism (Prunier *et al.* 2013). The number of locations and individuals we sampled (128 locations; mean of 4.2 individuals per location) should be sufficient to detect genetic structure when maximum dispersal distances are moderate (defined by Prunier *et al.* (2013) as 20% of maximum distance across the study area). However, the detectability of genetic structure using the samples we obtained becomes unreliable at greater maximum dispersal distances (Prunier *et al.* 2013). Because typical dispersal for reproductives of *B. bifarius* is unknown and estimates for other *Bombus* species vary widely (Lepais *et al.* 2010; Schmid-Hempel *et al.* 2014), it is unknown if genetic structure does not exist or if we were unable to detect it with the samples we obtained. There is, however, no substantial barrier to gene flow among sampled regions. Both population and individual based analyses corroborate data from Lozier *et al.* (2011) and Cameron *et al.* (2011) who determined this region to be a single genetic cluster when examined with the program STRUCTURE.

Patterns of genetic differentiation in the Palouse region

No isolation by distance was observed among individuals in the Palouse region, however, correlations were found between individual genetic distance and resistance distance derived from land cover classification maps (Table 4.5, Figure 4.1) indicating intensive agriculture serves as a weak barrier to *B. bifarius* dispersal and gene flow. Data on decreased gene flow through areas of intensive agriculture is corroborated by an inverse correlation between individual genetic diversity and proportion of suitable habitat in the surrounding 1-20 km (Table 4.4) which may lead to diversity related issues for bee species in regions of intensive agriculture, such as sterile male production, increased susceptibility to parasites and reduced colony size (Baer & Schmid-Hempel 1999; Zayed *et al.* 2004; Herrmann *et al.* 2007). Human altered land use inhibits dispersal and gene flow in *B. bifarius* in the Palouse Prairie but effects are weak. In both Mantel tests (Table 4.5) and linear regressions (Table 4.4), the amount of variation explained by the resistance surface or by the amount of suitable habitat is very low. It can be concluded that substantial gene flow still occurs within and through the highly agricultural Palouse region. Across a 1,000 km region in California, cropland and impervious cover (urban land) together induced genetic structure in *B. vosnesenskii* (Jha 2015). Our results indicate intensive agriculture can induce genetic structure on a much more fine spatial scale.

Reductions in gene flow across regions of inhospitable land cover such as open water or urban areas has been observed for *Bombus* species before (Goulson *et al.* 2011; Jha & Kremen 2013a; Lozier *et al.* 2013; Jha 2015). While as little as ten km of open water induced significant structure in populations of *B. muscorum* on Scottish islands (Darvill *et al.* 2006), reductions in gene flow in the Palouse region are probably not caused by an inability of dispersing reproductives to reach habitat fragments within the region of intensive agriculture. A survey of about 1000 prairie remnants in the central Palouse Prairie found the majority of remnants are within 150m of another remnant, and the most isolated remnant is 2,250m from its nearest neighbor (Looney & Eigenbrode 2012). Estimates for *Bombus* queen dispersal range from 3 km (Lepais *et al.* 2010) using a method unable to detect rare long-distance dispersal events, to 200 km inferred from tracing the colonization extent of an exotic bumble bee (Schmid-Hempel *et al.* 2014). While queen dispersal distance varies by species (Darvill *et al.* 2010), all Palouse Prairie remnants should be accessible even by low estimates. Indeed, by some estimates of queen dispersal, large portions of the Palouse agricultural region could be traversed by dispersing queens. Wide variation in estimates of dispersal distance for bumble bees mirror variations in estimates of foraging distance which range from several hundred meters (Osborne *et al.* 1999; Knight *et al.* 2005; Wolf & Moritz 2008) to 11.6 km (Rao & Strange 2012) for females. This may be due in part to species differences, but foraging range is highly plastic and dependent on habitat attributes (Jha & Kremen 2013b). Dispersal distance of *Bombus* reproductives may be similarly plastic and vary with landscape characteristics.

So, while habitat remnants within the Palouse agricultural matrix are likely accessible there does seem to be some barrier to immigration. It is unclear if this is caused by reluctance of *B. bifarius* individuals to disperse across regions of intensive agriculture or a lack of survival among immigrating individuals. Intensive agricultural land can reduce nest survival relative to other land (Goulson *et al.* 2010) and loss of nesting and foraging habitat is a potential cause of pollinator decline in agricultural landscapes (Williams *et al.* 2010). Therefore, in addition to the difficulties of finding suitable habitat, a significant proportion of individuals emigrating from continuous habitat on the periphery of the Palouse agricultural region may not survive to reproduce.

Conclusion

This research shows that even modern intensive agriculture with little unfarmed land can allow bumble bee persistence and gene flow. At least in this species genetic isolation, loss of genetic diversity and consequent issues are not significant concerns as long as some habitat is conserved. While large amounts of gene flow may be occurring among populations of *B. bifarius,* this species is a large and abundant bee. Among all bees detected in Palouse Prairie fragments *B. bifarius* was in the 85th percentile in terms of abundance and the 93rd percentile in terms of size (Rhoades *et al.* in review; Rhoades unpublished data). Since gene flow depends on not only dispersal but on successful reproduction, large and abundant species will not only be able to cross long stretches of inhospitable habitat but are more likely to find breeding partners at these locations (Slatkin 1987; Greenleaf *et al.* 2007). Dispersal distance and consequent susceptibility to habitat fragmentation varies by species (Darvill *et al.* 2010), and species that are smaller and/or less abundant may have more difficulty traversing the matrix between habitat fragments. Additionally, a smaller effective population can lead to reduced genetic diversity (Frankham 1996). Given that the majority of bee species in the Palouse region are smaller and less abundant than *B. Bifarius,* it is likely that the modest genetic structure detected for this species represents a lower limit for bees in the Palouse region. Although observed reductions in genetic diversity for *B. bifarius* are slight and likely aren’t associated with deleterious effects of reduced genetic diversity in bees such as sterile male production, reduced colony size or increased susceptibility to parasites (Baer & Schmid-Hempel 1999; Zayed *et al.* 2004; Herrmann *et al.* 2007), other smaller and more rare species may have undetected problems of this nature.

This work corroborates other data suggesting land conversion decreases gene flow of *Bombus* species in intensive agricultural systems (Dreier *et al.* 2014; Jha 2015). We found increasing the proportion of land cover composed of suitable habitat increased gene flow across intensive agricultural areas. Increasing the proportion of suitable habitat in an area has been found to increase species richness of bee communities in the area as well as increase pollination service to adjacent crops (Kleijn & Van Langevelde 2006; Lonsdorf *et al.* 2009). These data provide another reason to encourage retention of some non-farmed area in agricultural systems, particularly as other wild bee species will likely encounter greater barriers to gene flow across similar habitat.

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Table 4.1a P-values of chi-square tests for departure from Hardy-Weinberg equilibrium by population. Bold entries indicate significant departure from Hardy-Weinberg equilibrium at p=0.05.

Loci	Pop 1	Pop 2	Pop 3	Pop 4	Pop 5	Pop 6	Pop 7	Pop 8	Pop 9
BL13	**0.048**	0.366	0.700	0.726	0.256	0.992	0.090	0.532	0.495
B124	**0.000**	0.773	0.288	0.289	0.215	**0.049**	0.731	0.707	0.922
BTERN01	**0.001**	0.960	0.829	0.477	0.652	0.764	0.751	0.834	0.643
BT10	0.979	0.971	0.322	**0.018**	0.475	**0.002**	**0.019**	0.232	0.512
BT30	0.998	0.498	0.124	0.925	0.999	0.186	**0.019**	**0.001**	0.166
B96	0.124	0.176	0.842	0.439	0.203	0.795	0.562	0.593	0.492
BL11	**0.000**	0.471	0.544	0.502	0.700	0.636	0.876	0.390	0.265
BT28	**0.000**	0.933	**0.000**	0.965	0.999	0.904	0.960	0.955	0.948

Table 4.1b P-values of chi-square tests for departure from Hardy-Weinberg equilibrium for the Palouse population split by collection year. Bold entries indicate significant departure from Hardy-Weinberg equilibrium at p=0.05.

Loci	Pop1-2012	Pop1-2013	Pop1-2014
BL13	**0.000**	0.059	0.789
B124	**0.000**	0.856	0.983
BTERN01	0.086	0.038	**0.025**
BT10	0.994	0.923	0.898
BT30	0.392	1.000	**0.039**
B96	0.407	0.054	0.212
BL11	**0.000**	0.727	0.295
BT28	**0.000**	**0.045**	0.642

Table 4.2 Number of individuals in each putative population (*n*), Nei's unbiased expected heterozygosity (uH_e), allelic richness rarefied to 8 individuals (AR), private allelic richness per locus rarefied to 8 individuals (PAR).

	n	uH_e	AR	PAR
Pop1	128	0.78	6.99	0.33
Pop2	59	0.79	7.04	0.27
Pop3	87	0.78	7.12	0.38
Pop4	11	0.78	7.33	0.28
Pop5	28	0.77	7.12	0.30
Pop6	162	0.79	6.99	0.31
Pop7	17	0.78	6.73	0.29
Pop8	31	0.80	7.39	0.44
Pop9	8	0.81	7.88	0.48

Table 4.3a G’st among all putative populations. G'_{ST} values are below the diagonal, probability, P (rand >= data) based on 999 permutations, is shown above diagonal.

G'_{ST}	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9
Pop1	-	0.439	0.707	0.497	0.900	0.862	0.797	0.934	0.579
Pop2	0.000	-	0.504	0.509	0.778	0.831	0.622	0.445	0.614
Pop3	-0.001	0.000	-	0.474	0.856	0.388	0.312	0.472	0.675
Pop4	-0.001	-0.001	0.000	-	0.340	0.301	0.357	0.540	0.401
Pop5	-0.003	-0.002	-0.003	0.002	-	0.887	0.466	0.609	0.741
Pop6	-0.001	-0.001	0.000	0.003	-0.002	-	0.526	0.724	0.535
Pop7	-0.003	-0.002	0.001	0.002	0.000	-0.001	-	0.331	0.255
Pop8	-0.003	0.000	0.000	-0.001	-0.001	-0.001	0.001	-	0.810
Pop9	-0.002	-0.002	-0.003	0.001	-0.005	-0.001	0.005	-0.005	-

Table 4.3b D_{est} among all putative populations. D_{est} values are below the diagonal, probability, P (rand >= data) based on 999 permutations, is shown above diagonal.

D_{est}	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9
Pop1	-	0.411	0.698	0.508	0.888	0.863	0.778	0.938	0.616
Pop2	0.001	-	0.507	0.471	0.846	0.798	0.635	0.425	0.624
Pop3	-0.002	0.000	-	0.465	0.911	0.377	0.311	0.440	0.666
Pop4	-0.003	-0.002	-0.001	-	0.430	0.275	0.312	0.557	0.441
Pop5	-0.009	-0.011	-0.011	0.005	-	0.911	0.467	0.661	0.772
Pop6	-0.003	-0.004	0.001	0.008	-0.010	-	0.546	0.754	0.518
Pop7	-0.010	-0.006	0.005	0.009	0.000	-0.003	-	0.358	0.252
Pop8	-0.010	0.001	0.000	-0.005	-0.006	-0.005	0.005	-	0.763
Pop9	-0.007	-0.009	-0.012	0.006	-0.022	-0.004	0.020	-0.021	-

Table 4.4 Products of regression analysis between homozygosity by locus (HL) or internal relatedness (IR) and the proportion of bumblebee habitat within 1 km, 2 km, 5 km, 10 km, or 20 km.

	1 km	2 km	5 km	10 km	20 km
HL	$p=0.046$ $r^2=0.0098$	$p=0.026$ $r^2=0.0122$	$p=0.027$ $r^2=0.0121$	$p=0.021$ $r^2=0.0131$	$p=0.045$ $r^2=0.0098$
IR	$p=0.042$ $r^2=0.0102$	$p=0.026$ $r^2=0.0121$	$p=0.030$ $r^2=0.0115$	$p=0.020$ $r^2=0.0132$	$p=0.045$ $r^2=0.0099$

Table 4.5 Results of Mantel and partial Mantel tests of the relationship between genetic and environmental resistance or geographic distance. For partial Mantel tests the variable being partialed follows the + symbol. Entries in bold are significant at p=0.05

	Resistance model	Mantel r	Significance
Palouse region	distance	0.007	0.374
	precipitation	-0.017	0.703
	suitable habitat	0.060	**0.032**
	precipitation + distance	0.016	0.23
	suitable habitat + distance	0.059	**0.041**
Northwest region	distance	0.001	0.501
	precipitation	-0.011	0.641
	canopy	0.012	0.303
	precipitation + distance	-0.024	0.809
	canopy cover + distance	0.012	0.349

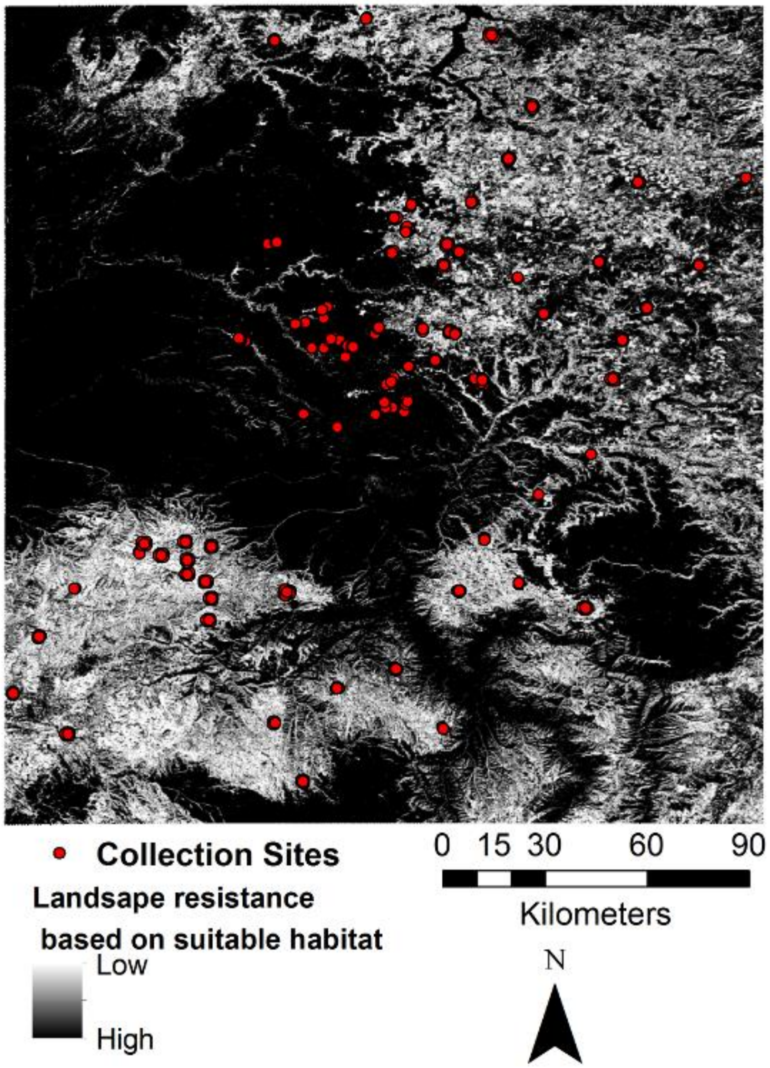

Figure 4.1 Collection sites in the Palouse agricultural region. Shading denotes hypothesized landscape resistance produced using proportion of suitable habitat with darker colors representing areas with less suitable habitat therefore hypothesized to be more resistant to *B. bifarius* dispersal and gene flow.

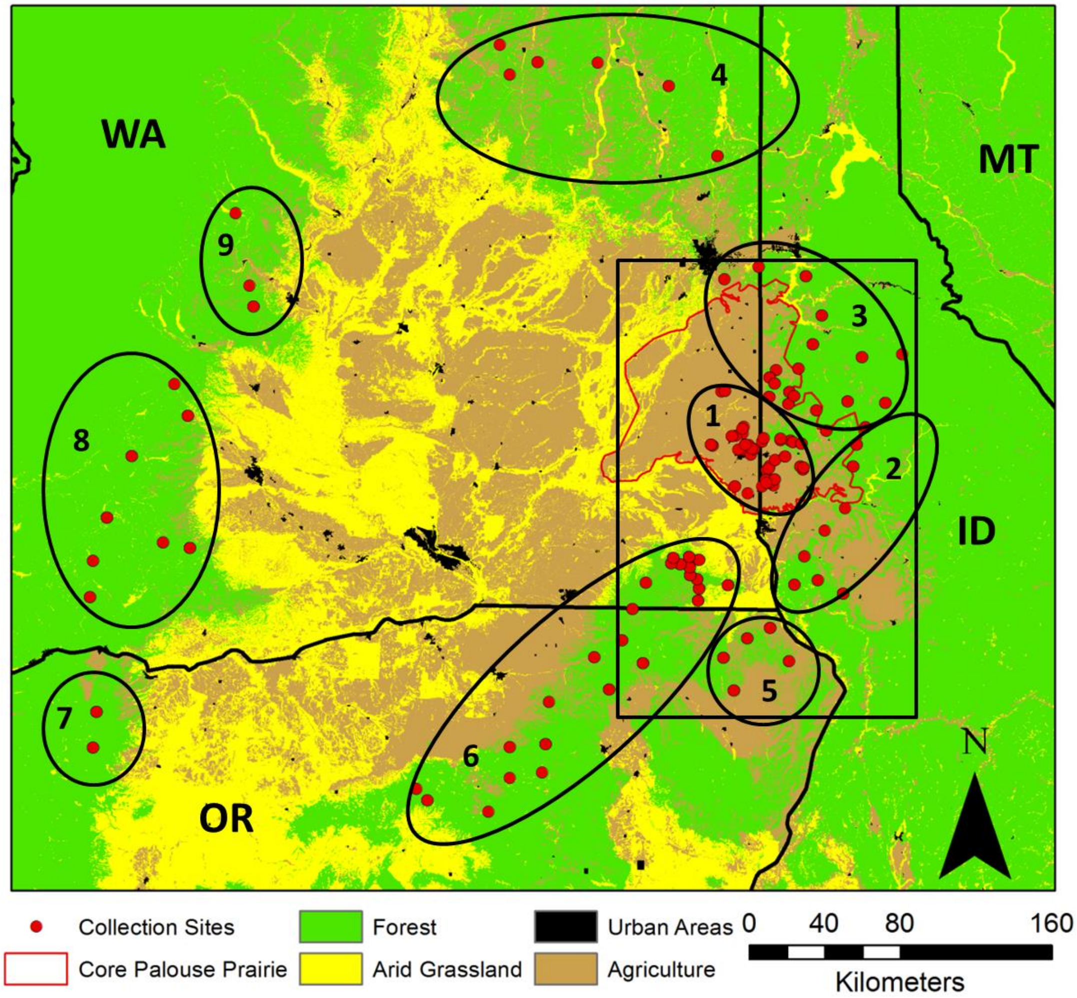

Figure 4.2 Collection sites in the inland Pacific Northwest. Ellipses denote putative populations 1-9 (Tables 4.1 and 4.2). Rectangle delineates area represented in Figure 4.1.

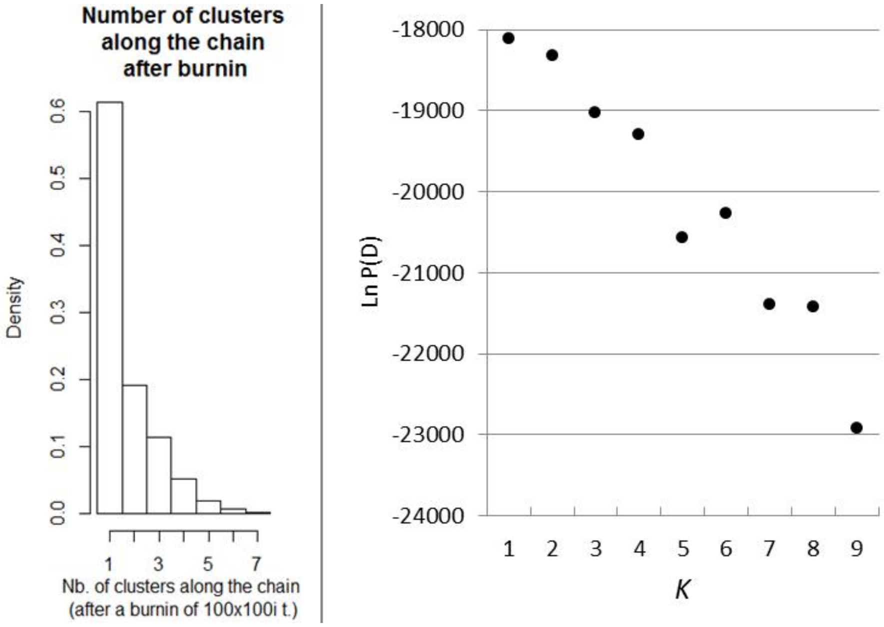

Figure 4.3 Output of Bayesian clustering programs used to search for genetic substructure. Both methods suggest a single cluster (population) across the entire study area. On the left, output from program GENELAND shows a clear mode of 1 on the histogram of simulated values. On the right, a plot of Ln P (D) vs. *K* (number of populations) produced using program STRUCTURE. *K* = 1 has the highest posterior probability.

CHAPTER 5: INFLUENCE OF EXOTIC GRASSES ON BEE COMMUNITY STRUCTURE IN FRAGMENTS OF PALOUSE PRAIRIE

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Abstract

Exotic grass invasion is a serious and persistent problem across western North America. Although invasive organisms are an important cause of species extinctions, the influence of invasive grass on bee communities has not been assessed. We use canonical correlation analysis and linear regression to evaluate alterations to bee community metrics associated with exotic grass and other habitat variables known to mediate bee communities including amount of habitat and plant species richness and diversity. Analyses were performed on the entire bee community as well as on functional subsets including large bees, small bees, ground nesting bees, and aerially nesting bees. We found exotic invasion grass reduces species richness of small bees and ground nesting bees while large bees and aerially nesting bees are mostly unaffected.

Introduction

Bee conservation is important for natural ecosystems and agricultural production (Fontaine *et al.* 2005; Klein *et al.* 2007). Wild bee populations are currently threatened by a variety of factors, including pesticides and pathogens, but are most significantly impacted by habitat loss caused by changing land use (Potts *et al.* 2010; Goulson *et al.* 2015).

Due to their suitability for agriculture, grassland systems have undergone dramatic land use change over the last century (Hassan, Scholes & Ash 2005) leading to reduced bee species richness in these environments (Kremen, Williams & Thorp 2002). Influence of land use change has been assessed in most cases by sampling bee communities in different types of land use (e.g., organic farms, conventional farms, and natural land as in Forrest *et al.* 2015), or by comparing bee communities in regions with differing amounts of natural land in the surrounding area (Steffan-Dewenter 2003). In general, the amount of natural (non-agricultural) land in the area and the plant diversity at the bee collection site are important factors mediating bee community attributes in fragmented agricultural regions (Potts *et al.* 2003; Steffan-Dewenter 2003; Kremen *et al.* 2004), but this may not be true in all cases (Winfree *et al.* 2008; Carré *et al.* 2009). Discrepancies could be caused by regional differences among study areas but also a failure to characterize the utility of natural land for wild bees. Natural land exists on a spectrum of utility for bees (Potts *et al.* 2003), and degraded land may not function the same as high quality land in supporting bee populations.

Exotic Mediterranean grasses are very well suited to the environment in Western North America and are a significant cause of habitat degradation throughout the region (Vitousek *et al.* 1997; Norton, Monaco & Norton 2007). Although invasive organisms are an important cause of species extinction, perhaps only second to land use change (Wilcove *et al.* 1998), the influence of exotic grasses on native bee communities has not been evaluated. Invasive grasses can decrease species richness of native plant communities (Levine *et al.* 2003) and plant communities and bee communities are closely linked (Potts *et al.* 2003). Therefore we expect the level of exotic grass invasion to be inversely correlated with bee species richness and diversity. However, it is unlikely all bee species will be affected similarly; rather, effects of exotic grass invasion will be mediated by species specific traits (Roulston & Goodell 2011). For example, oligolectic bees may find their preferred food plant to be exceptionally sensitive to plant invasion or small bees may find it difficult to travel across patches of invasive grass to locate and exploit food or nesting resources.

The Palouse Prairie of northern Idaho and eastern Washington State has experienced large amounts of habitat loss, fragmentation, and exotic grass invasion. First, conversion to agriculture significantly reduced its distribution to approximately 1% of its original area in the last century (Black *et al.* 1998). The remaining prairie occurs almost entirely in small remnants, less than 2 ha in size with high perimeter-to-area ratios (Looney & Eigenbrode 2012). Based on this drastic reduction, Palouse prairie could be classified as a 'Critically Endangered' ecosystem (Keith *et al.* 2013). Second, in more recent decades, the Palouse Prairie has been experiencing invasion by exotic plant species, including grasses. As a result, plant communities in Palouse Prairie fragments range from diverse communities of native forbs, grasses and shrubs to highly invaded patches dominated by exotic grasses (Davis 2015).

The purpose of this paper is to examine how wild bee communities are influenced by exotic grass invasion in fragments of natural land (Palouse Prairie) existing in a matrix of intense agriculture. Our hypotheses are: 1. Prairie sites with higher levels of exotic grass invasion in the surrounding landscape will have lower wild bee species richness and diversity. 2. Collection sites with larger amounts of natural land in the surrounding landscape will have higher wild bee species richness and diversity. 3. Collection sites with higher plant species richness and diversity will have higher wild bee species richness. 4. Small bees and aerially nesting bees will be more responsive to reduced plant diversity or natural land or increased non-native grass invasion.

To test these hypotheses we collected bees and characterized the plant community by quantifying bee species richness, evenness and the number of rare bee species. Functional traits of size and nesting location were assigned to bee species subdividing the bee community into functional groups. Habitat variables relevant to bee communities were also quantified including plant diversity and non-native grass cover within 1km, plant species richness at the bee collection site, and the amount of natural land within 1.25km. Linear regression, Wardle's V, and canonical correlation analysis were used to evaluate relationships between bee community metrics, bee functional groups and the measured habitat variables.

Materials and Methods

Sample collection

Bee collection occurred at 29 sites in 26 fragments of Palouse Prairie in 2012 and 2013. Each site was sampled four times in each year, with sampling intervals of approximately three weeks. Sampling location within the fragment was determined by generating a random point within each prairie fragment at least 10 meters from the fragment edge using ArcMap10.0 (ESRI, Redlands CA). If the sampling point fell within a thicket of shrubs or small trees, which would inhibit trap placement, the sampling location was moved 5 meters beyond the nearest edge of the thicket. These sampling points were used for all 8 sampling events. At each visit, pan traps, blue vane traps, and aerial net collection were used for bee sampling. Three colored pan traps (3.25 oz. soufflé cups, Solo model #p325w-0007) filled with soapy water were placed on the ground in areas without dense plant growth that would decrease visibility. Pan colors (one yellow, one blue and one white) were randomized and were placed in a transect leading away from the sampling point about three meters apart. Blue vane traps were filled with 500 ml of soapy water and hung about 2 meters off the ground from a bamboo tripod. Net collection occurred at flowers within 50 meters of the sampling point for 5 minutes at the time of trap placement and removal for a total of 10 minutes of net collection for each site at each sampling period.

Sampling occurred on mostly sunny days with highs above 16°C. The average high temperature for sampling days was 22.7°C in 2012 and 27°C in 2013; the average low temperature was 5.4°C in 2012 and 6.2°C in 2013; 1.16 cm of precipitation fell over four sampling days in 2012 and 0.15 cm fell over two days in 2013.

Net-collected bees were kept frozen before pinning and identification. Bees collected in blue vane traps or pan traps were rinsed in ethanol and then placed in a Whirl-Pak bag (Nasco, Fort Atkinson, WI), covered with ethanol and eventually washed and dried before pinning and identification (methods adapted from Droege (2009). Bees were identified to species except for *Nomada, Epeolus, Sphecodes,* and *Lasioglossum* in the *Hemihalictus* series, which were only identified to genus or series. In the first year of collection all bees were pinned and identified. In the second year a few very common and easily identifiable species including *Agapostemon angelicus* Cockerell, *A. virescens* (Fabricius), *A. femoratus* Crawford and *Halictus tripartitus* Cockerell were identified without pinning. *Lasioglosum* in the *Hemihalictus* series were identified as such and stored without pinning. Voucher specimens reside in the William F. Barr Insect Museum at the University of Idaho and the U. S. National Pollinating Insects Collection, USDA Bee Biology and Systematics Laboratory housed on the campus of the Utah State University

Site and landscape characterization

Landscape composition was characterized using the CropScape (USDA-NASS) remotely sensed land cover classification supplemented with National Agricultural Imagery Program (NAIP) imagery and high resolution Google Maps data. Landscape features were heads-up digitized into polygons using ArcView 10.0 and classified into 16 categories: dense forest, open forest, highly developed land, lightly developed land, hay/pasture/CRP, natural land, spring wheat, winter wheat, canola, garbanzos, dry peas, lentils, barley, grass seed, and alfalfa. Hay fields, pasture, land enrolled in the USDA Conservation Reserve Program (CRP), and Palouse Prairie fragments were all classified similarly in the CropScape data layer. Polygons were assigned to either hay/pasture/CRP or natural land by evaluating the heterogeneity of the plant cover using high-resolution aerial photographs. Land with high plant heterogeneity was judged to have large amounts of floral resources to support bee populations and considered natural land. This method was calibrated by comparing aerial photos with plant community data at collection sites. The 16 categories were aggregated into two categories: useful habitat for bees (open forest, natural land, and lightly developed land) and not useful habitat for bees (everything else); polygons were then reclassified into these two categories in ArcView using the Reclass tool. Concentric rings were generated around the sampling point at ten distances (50m, 100m, 150m, 250m, 400m, 600m, 800m, 1,000m, 1,250m, and 1,500m) using the Multiple Ring Buffer tool. The Iterate Feature Selection tool in model builder in ArcView was used to automate the process of quantifying the area of land cover type at each sampling point/radius combination by using the Tabulate Area tool and the Append tool at each radius for each sampling point. A measure of area of each land cover type within each concentric ring (e.g., between 1,000m and 1,250m) was obtained by subtracting the area of each land cover type for each radius from that of the next largest radius. These data were then log transformed.

The plant community was characterized in 2 ways. 1. Plant species richness was evaluated by identifying all entomophilous plants in flower within 50m of the bee collection site at the time of trap placement. Plants were either identified in the field or in consultation with a botanist (Cleve Davis) using photos. 2. Percent foliar cover by species was estimated using quadrat/transect-based methods at each bee collection site and at 75 additional plots all within 1 km of a bee collection site. The number of additional plant cover assessment plots was proportional to the fragment size on which sampling took place and varied between zero and nine additional sites within 1km. Locations of additional assessment plots were randomly selected using the Create Random Points tool in ArcGis but, because aspect and elevation are major drivers affecting plant species composition on the Palouse prairie (Hanson *et al.* 2008), selection of additional plots was constrained to sample the range of nearby aspect and elevation. These data were collected May-July during 2012 and 2013 when a majority of plant species could be easily identified. Plant species cover was estimated following the Daubenmire (1959) canopy-coverage method, which was modified by using 0.50 by 0.25 m rectangular quadrats instead of 0.50 by 0.20m quadrats. Twelve quadrats were read spaced 1 m apart on a twelve meter transect. Quadrats were oriented so the 0.20m section was parallel to the transect direction. Within in each quadrat, percent foliar cover of each species was classified into six categories: 1 = 0.01-5%, 2 = 5-12.5%, 3 = 12.5-25%, 4 = 25-50%, 5 = 50-75%, 6 = 75-100%. The mid-point value of these cover class estimates was used to determine cover by species. Species were identified in the field or collected and identified by comparison with herbarium specimens at the University of

Idaho Stillinger and Washington State University Marion Ownbey herbaria. Only one observer was used to make estimations. For each plot, total cover of nonnative grass species and Shannon's diversity index for total flora based on coverage were calculated in R (R Core Development Team 2015). Values for nonnative grass cover and Shannon's plant diversity were averaged for all plots within 1 km of each bee collection site.

Data analysis

Spatial variograms were constructed using the geoR package in R (Ribeiro Jr & Diggle 2001; Team 2015) to evaluate spatial autocorrelation among sites for bee species richness, diversity, plant species richness, and amount of habitat suitable for bees at each radius.

Bee species were classified into functional groups based on nesting strategy and size. Because bees in the *Hemihalictus* series represent an unknown number of species [possibly as many as 37 (Kimoto *et al.* 2012)], they were not included in any analysis. Nesting strategy classes were ground and aerial nesting. Parasitic species and species for which the nesting strategy is unknown were left unclassified. Size classes were based on intertegular distance (Cane 1987), and divided into large and small groups at the median intertegular distance of 2.0mm. Together these two classification dimensions produced four functional groupings: small and large ground nesting; small and large aerial nesting. A Chi-squared test indicates bee size is independent of nesting strategy ($\chi^2=0$, df=1, p=1).

Canonical correlation analysis (CCA) was used to examine relative influence of different habitat variables on bee community metrics. Five CCAs were run using community metrics; one for the entire community and one for each of the four functional groupings. Bee community metrics were the number of rare bees detected at each site (defined as species with fewer than ten individuals detected, 107 species were classified as rare), bee species richness and bee species evenness. Habitat variables included plant species richness at the collection site, the amount of natural land within 1250m, and nonnative grass cover and plant diversity within 1,000m. Significance of CCA axes was determined using Wilk's λ. All CCA operations were performed using Proc CANCORR in SAS 9.2 (Cary, North Carolina). Individual linear regressions were performed among the community metrics and habitat variables to clarify the results of the CCA by examining relationships between variables in isolation.

Wardle's V (Wardle 1995) is a metric describing habitat association of each species in a sample:

$$V = \frac{2M_a}{M_b + M_a} - 1$$

Where M_a is the number of occurrences for a species in habitat type 'a' and M_b is the number of occurrences in habitat type 'b'. It scales from 1 (only encountered in habitat type 'a') to -1 (only encountered in habitat type 'b'). All collection sites were ranked based on the four habitat attributes used in the CCA: plant species richness at the collection site, the amount of natural land within 1,250m, and nonnative grass cover and plant diversity within 1 km. Collection sites were then divided into high (upper 2/5) or low (lower 2/5) for each of the 4 habitat attributes (e.g., high plant species richness or low plant species richness). Sites falling into the middle 1/5 for a particular habitat attribute were not included in that analysis. Wardle's V values were calculated for each species for each of the four habitat attributes. A sign test was then used to assess whether each of four bee families: Andrenidae, Apidae, Halictidae, Megachilidae, (Colletidae had too few individuals to analyze) and each of the four functional groups were significantly associated with each habitat type.

Results

Not counting the *Hemihalictus* series, 9,200 bees representing 172 species and 7 morphospecies in 29 genera were collected over the two years of sampling (Table 1.1). Nesting strategy was determined for 160 species and morphospecies. Of these, 47 species and 1,732 individuals are aerial nesters and 113 species and 7,468 individuals are ground nesters. Intertegular distance ranged from 0.9 mm for *Hylaeus verticalis* to 6.2 mm for *Bombus californicus.* All species were binned by size: 56 species and morphospecies were binned into the small category and 128 species and morphospecies were classified as large. The most abundant small genera were *Ceratina*, *Halictus* and *Lasioglossum.* The most speciose small genera were *Andrena* and *Lasioglossum*. The most abundant large genera were *Osmia*, *Lasioglossum*, *Agapostemon*, and *Bombus.* *Osmia*, *Bombus* and *Andrena* were the most speciose large genera.

Plant species richness at bee collection sites ranged from 7 to 38 species with a mean of 22.3. At the 104 plant cover transects, cover values were recorded for 239 species of vascular plants, 12 lichens, 13 bryophytes, 1 fungus, 1 club moss and 1 liverwort species. Plant Shannon's diversity averaged over all transects within 1 km of the bee collection site ranged from 0.94 to 2.55 with a mean of 1.9. Non-native grass species were present at every site. Total coverage of invasive grass ranged from 2.3% to 69.3%. Nineteen non-native grass species were observed in this study. The most common were *Ventenata dubia* (present in 75% of plots), *Bromus tectorum* (present at 59% of plots), *Bromus hordeaceus* ssp.

hordeaceus (*Bromus mollis*) (present in 34% of plots) and *Bromus racemosus* (*Bromus commutatus*) (present in 33% of plots).

Canonical correlation analysis on the entire bee community and 4 functional groupings of the bee community yielded three significant models (Table 5.1). Models using community metrics from only large bees or aerially nesting bees did not have canonical correlations significantly different from zero and therefore are not as sensitive to the measured environmental variables compared to small bees or ground nesting bees (Table 5.1). However, the first canonical axis was significant in models using community metrics from only small bees or ground nesting bees as well as the entire bee community (Table 5.1).

In all significant models bee species richness has the highest canonical coefficients and highest loadings and so is the most responsive to habitat variables (Tables 5.2-5.4). Plant diversity and the amount of natural land have consistently high canonical coefficients and loadings, indicating consistent importance of these habitat variables on the measured bee community metrics. The importance of non-native grass varies, showing a higher canonical coefficient and loading in the model using only the subset of small bees where it has importance similar to plant species richness at the collection site (Table 5.3).

Individual linear regressions among the variables used in the CCA further clarify these relationships (Table 5.5). Plant species richness at the bee collection site was the most consistently important habitat variable being correlated with bee species richness for the entire bee community as well as for all four subsets of the bee community. Plant species richness at the collection site is also correlated with the number of rare bees detected in the entire bee community as well as for small bees and ground nesting bees, but not large bees or aerially nesting bees. The amount of useful habitat within 1.25 km was the next most consistently important habitat variable, correlated with the number of rare bees detected for small bees and ground nesting bees and with bee species richness for all bees, small bees, and ground nesting bees. Plant diversity within 1km of the bee collection site is correlated with bee species richness for the entire bee community, for small bees, and for ground nesting bees. Non-native grass cover was correlated with the species richness of small bees and the number of detected rare species for ground nesting bees.

Wardle's V values were generally consistent with the CCA and regression analysis in that small bee species and ground nesting bee species were more sensitive to the included habitat attributes. Bee species in the Andrenidae were associated with sites with low non-native grass and large amounts of useful habitat, high plant species richness at the collection site and high plant diversity within 1 km. The Halictidae and Megachilidae were mostly not responsive to the measured habitat attributes.

Discussion

Human-caused elimination of biogeographical barriers to dispersal has created contact among taxa that were previously isolated, altering ecosystem services and processes throughout the world (Hobbs & Mooney 2000). Mechanisms facilitating exotic grass invasion and the effects these invaders have is not well understood (Levine *et al.* 2003). Frequently, exploitative competition for light, water, nutrients or pollination has been hypothesized as contributing to invasive plant success (Levine *et al.* 2003). Our data shows exotic grass invasion can reduce bee species richness and alter bee community structure, possibly altering native plant pollination. Alterations to ecologic processes caused by invasive plants are significant because of wide ranging ecosystem level effects, which are less well understood than community or population level effects (D'Antonio & Vitousek 1992; Hulme *et al.* 2013). As pollination is often crucial for reproductive success and presumably eventual plant recruitment (Burd 1994; Aguilar *et al.* 2006), reductions in bee species richness caused by exotic grass invasion could facilitate further invasion. These data will increase our understanding of the response of bee communities to both fragmented agricultural habitats and exotic grass invasion.

In general, relationships we found between community metrics of the entire bee community and landscape and site attributes are in line with other work. Plant species richness in Palouse Prairie fragments is correlated with bee species richness (Table 5.5). The hypothesis that the diversity of a guild of consumers is related to the diversity of their resource base is well established (MacArthur 1972; Tepedino & Stanton 1981) and this phenomenon has been observed many times in bee communities (Potts *et al.* 2003; Hines & Hendrix 2005; Hatfield & LeBuhn 2007). Although not discussed in the methods, we did find remnant size to be uncorrelated with bee species richness (data not shown). Although this departs from traditional ecological theory (i.e., Island Biogeography Theory, MacArthur & Wilson 1967), the utility of Island Biogeography Theory in terrestrial environments is uncertain (Simberloff & Abele 1976; Higgs 1981) and the lack of influence of fragment size on bee community metrics has been observed before (Brosi *et al.* 2008; Hendrix, Kwaiser & Heard 2010). On the other hand, bee species richness was positively correlated with the amount of natural land (Table 5.5). This relationship has been observed often (Steffan-Dewenter *et al.* 2002; Kremen *et al.* 2004; Kleijn & Van Langevelde 2006).

Although the amount of natural land and plant species richness and diversity has a more pronounced effect on native bee community metrics, our data is the first to show exotic grass invasion can alter bee communities by reducing species richness of small bees and reducing the number of rare ground nesting bee species (Table 5.5). Invasive organisms, along with habitat disturbance, loss, and fragmentation, are noted as one of the primary threats confronting native bees (Kremen & Ricketts 2000). However, most research on interactions between bees and invasive plants has focused on pollinator-mediated effects of exotic entomophilous plants on native plant communities. Entomophilous invaders can alter pollination regimes of native plants by distracting pollinators away from less visually prominent native plants (Chittka & Schürkens 2001; Brown, Mitchell & Graham 2002) or by prompting inconsistent foraging (Brown & Mitchell 2001), which can lead to a variety of deleterious effects associated with mixed pollen deposition (reviewed in Brown *et al.* 2002). While these invasive entomophilous plants can negatively impact native bees by disrupting mutual relationships between native bees and native plants (Aizen, Morales & Morales 2008), neutral and positive effects are also seen (Stout & Morales 2009). Effects of invasive grass are wholly different and the mechanism of influence on bee communities may more closely resemble those of herbicides (Le Féon *et al.* 2013) or grazing (Sugden 1985) by reducing the amount of bee forage. Unlike entomophilous invasive plants, which in some cases can actually increase the carrying capacity of the land for bees, [especially for generalist bee species (Tepedino, Bradley & Griswold 2008)], invasive grasses seem to have an entirely negative effect.

Large bees and aerially nesting bees are comparatively insensitive to exotic grass invasion. Both functional classes are generally less sensitive to the habitat variables we measured and are only correlated with plant species richness at the collection site (Tables 5.1 and 5.6). Reports of sensitivity of large bees to habitat degradation or loss vary. Previous work has found large bees to be both more sensitive (Larsen, Williams & Kremen 2005; Hinners 2008; Bommarco *et al.* 2010) and less sensitive (Bommarco *et al.* 2010; Williams *et al.* 2010; Jauker *et al.* 2013) to declining habitat quality relative to small bees. On one hand, body size is correlated with flight range (Greenleaf *et al.* 2007), and so large bees may be able to locate and exploit food and nesting resources spatially separated by habitat fragmentation. On the other hand, large bee species may have larger resource requirements and smaller population sizes making then more vulnerable to habitat degradation and more susceptible to local extirpation. As patterns of grass invasion can be spatially heterogeneous (Taylor, Hastings & Sherratt 2005), large bees may be able to traverse areas of poor habitat quality more easily than small bees. Indeed, some large bees may be able to cross relatively expansive patches of agricultural land (Rao & Strange 2012) resulting in apparent insensitivity to the landscape scale variables we measured (exotic grass invasion, amount of natural land, and plant diversity).

Aerially nesting bees were also not sensitive to exotic grass or most of the measured habitat variables (Tables 5.1 and 5.6), possibly because nest site availability was the most important limiting factor for these bee species (Steffan-Dewenter & Schiele 2008). While others have found aerially nesting bees to be more influenced by habitat loss than ground nesting bees (Williams *et al.* 2010; Forrest *et al.* 2015) the abundance of plants useful for nesting may not be correlated with habitat area. However, we did not measure abundance of nesting resources for aerially nesting bees and our plant surveys were too patchy in nature to adequately assess the presence of the shrubs, small trees and pithy-stemmed plants used for nesting. Exotic grass invasion may not alter the composition of forb and woody shrub communities at the same temporal scale, and in certain cases invasive grass litter may actually facilitate mature native shrubs useful for nesting (Wolkovich, Bolger & Cottingham 2009). In the study area, it is likely that nesting resources are the primary limitation to aerially nesting bees relative to invasive grass, amount of natural land, and plant diversity.

The families Andrenidae, and to a lesser extent Apidae, were more sensitive to the habitat variables measured compared to Megachilidae and Halictidae (Table 5.6). This may be because Andrenidae and Apidae contain a high proportion of ground nesting bee species and Andrenidae are nearly all small bees. Conversely the Halictidae are also small, ground nesting bees yet they seem relatively insensitive to the measured habitat variables. Although species known to be oligolectic were not abundant enough to analyze using the above methods, many oligolectic species are Andrena and may therefore be more sensitive to habitat degradation. This may explain the discrepancy in sensitivity between Andrenidae and Halictidae, which are mostly generalist species. Megachilidae has many aerially nesting species so these bees may not respond to the measured variables.

The fact that not all genera and functional classes were affected similarly, suggests species composition as well as species richness changes with changing landscape and habitat structure (McKinney & Lockwood 1999). Functional complementarity enhances pollination and may contribute to plant community diversity (Fontaine *et al.* 2005; Martins, Gonzalez & Lechowicz 2015). Altering the composition of bee communities could alter the composition of associated plant communities and their non-bee associated fauna, possibly changing the functioning of the entire ecosystem (Fortuna & Bascompte 2006).

These results have relevance to both native bee and native plant conservation in fragmented systems. For example, proximity and amount of natural habitat is an often cited correlate of pollination service and bee species richness (Kremen *et al.* 2002; Morandin & Winston 2006; Greenleaf & Kremen 2006; Winfree 2010). This work demonstrates the importance of verifying the utility of the natural habitat for bees.

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Table 5.1 Eigenvalues and Wilks λ significance test for the first axis of Canonical Correlation Analysis performed on different subsets of the Palouse bee community. All bees, small bees (<2mm intertegular distance), large bees (>2mm intertegular distance), ground nesting bees and aerial nesting bees were analyzed using variables listed in Tables 2-4. The first axis was significant using the entire bee community, small bees, and ground nesting bees. The remaining axes were never significant. The lack of significant axes for large bees and ground nesting bees suggest these bees are not as sensitive to the measured environmental variables.

	Eigenvalue	Wilks λ	F value	P>f
All bees	**0.79**	**0.37**	**2.21**	**0.023**
Small bees	**1.75**	**0.30**	**2.81**	**0.004**
Large bees	0.30	0.72	0.63	0.804
Ground nesting	**0.85**	**0.37**	**2.20**	**0.024**
Aerial nesting	0.38	0.62	0.97	0.489

Table 5.2 List of canonical coefficients and canonical correlations for the entire bee community. Canonical coefficients describe the contribution of each variable to the axis. Canonical correlations describe what each axis represents.

		Standardized canonical coefficients	Canonical correlation
Bee community variables	Number of rare bees	-0.42	0.42
	Bee species richness	1.06	0.84
	Bee species evenness	0.46	0.64
Environmental variables	Plant diversity	0.73	0.86
	Non-native grass cover	0.37	-0.25
	Natural land	0.46	0.85
	Plant species richness	0.11	0.64

Table 5.3 List of canonical coefficients and canonical correlations for the community of small bees. Canonical coefficients describe the contribution of each variable to the axis. Canonical correlations describe what each axis represents.

		Standardized canonical coefficients	Canonical correlation
Bee community variables	Number of rare bees	-0.43	0.38
	Bee species richness	1.22	0.93
	Bee species evenness	0.17	0.48
Environmental variables	Plant diversity	0.23	0.83
	Non-native grass cover	-0.25	-0.62
	Natural land	0.64	0.90
	Plant species richness	0.11	0.69

Table 5.4 List of canonical coefficients and canonical correlations for the community of ground nesting bees. Canonical coefficients describe the contribution of each variable to the axis. Canonical correlations describe what each axis represents.

		Standardized canonical coefficients	Canonical correlation
Bee community variables	Number of rare bees	-0.12	0.56
	Bee species richness	0.93	0.51
	Bee species evenness	0.50	0.86
Environmental variables	Plant diversity	0.59	0.85
	Non-native grass cover	0.24	-0.33
	Natural land	0.55	0.90
	Plant species richness	0.13	0.67

Table 5.5 Products of correlations between bee community metrics and habitat variables. Community metrics were calculated for all bees and for four subsets of the entire community defined by functional traits. Values significant at p=0.05 are in bold.

		Non-native grass	Natural land	Plant species richness	Plant diversity
All bees	Bee species richness	p=0.10 r^2=0.10	**p<0.01 r^2=0.28**	**p=0.01 r^2=0.21**	**p=0.01 r^2=0.26**
	Evenness	p=0.43 r^2=0.02	p=0.12 r^2=0.08	p=0.13 r^2=0.08	p=0.15 r^2=0.07
	Number of rare bees	p=0.25 r^2=0.05	p=0.11 r^2=0.09	**p=0.01 r^2=0.21**	p=0.22 r^2=0.05
Small bees	Bee species richness	**p=0.01 r^2=0.22**	**p<0.01 r^2=0.46**	**p<0.01 r^2=0.28**	**p<0.01 r^2=0.36**
	Evenness	p=0.98 r^2=0.0	p=0.10 r^2=0.10	p=0.08 r^2=0.10	p=0.10 r^2=0.10
	Number of rare bees	p=0.34 r^2=0.03	**p=0.05 r^2=0.14**	**p=0.05 r^2=0.14**	p=0.15 r^2=0.08
Large bees	Bee species richness	p=0.42 r^2=0.02	p=0.11 r^2=0.08	**p=0.02 r^2=0.18**	p=0.06 r^2=0.12
	Evenness	p=0.43 r^2=0.02	p=0.82 r^2=0.01	p=0.85 r^2=0.01	p=0.85 r^2=0.01
	Number of rare bees	p=0.44 r^2=0.02	p=0.53 r^2=0.01	p=0.06 r^2=0.12	p=0.68 r^2=0.00
Ground nest	Bee species richness	p=0.11 r^2=0.09	**p<0.01 r^2=0.33**	**P=0.03 r^2=0.16**	**p<0.01 r^2=0.24**
	Evenness	p=0.85 r^2=0.0	p=0.25 r^2=0.05	p=0.18 r^2=0.07	p=0.10 r^2=0.10
	Number of rare bees	**p=0.04 r^2=0.15**	**p=0.04 r^2=0.14**	**p=0.01 r^2=0.24**	p=0.07 r^2=0.12
Aerial nest	Species Richness	p=0.49 r^2=0.02	p=0.70 r^2=0.01	**p=0.04 r^2=0.13**	p=0.21 r^2=0.06
	Evenness	p=0.69 r^2=0.01	p=0.25 r^2=0.04	p=0.22 r^2=0.05	p=0.34 r^2=0.04
	Number of rare bees	p=0.28 r^2=0.03	p=0.38 r^2=0.03	p=0.73 r^2=0.03	p=0.39 r^2=0.03

Table 5.6 Wardle's V values averaged by functional group or family for each of four habitat types. Values closer to one indicate an affinity for the listed habitat type. Values in bold are significantly different from zero by a sign test (p<0.1).

	Low nonnative grass	High natural land	High plant species richness	High plant diversity
Small bees	**0.27**	0.23	**0.22**	**0.26**
Large bees	0.09	0.14	**0.15**	0.07
Aerial nester	0.05	0.04	0.05	0.08
Ground nester	**0.19**	**0.23**	**0.23**	0.14
Halictidae	0.04	0.03	0.13	0.00
Andrenidae	**0.55**	**0.46**	**0.37**	**0.40**
Apidae	0.10	**0.25**	**0.24**	0.11
Megachilidae	0.06	0.06	0.08	0.07

CHAPTER 6: SOCIAL VALUES OF CULTURALLY SIGNIFICANT PLANTS ON THE PALOUSE PRAIRIE

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Abstract

Although economic valuations of ecosystem services can account for externalities and be useful for conveying costs, these valuations can be considered inadequate by certain stakeholders or when viewed from certain social perspectives. This study sought to assess the social value of culturally significant plants on the Palouse Prairie in northern Idaho and southeastern Washington through interviews and surveys. The purpose of the study was to identify potential social support for the conservation of culturally significant plants on the Palouse Prairie. A mail survey and semi-structured interviews found that Native Americans of the region and 36 percent of the respondents from the local population considered culturally significant plants valuable. In addition, the demographic factors gender, level of conservatism, economic level, self-identified heritage connection with the study area, and views on basing conservation decisions upon a dollar amount each had a statistically significant effect on respondents' views on valuing culturally significant plants. The study differs from other valuations by focusing on the perspectives of both Indigenous and non-Indigenous populations and assessing the social value of culturally significant plants using quantitative and qualitative approaches.

Introduction

The ecosystem services framework involves quantifying and valuing conditions and processes through which ecosystems and biodiversity sustain, benefit, and fulfill human life.

The global benefits of ecosystem services (ES) are enormous because human societies could not exist without them (Daily 1997). Ecosystem services can include provisioning, regulating, habitat, supporting, cultural and amenity services (Sarukhán and Whyte 2005; de Groot et al. 2010). Scientists and policy makers are increasingly describing ecosystems and biodiversity as “environmental capital” or “natural capital” (Holdren et al. 2011; Kareiva et al. 2011). Economic valuations of ES can be used to prioritize conservation (van Berkel and Verburg 2012) and can be incorporated into markets to inform policy decisions (Constanza et al. 1997). Accounting for the value of ES may help guide society in assessing the impacts of degradation and loss of these services (Pascual et al. 2010). The need to quantify and value ES is widely accepted by scientists and policy makers (Daniel et al. 2012).

Despite the acknowledged importance of economic valuations of ES, economic valuations alone are considered by some to be inadequate or insensitive to the perspectives of certain stakeholders with differing social views (Johansson-Stenman 1998; Burger 2011). For example, stakeholders who consider the natural environment sacred may reject the notion of quantifying how much they would be willing to pay to sustain a particular ES (Chan et al. 2012). Indeed, indigenous cultures could view economic valuations as part of the colonial process premised on commoditization of the natural world and hence fundamentally unacceptable. How to value ES in a manner that is sensitive to these social and cultural perspectives but compatible with global economic forces is a significant challenge. To address the inadequacy of a strictly economic valuation of cultural ES, Chan et al. (2012) proposed a multi-metric approach that included non-monetary variables. Our study applied a non-monetary approach to value culturally significant plants, as they do not fit well into any one category of ecosystem services and fact that economic valuation of such plants may be considered insensitive to certain social perspectives.

“Culturally significant plants” are defined here as any native plant, lichen, moss, or fungus that can be used for food, teas, medicine, in ceremonies, or materials used in artisan craft. The value of certain culturally significant plants can be difficult to ascertain due to their wide variety of potential uses (e.g., food, medicine, spiritual enrichment, etc.) that do not fit well into any one ES category. For example, determining the value of a plant used in religious ceremonies may be particularly challenging because its importance to people is not easily translatable to economies and markets. Due to the level of intangibility the value supplied can be difficult to quantify monetarily or even biophysically (Daniel et al. 2012, Milcu et al. 2013).

Culturally significant plants are valuable for the cultural services they provide, as defined by Sarukhán and Whyte (2005:40): “nonmaterial benefits people obtain from ecosystems through spiritual enrichment, cognitive development, reflection, recreation, and aesthetic experiences”. Furthermore, their conservation constitutes a cultural legacy from past to future generations. For example, Native Americans utilize culturally significant plants in religious ceremonies, as religious symbols, or as items of inspiration or spiritual enrichment (Stewart 1987; Moerman 1998). Culturally significant plants can also provide provisioning, regulating, and habitat support services. For example, culturally significant plants and plant communities can provide aesthetic beauty or natural scenery, and provisioning ES as sources of wild food, medicine, and raw materials. They can also provide regulating or supporting ES through carbon sequestration and through their effect on minimizing soil erosion and increasing soil fertility. As part of a native plant community, culturally significant plants provide habitat and supporting services to wildlife.

Due to the difficulties with placing an economic value upon culturally significant plants, this study sought to examine a non-monetary approach to their valuation through semi-structured interviews and a general population sample survey. The focus area of the study was the Palouse region of northern Idaho and southeastern Washington (Figure 6.1). The Palouse region provides an ideal locale for examining non-monetary approaches to assessing the value of culturally significant plants, first, since the region was once considered a vast garden for culturally significant plants by the native inhabitants (Scheuerman and Finley 2008), and second, because plant biodiversity is now severely at risk in the region due to the spread of invasive plants and loss of habitat from agricultural conversion (Noss and Peters 1995; Black et al. 2000; Nyamai et al. 2011; Looney and Eigenbrode 2012). Therefore, the goal of the study was to identify potential social synergies that could be used to influence conservation of culturally significant plants on the Palouse prairie. A social synergy is the interaction of social elements or common values that when combined produce a total effect that is greater than the sum of the individual elements or contributions to achieve a desired outcome.

Study Region

The Palouse Prairie grassland is critically endangered because most of its former extent is now dedicated almost exclusively to rainfed farming, mostly grain and pulse crops (Hanson et al. 2008; Looney and Eigenbrode 2012; Donovan et al. 2009). The Palouse region has an extensive and significant prehistory. Some of the earliest records of humankind in North

America have been uncovered in the nearby basalt canyons along the Snake River, so it is likely that there has been some human presence in the Palouse Prairie for at least 12,000 years (Breckenridge 1986; Black et al. 2000; Chatters 2004). When Lewis and Clark of the Corps of Discovery entered the region in 1805, it was inhabited by Palouse (*Naha’ùumpùu*), Nez Perce (*Niimìipuu*), Spokane (*Sqeliz*), and Coeur d’Alene (*Schitsu’umsh*) peoples (Walker 1998; Sprague 1998; Frey 2001; Scheuerman and Finley 2008). The Palouse and Nez Perce speak the Sahaptin language and are culturally related.

Subsistence practices of the indigenous populations were based upon hunting, fishing, and gathering, as well as low-impact agriculture of native plant species (Black et al. 2000). The Palouse Prairie was particularly important for the gathering of edible and medicinal plants by the indigenous populations (Sprague 1998; Frey 2001; Scheuerman and Finley 2008). The seasonally wet meadows and prairies of the Palouse Prairie supported high densities of the edible blue camas (*Camassia quamash*). When the horse was acquired in the 1700s, use of the area by Indigenous people diversified to stock raising (Black et al. 2000). However, most of the Indigenous population was severely reduced in size by 1860 through war, disease, and famine that resulted from Euro-American invasion and settlement (Sprague 1998).

In the late 1800s, the Palouse Prairie underwent an extensive and profound transformation. Euro-Americans used the region in a dramatically different way from that of the Indigenous peoples (Black et al. 2000). Initially, Euro-Americans pastured livestock and grew tree fruits (Williams 1991). Within a few decades, competition from areas better suited for fruit production and high returns for wheat production drove a nearly complete transition to grain farming (Williams 1991). Since 1900 it has been estimated that as little as one tenth of one percent of the Palouse Prairie grassland remains (Noss and Peters 1995). Today, the region is considered to be one of the United States' most productive dryland farming areas (Duffin 2005). What remains of the natural Palouse Prairie is considered an endangered ecosystem (Noss and Peters 1995), and a large majority of it under private ownership (Black et al. 2000).

Valuations of culturally significant and wild plants involve ascribing value to a particular species using an index scoring system or economic valuation. The aim of many valuation methods for culturally significant plants is to identify the importance of plant species without investigator bias, often with a focus on subsistence use by Indigenous peoples (Hunn 1982; Turner 1988; Stoffle et al. 1990; Godoy et al. 1993; Phillips et al. 1994; Pieroni 2001; Cocks and Wiersum 2003; Reyes-García et al. 2006; Thomas et al. 2009). However, we were unable to find any study that valued the importance of culturally significant plants using an integrated analysis of both Indigenous and a somewhat recent dominant immigrant population of Euro-Americans descent populations. In the Palouse, this sort of integrated assessment is appropriate because of the co-occurrence of both Indigenous and non-Indigenous populations.

Methods

The overall approach for this analysis included conducting semi-structured interviews with Nez Perce Tribal members to gain a better understanding of Indigenous eco-cultural priorities, concerns, and perspectives of the Palouse Prairie; as well as an analysis of sample survey responses by the local populations of the Palouse region. Information gathered via interviews was used to develop survey questions for a quantitative survey of the regional population that included both the Indigenous and non-Indigenous community.

Semi-structured Interviews with Tribal Members

The Nez Perce and Coeur d’Alene Reservations span portions of what we are defining as the core area of the Palouse Prairie landscape. To gain approval to conduct interviews with Native American Tribes, meetings were held with the Nez Perce and Coeur d’Alene Tribal representatives. The interview protocol was approved as posing no significant risks to human subjects by the University of Idaho Institutional Review Board on August 27, 2012. A research permit with the Nez Perce Tribe was approved on June 26, 2012 by the Nez Perce Tribe Executive Committee. Permission to conduct semi-structured interviews with the Coeur d’Alene Tribe was not obtained. Six semi-structured interview sessions were conducted with multiple Nez Perce Tribal members who were identified by the local community as being knowledgeable of traditional language and culture. The interviews took place in 2012 and 2013 on the Nez Perce Reservation in northern Idaho. The questions posed during interviews were standardized (Appendix 1), but recorded responses included extended discussions, consistent with semi-structured interview methods.

Responses to questions were recorded and coded based upon theme of the response. As part of the Nez Perce Research Permit, the Tribe was provided an opportunity to review information summarized and a draft of this manuscript to ensure protection of sensitive information of the Tribe. This included two reviews of earlier drafts of this manuscript and a printed hard copy delivered to the Cultural Department of the Nez Perce Tribe.

Sample Survey

Based upon the semi-structured interviews, three survey questions were developed related to valuing culturally significant plants and included in a more comprehensive survey aimed at valuing ES on the Palouse Prairie. The questions were number 28, 29, and 32 of a larger survey. These questions and the response options and provided (Table 6.1). The hypotheses of the sample survey analysis included: 1) culturally significant plants provide little value (i.e., less than 10 percent through sample survey) to the local community; and 2) demographic factors can be used to predict how the respondent values culturally significant plants. To test the first hypothesis respondents were asked to rate the importance of culturally significant plants on a scale of 1 to 5, with 1 being not valuable at all to themselves and their families and 5 being extremely valuable to themselves and their families. Due to low response rates for each level in the scale, responses were aggregated as follows: scores of 1-2 were considered Not Valuable, a score of 3 was considered Neutral, and scores of 4-5 were classified as Valuable. To test the second hypothesis, the importance rating of culturally significant plants was modeled by five demographic variables (i.e., Age Category, Education Level, Gender, Income Level, Political View, Heritage, and Dollar Appropriateness) and responses to questions 29 and 32. Responses to question number 29 were scored directly and not aggregated; the variable is termed “Dollar Appropriateness” from this point forward.

Question 32 was posed in the survey to identify if the respondent considered the Palouse Prairie as part of their heritage. Respondents were also asked to identify if they “strongly agree”, “somewhat agree”, “neither agree nor disagree”, “somewhat disagree”, and “strongly disagree” with the statement “The Palouse Prairie is part of my heritage”. From this point forward this variable is referred to as “Heritage”. Responses to the question 32 also scored directly and not aggregated. For this point forward this variable is referred to as “Heritage”.

Demographic profile information was also collected to identify the respondents: age category, gender, education level, income level, and political view. Age of the respondent was collected with the question, “What year were you born (YYYY)?” with a blank space for the respondent to fill in. Responses were then categorized by taking the age difference from the year 2014 and tabulating the number of responses within the age categories: 18-25, >25-35, >35-45, >45-55, >55-65, and >65-93. Male or female gender was identified by the respondent selecting a box with “Male” or “Female” below the question “What is your gender?”. Political view was collected with the question, “On a scale of 1 to 7 where 1 is very liberal, 4 is moderate and 7 is very conservative, how would you describe your political views?”. Education level of the respondent was collected with question “What is the highest grade or year of school you completed?”. The respondent could identify education level by selecting the appropriate box with the following categories: “12th grade or less, no diploma”, “High school graduate or GED”, “Some college, no degree”, “Associate’s degree”, “Bachelor’s degree”, “Graduate or professional degree”. Gender, political view, income level, education level, heritage, age category, and dollar appropriateness were verified for homoscedasticity using the Bartlett test and Fligner-Killeen test (alpha 0.05). An eighth demographic factor, “ethnicity/race”, could not be assessed statistically because of a low response rate from minority groups and heteroscedasticity of the data.

The population sampled was people residing within the core area of the Palouse Prairie. This area included all of Latah County, Idaho and Whitman County, Washington. One thousand three hundred households were drawn proportionate to the population size in the two counties. Samples of 100 households were also drawn from Plummer (Benewah County, Idaho), Worley (Kootenai County, Idaho), and Lapwai (Nez Perce County, Idaho) for a total of 300 additional samples. Therefore the frame of this study was an address based sample of 1,600 residents in the Palouse Prairie area. Addresses were purchased from Survey Sampling Inc. of Connecticut. The address purchase was done to capture the perspectives of hard-to-reach populations such as ethnic minorities and those of lower socio-economic status. The towns of Plummer, Worley, and Lapwai are located within the Nez Perce and Coeur d'Alene Indian Reservations. Data collection was conducted by the University of Idaho Social Science Research Unit using a modified Dillman method (Dillman 1978; Dillman et al. 2008). This method has proven useful for increasing response rates (Hoddinott and Bass 1986).

The modified method included four stages. At the first stage, a preselected postcard was mailed to all 1,600 households with a notification to expect a survey letter with an internet link to a web-based survey. The postcard with the world-wide-web based survey link followed within a few days. From this sample, 208 were returned as undeliverable. A $1.00 incentive was sent a week and half later to all non-responsive households to complete the survey. A paper survey with prepaid return envelope was sent to all remaining non-respondents as a final measure to increase response rate. Overall, the sampling strategy resulted in 241 surveys completed online and 180 completed paper copies for a total of 421 completed surveys ($n = 421$).

The sample survey was administered during the summer in a region that includes two large land grant universities. As a result the sample does not necessarily conform to the population demographics of the region when school is in session, but does primarily represent the perspectives of permanent residents of the region.

Data Analysis

Survey data were analyzed using the R program version 3.1.1. The analysis focused on the effect of seven demographic factors: Gender, Political View, Income Level, Education, Heritage, Age Category, and Dollar Appropriateness. We modeled these seven factors for predicting a response to a survey question on the value of culturally significant plants to the respondent and the respondent's family, on a scale ranging from Not Valuable (1), Neutral (2), and Valuable (3).

The ordered logistic regression (OLR) was executed using the *polr* command from the MASS package to estimate a model. This analysis is a proportional odds logistic regression to assess how demographic factors influenced the assessed importance of valuing culturally significant plants. P-values were calculated by comparing the regression t-value against the standard normal distribution. The logistic model used to show the function of the probabilities results in a linear combination of parameters is

$$ln\left(\frac{prob\ (event)}{(1-prob(event))}\right)=\beta_0+\beta_1X_1+\beta_2X_2+\cdots+\beta_kX_k\ .$$

The logit in this case is the ratio of the number of people who placed a value (i.e., Not Valuable, Neutral, Valuable) on culturally significant plants against those who held a different value. The resulting coefficients on the OLR model tell how much the logit changes based on the values of the predictor variables. The purpose of the model was to identify the relationship between value placed on culturally significant plants by gender, political views, income level, education, heritage, age category, and appropriateness of using a dollar value in conservation decisions related to valuing the importance of culturally significant plants.

Proportional probabilities were calculated independently upon predictor variables found to be significant. This was done by using the *polr* command fitting the value category by the variables found to be significant. These variables included: gender, income, political view, heritage, and appropriateness of using dollar value in conservation decisions. Model predictions were done using *predict* command of the Stats package of R.

Results

Interviews

The number of times reoccurring themes and issues identified during interviews with Nez Perce Tribal members is provided in Table 6.2. "The importance and names of natural foods" was the most frequently occurring theme. The traditional uses, practices, and values were the second most reoccurring theme. Two individuals also repeatedly identified a distinct difference in culture between the Nez Perce and dominant Western Society.

The use, knowledge, and importance of natural foods and traditional culture to interviewees were articulated during interviews. It was also revealed through the interviews that the epistemological perspectives of interviewees guided harvesting and use practices of the natural products on Palouse Prairie. One interviewee stated that, "The elders say the importance of one plant or animal should not be called out over another. All are important". Another interviewee stated that, "When you die and your body decays to dust you return to mother earth. This is why the Nez Perce think land is sacred". Another major theme and issue identified through the interviews was that there is a perceived difference in culture between Indigenous and non-Indigenous people. One interviewee stated:

> The dominant society wants to make the world like Europe. What is there you want to protect? Our natural foods and medicines are important to protect. Once these are gone they are irreplaceable. Where else can you get the natural foods? Nowhere. All our land, food, fish, and forest have been taken away.

Another interviewee stated, “White man thinks only certain points in the system are important, when the whole system is important”. There was concern that natural foods and medicines are being lost through environmental degradation and agricultural production activities. Concerns about the use of pesticides were identified by the interviewees. There is perception that when pesticides are applied that “poison is still there” and pesticide use has eliminated certain plant species.

The Palouse Prairie also represented a sense of place for several of the interviewees, either through knowledge of traditional use or features at a particular location. For example, the region now known as Moscow, Idaho, was known by Sahaptin speaking people as *Tatxinme* the “Fawn Place”. It was called the fawn place because it was a known fawning area for deer. It was also an important trading place for Indigenous people. There is also a very old oral tradition about Steptoe Butte and how the butte was once used to escape a flood. Several of the interviewees also stated that in the past the Palouse Prairie was also important for horse pasture.

Of all the plant species identified by the interviewees, camas was mentioned the most. Loss of camas due to agricultural practices (plowing, livestock grazing) and activities (pesticide use) was a major concern. Due to the loss of camas and worry of ingesting pesticides access to “pure” camas has diminished. One interviewee stated that traditional harvesting of camas is beneficial to camas because the digging tills up the soil. The traditional method of harvesting camas by the Nez Perce is to do so after seed ripening (late summer), and dropping seed into the disturbed soil after harvesting bulbs. Another interviewee stated that only large bulbs are harvested and smaller bulbs are left to grow.

Nez Perce elders expressed the importance of educating tribal youth in traditional language and culture, but there is some concern about sharing the knowledge with outsiders. Access to harvesting and use of natural foods and medicines is becoming more difficult due to development, landownership, and spread of invasive non-native species. Tribal members are traveling further to find harvest locations, usually on public land, that have not been impacted by agricultural activities. Due to the losses associated with agricultural production and invasive species there is a need for restoration and more sustainable use of the landscape.

There is also a perception that climate change has impacted water availability and has made wind and temperature more extreme. Oral traditions maintain that rivers used to freeze over and now they never freeze over. There is also a perception that climate change is altering the seasons and harvest times of natural foods and medicines.

There is a perceived lack of support of Tribal interests by local non-Indian politicians and federal land managers, despite the Tribes’ right to exercise off-reservation treaty rights on both federal and private lands. One Tribal member also expressed the desire to reconnect to the Palouse Prairie through hunting, fishing and gathering.

Although some of the respondents stated that all native plant or animal species are considered important, some species were specifically identified as being culturally significant (Table 6.3). Native American's interviewed also identified several locations on the Palouse Prairie where culturally significant plants can be harvested.

Likert plots were used to show sample survey results by demographic groupings (i.e., age category, gender, education level, income level, and political view), heritage, and dollar appropriateness. The age distribution, category of ">45-55" had the highest percentage of respondents who considered culturally significant plants as valuable, while the age-category of ">65-93" years had the lowest (Figure 6.2). Female respondents had a higher percentage that considered culturally significant plants as valuable (Figure 6.3). Males were nearly equally divided between not valuable, neutral, and valuable. Within the education category (Figure 6.4) the group that had "Some College, No Degree" had the highest percentage that considered culturally significant plants valuable, while those with "12th Grade or Less" of "High School Graduate/GED" had the lowest percentage. Overall, the value of culturally significant plants increased with income up to the "$25,000-$34,999" level, but decreased with increasing income levels thereafter (Figure 6.5). Among income level categories those with household incomes of "$25,000-34,999" had the highest percentage considering culturally significant plants valuable, while those with the highest income (i.e., "greater than $100,000") valued culturally significant plants the least. Among the political view demographic (Figure 6.6), the "Strongly Liberal" category had the highest percentage of respondents who considered culturally significant plants as valuable, while the "Strongly Conservative" group had the lowest. Those respondents who strongly agreed that the Palouse Prairie was part of their heritage had the highest percentage who considering culturally significant plants valuable, while those who “Somewhat Disagreed” had the lowest (Figure 6.7). Respondents who selected “Somewhat Appropriate” for using a dollar amount to inform conservation decisions about the Palouse Prairie had the highest percentage that considered culturally significant plants valuable, while those that selected “Not Appropriate” had the lowest (Figure 6.8). In regard to ethnicity/race group (Figure 6.9), the “Hispanic/Latino” category had the highest percentage of respondents who considered culturally significant plants as valuable, while the “Asian/Pacific Islander” group had the lowest.

Overall 384 individuals or 36 percent of all respondents to the survey considered culturally significant plants as valuable and 64 percent were equally divided between neutral and not-valuable. In other words, one-third of the population in Latah County, Idaho, Whitman County, Washington, and three towns (i.e., Plummer, Worley, and Lapwai) located on Native American Reservations consider culturally significant plants valuable. The OLR output modeling the value of culturally significant plants by Age Category, Education Level, Gender, Income Level, Political View, Heritage, and Dollar Appropriateness is provided in Table 6.4.

The odds ratios and confidence intervals for the significant factors are provided in Table 6.5. Based upon the OLR model females are 1.8 times more likely than males to value culturally significant plants as being “Valuable” rather than being “Neutral” or “Not Valuable”, given that all of the other variables in the model are held constant. The second highest odds ratio was dollar appropriateness; as a dollar appropriateness level moved 1 unit, the odds of moving from “Valuable” to “Neutral” or “Not Valuable” (or from the “Valuable” and “Neutral” categories to the “Not Valuable” category) was 1.3 time greater. Income had a moderate odds ratio of 0.8. The lowest odds ratios were Heritage and Political View, with an odds ratio of 0.7.

Based upon the estimated response probabilities for gender (Table 6.6), males had a higher probability than females of considering culturally significant plants as not valuable while females had higher probability for considering culturally significant plants as valuable; the finding supports a sex-specific difference in ethnobotanical valuation.

The estimated response probabilities indicate lower income levels place a higher value upon culturally significant plants than higher income levels (Table 6.7). The "<$15,000" income level group had the highest response probability for considering culturally significant plants as valuable. While the lowest income level group valued culturally significant plants the most the highest income level group valued culturally significant plants the least. The income analysis suggests culturally significant plants within the study area are mostly a good valued by the poor.

The estimated probabilities according to political views are provided in Table 6.8. Overall, there was a strong difference in response between self-defined liberals and conservatives. Based upon the estimated response probabilities, "Very Liberal" respondents had the highest probability for considering culturally significant plants as valuable, while "Very Conservative" respondents had the lowest probability for valuing culturally significant plants. The political view analysis suggests that culturally significant plants are mostly valued by individuals with liberal political views, the value of culturally significant plants decreases considerably with conservatism.

The estimated response probabilities for valuing culturally significant plants in relation to how respondent rated their heritage affiliation to the Palouse Prairie is provided in Table 6.9. The response probabilities for considering culturally significant plants as valuable were highest for respondents who selected “Strongly Agree” that the Palouse Prairie was part of their heritage and lowest for the “Strongly Disagree” group. The analysis on heritage affiliation with the Palouse Prairie suggests that those who consider it to be part of their heritage, value culturally significant plants the most, while those who do not consider the Palouse Prairie as part of their heritage value culturally significant plants the least.

The estimated response probabilities for valuing culturally significant plants for the question that examined how the respondent felt about basing conservation decisions upon a dollar amount is provided in Table 6.10. The response probability for considering culturally significant plants as valuable was highest for those respondents who selected “Very Appropriate” for using a dollar amount to inform conservation decisions. The response probability was lowest for those individuals who considered it “Not Appropriate” to use a dollar amount to inform conservation decisions.

Discussion

Interviews

Although little remains of the natural Palouse Prairie grassland, this study found that Native Americans of the region, as assessed through semi-structured interviews, and 36% of the general population surveyed considered culturally significant plants, lichens, mosses, and fungi of the Palouse Prairie to be valuable. Plant species such as camas were repeatedly identified as being important to the Nez Perce. The Nez Perce also conveyed during interviews that the importance of plants must be viewed in broader context of its function and contribution to the ecosystem. Furthermore, resource policy should consider input from Native Americans, as their knowledge, values, and use of the ecosystem may contribute to replenishment of natural plant foods and ecosystem services. For example, the Nez Perce method of harvesting camas after seed set and replanting of seed after harvesting disturbance or the practice of only taking the larger bulbs and replanting of the smaller bulbs. In some instances ecosystem conservation initiatives of Western Societies may be too quick to strictly exclude Native American human uses, without fully understanding how the practices or use contributes to ecosystem function and resiliency. The urgency of adopting aspects of traditional ecological knowledge of Native Americans into modern policy development may be a powerful tool to combat the profound and widespread ecocide and pollution we are experiencing today (Wildcat 2009).

Sample Surveys

Ethnobotanical valuations among people are thought to be dependent upon many factors. These factors can include: ethnicity, gender, age, education level, religious and cultural beliefs, abundance and usefulness of plant species, social status, income level, profession or role in the community and at home, mental capacity, as well as control and access to natural resources (Holt 2005, Ayantunde et al. 2008; Sop et al. 2011). There was no significant difference in value responses for the age and education level demographic variables. However, we did find a pattern that females more frequently agreed that culturally significant plants are valuable. Previous research has indicated that gender difference may be attributed to women’s roles in the local community or at home, and profession (Voeks

2006; Rangel de Almeida et al. 2010). However, further research would be necessary to identify why there was a difference between genders.

There was a valuation difference among differing income levels, as individuals in lower income classes placed a higher value upon culturally significant plants. Our finding is similar to the finding of Benz et al. (2000) who identified the most marginal of the communities in Manantlan, Mexico who used a wider diversity of plants and had more uses of individual species. However, our finding was different in that we assessed how varying income levels valued cultural significant plants as whole. As approximately one third of the local community considered culturally significant plants as valuable, further research towards identifying individual species of the local community and their uses would be highly important for conservation purposes.

The political view analysis suggested that culturally significant plants are mostly valued by individuals with liberal political views and their value decreases considerably with conservatism. We were unable to find any valuation of culturally significant plants that assessed how political views influenced value choices. However, based upon surveys from the World and European Values Surveys, Neumayer (2004) found left-wing orientations embraced pro-environmental issues. If valuing culturally significant plants is considered pro-environmental, our findings that self-identified liberals value culturally significant plants more than conservatives support the findings of Neumayer (2004).

We were unable to find any published study that valued culturally significant plants by assessing how the respondent self-identified heritage connection with the study area. Our findings suggest that individuals with a heritage connection with a region value culturally significant plants higher than those who do not have such a connection. In regard to the valuation of culturally significant plants based upon how the respondent felt about basing conservation decisions upon a dollar amount, individuals who valued culturally significant plants are also concerned about costs associated with conservation. As a result, conservation costs are an important factor to consider during policy development to conserve culturally significant plants.

Conclusions

Previously reported valuation systems of culturally significant plants are often based upon one or more of the following attributes: number of potential uses, number of participants identifying a particular species, utilitarian purposes, taste appreciation, perceived quality, financial benefits provided, contingent valuations, marginal costs, time and travel spent harvesting and processing, selling price on the market, and value in local markets (Hunn 1982; Turner 1988; Stoffle et al. 1990; Phillips et al. 1994; Godoy et al. 1993; Pieroni 2001; Cocks and Wiersum 2003; Reyes-García et al. 2006; Thomas et al. 2009). Many of these studies have made an attempt to prioritize value to individual species, and focus on how a single social group (e.g., indigenous hunter gatherer society) values a particular plant taxon. This study differs from most of the previous work on valuation of culturally significant plants in two important respects. Although many prior studies focus on a single social group (e.g., *mestizos* from Tambopata area, Peru; Paiute and Shoshone of the Western, United States) and how its members value a particular plant taxon, this study considers the culturally significant plant community as a whole, and it includes responses from Indigenous and non-indigenous populations of a single region.

Methods that focus on the value of an individual plant taxon in itself inherently reveal an epistemological difference between Western societies and Indigenous people, who tend to view natural systems holistically. For example, similar to the findings in this study, Turner (1988:274) noted that when asking which plants are most important, a knowledgeable Salish tribal elder responded “I’d pick them all – they’re all important”. On the other hand, existing valuation methods and research developed by Western scientists, which are often done in response to development imperatives, go to great efforts to define cultural significance of a plant taxon based upon the researcher’s perceived role it plays within a particular culture.

Based upon the findings of this study, social support to conserve culturally significant plants exists is strongest among females, Nez Perce Tribal members, individuals with liberal political views, people who consider the Palouse Prairie as being part of their heritage, and people within lower income classes. However, in productive landscapes like the Palouse, the agricultural-dependent community is the primary steward of biodiversity, ideally generating co-benefits for regional biodiversity conservation and local peoples (Scherr and McNeely 2008). This presents a challenge in the Palouse where the agricultural sector demographic does not align with the demographics that most strongly value culturally significant plant. As the demographic groups that are likely best situated to make decisions about agricultural production on the Palouse include wealthy white male conservatives.

One potential strategy to conserve culturally significant plants would be to coordinate conservation efforts with local Indian Tribes. For example, Nez Perce Tribal members identified several locations along major roadways and natural areas on the Palouse Prairie where camas continues to thrive. These areas could be recognized as important features of the cultural and natural heritage of the region, justifying their management as remnants of biodiversity beneficial to the local community, including Native American Tribes. As part of management of culturally significant plants and biodiversity, the local community could also consider impacts associated with agricultural inputs and make efforts to minimize environmental pollution. This is especially important considering that some of the Native Americans interviewees identified a concern about the harvesting of natural foods that have been exposed to pesticides or pollution. Another potential strategy to benefit biodiversity and culturally significant plants of the region would be to analyze existing and potential habitat networks at a landscape scale that can be enhanced to improve ecosystem integrity, structure, and function (Freemark et al. 2002; Scherr and McNeely 2008). For example, plantings of desirable native species along roadways and field margins could promote habitat connectivity and have little or no impact upon agricultural production. Where possible these networks could be targeted within existing patches with highest biodiversity or natural areas.

Although this study did not emphasize economic valuation of culturally significant plants out of respect for social perspectives that would reject the notion of placing a dollar amount on something considered sacred, the general population survey revealed a tendency to agree with the appropriateness of using a dollar amount to inform conservation decisions. This finding suggests a need to identify restoration and maintenance costs as part of the ecosystem service valuation process, despite the views of some stakeholders that economic valuations can be inappropriate. Overall, the findings highlight the importance of the recommendation by Chan et al. (2012) of using a multi-metric approach to valuing ecosystem services. We therefore hope our result can facilitate valuation that is representative of the diversity of viewpoints among stakeholders of the Palouse Prairie and thus more readily and widely accepted. The results indicate that the importance of conserving culturally significant plants should incorporated into policy development in the Palouse region. Furthermore, this study illustrates the importance of considering the knowledge and philosophies of Indigenous peoples, and the role this knowledge can serve to protect ecosystem services, biodiversity, and culture.

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Table 6.1 Question 28, 29, and 32 of the Palouse Prairie sample survey instrument. Questions related to valuing culturally significant plants and were included in a more comprehensive survey aimed at valuing ES on the Palouse Prairie. [2-column fitting image]

Question No. on Survey	Text of Question	Response options
28	Culturally significant plants are defined as any native plant, lichen, moss, or fungus that can be used for food, teas, medicine, in ceremonies or materials in artisan craft. On a scale of 1 to 5, with 1 being not valuable at all and 5 being extremely valuable, please rate how valuable culturally significant plants, lichens, mosses and fungus of the Palouse Prairie are to you and your family	Likert 1-5 with 1 indicating Not Valuable At All and 5 indicating Extremely Valuable
29	How appropriate or inappropriate is it to use a dollar amount to inform conservation decisions about Palouse Prairie	Likert 1-5, with 1 indicating Not Appropriate, 3 indicating Neutral, and 5 indicating Very Appropriate
32	The Palouse Prairie is part of my heritage	Likert 1-5, with 1 indicating Strongly agree, 2 indicating Somewhat agree, 3 indicating Neither agree nor disagree, 4 indicating Somewhat Disagree, 5 indicating Strongly disagree

Table 6.2 The number of Palouse Prairie issues and themes identified as priority or concern during interviews with Nez Perce Tribal members.

Theme/Issue	Number of Times Mentioned by Interviewees
Importance and names of natural foods	19
Traditional uses, practices, and values	12
Differences in culture among Natives and non-Natives	10
Degradation/loss of traditional cultural landscape	9
Tribal epistemology (i.e., All of the natural environment is important/connected, Lessons from Animals/Nature, Gifts from Creator)	9
Camas	7
Sense of place	7
Importance of educating Tribal members of traditional knowledge and language	6
Loss of access	5
Natural medicines	4
Seasonal uses	3
Invasive species	3
Pesticide use	3
Need for restoration	3
Traditional methods to promote natural replenishment	3
Climate change	3
Maintenance of Treaty Rights	3
Self-belief(s)	3
Importance of horse pasture	2
Exercise of off-Reservation Treaty Rights	2
Degradation/loss of water	2
Dominant society failure to acknowledge impacts upon Native Americans and ecosystem	2
Importance of Treaty Right consultation	1
Importance of protecting traditional knowledge	1
Desire to reconnect to Palouse Prairie	1
Tactics of colonization	1

Table 6.3 List of specific native plants[1] of the Palouse Prairie identified as culturally significant to the Nez Perce Tribe during 2011 and 2013 semi-structured interviews.

Life Form Type	Scientific Name	Name Used by Interviewee
Bryophyte	Unknown	*Hoopop,* Pine moss
Vascular Plant	*Camassia quamash*	*Quem'es*, camas
Unknown	Unknown	Indian tea
Vascular Plant	*Vaccinium membranaceum*	Huckleberry
Vascular Plant	*Lomatium cous*	*Cous cous*
Vascular Plant	Unknown	*Qeqeite*
Fungus	Unknown	*Hepau*
Vascular Plant	Unknown	*Weim*, Celery
Vascular Plant	*Balsamorhiza sagittata*	*Pask*
Vascular Plant	Unknown	*Tetineze,* Shiners
Unknown	Unknown	Mountain tea
Vascular Plant	*Allium* sp.	Onion
Fungus	*Morchella* sp.	Morels

1Although these species were specifically identified during the interviews, Nez Perce Tribal members adhere to oral tradition that all native plants and animals are important.

Table 6.4 Ordered logistic regression output modeling value of culturally significant plants by Age Category, Education Level, Female Gender, Income, Political View, Heritage, and Dollar Appropriateness. Output includes coefficient table including the value of each coefficient, standard error, t-value, estimated p-value, and 95% confidence intervals.

Factor	Coefficient Value	Standard Error	t-value	p-value	2.5%	97.5%
Age Category	-0.01194	0.07332	-0.1628	0.8706	-0.1561	0.1318
Education Level	0.09393	0.08294	1.1325	0.2574	-0.0688	0.2569
Female Gender	0.58374	0.22883	2.5510	0.0107	0.1365	1.0346
Income Level	-0.18625	0.06240	-2.9847	0.0028	-0.3097	-0.0647
Political View	-0.28513	0.06947	-4.1046	<0.0000	-0.4231	-0.1503
Heritage	-0.41513	0.10037	-4.1362	<0.0000	-0.6150	-0.2208
Dollar Appropriateness	0.25683	0.09963	2.5778	0.0099	0.0623	0.4536

Table 6.5 Odds ratios and lower and upper confidence intervals.

Factor	**Odds Ratio**	**2.5%**	**97.5%**
Female Gender	1.7927	1.1462	2.8139
Income	0.8301	0.7337	0.9374
Political View	0.7519	0.6550	0.8605
Heritage	0.6603	0.5406	0.8019
Dollar Appropriateness	1.2928	1.0642	1.5740

Table 6.6 Estimated response probabilities for gender.

Culturally Significant Plants	Male	Female
Not Valuable	0.37	0.22
Neutral	0.34	0.32
Valuable	0.29	0.46

Table 6.7 Estimated response probabilities by income level.

Culturally Significant Plants	<$15,000	$15,000-$24,999	$25,000-$34,999	$35,000-$49,999	$50,000-$74,999	$75,000-$99,999	>$100,000
Not Valuable	0.19	0.22	0.25	0.28	0.32	0.37	0.41
Neutral	0.28	0.29	0.31	0.32	0.32	0.32	0.31
Valuable	0.53	0.49	0.44	0.40	0.35	0.31	0.28

Table 6.8 Estimated response probabilities by political view.

Culturally Significant Plants	Very Liberal	Liberal	Moderate Liberal	Moderate	Moderate Conservative	Conservative	Very Conservative
Not Valuable	0.14	0.18	0.24	0.30	0.38	0.46	0.54
Neutral	0.27	0.31	0.33	0.35	0.34	0.33	0.29
Valuable	0.59	0.51	0.43	0.35	0.28	0.21	0.17

Table 6.9 Estimated response probabilities that the Palouse Prairie is considered part of the respondent's heritage.

Value of Culturally Significant Plants	Strongly Agree	Somewhat Agree	Neither Agree Nor Disagree	Somewhat Disagree	Strongly Disagree
Not Valuable	0.16	0.23	0.31	0.41	0.51
Neutral	0.28	0.32	0.34	0.33	0.30
Valuable	0.56	0.45	0.35	0.26	0.19

Table 6.10 Estimate response probabilities on how appropriate or inappropriate it is to use a dollar amount to inform conservation decisions about the Palouse Prairie.

Value of Culturally Significant Plants	Not Appropriate	Somewhat Not Appropriate	Neutral	Appropriate	Very Appropriate
Not Valuable	0.49	0.42	0.34	0.27	0.21
Neutral	0.30	0.32	0.33	0.32	0.30
Valuable	0.21	0.26	0.33	0.41	0.49

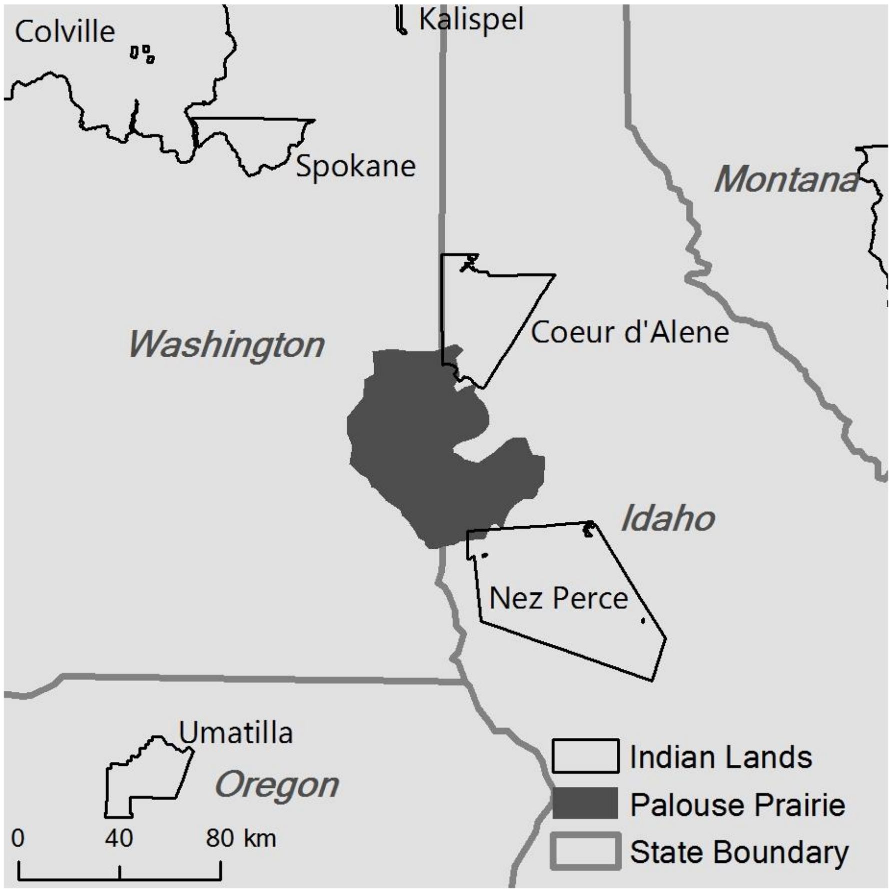

Figure 6.1 The core area of the Palouse prairie region in northern Idaho and southeastern Washington.

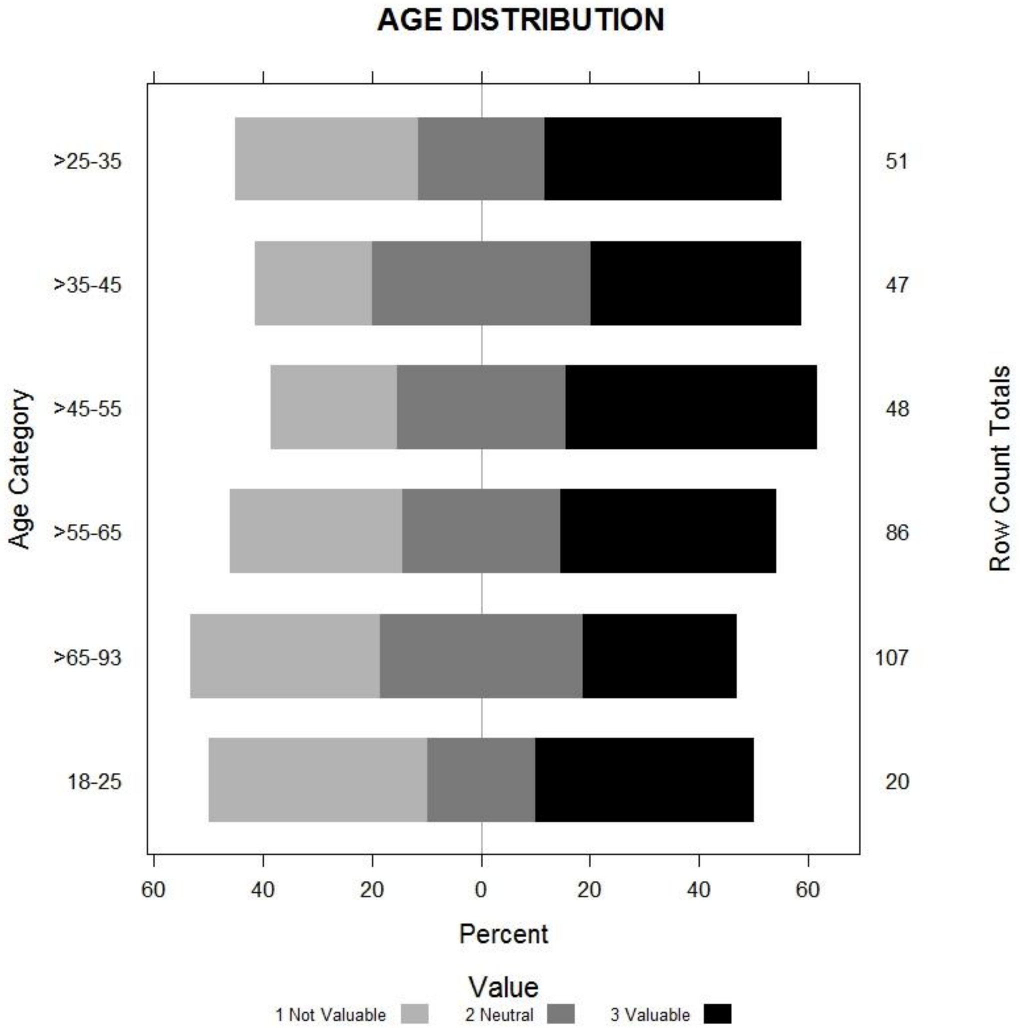

Figure 6.2 Likert plot of the proportions of survey respondents indicating culturally significant plants are not valuable, neutral, or valuable to themselves and their families, sorted by age category. Percentage of responses is provided on the x-axis, age category on the left y-axis and row count totals (tabulations) on the right y-axis.

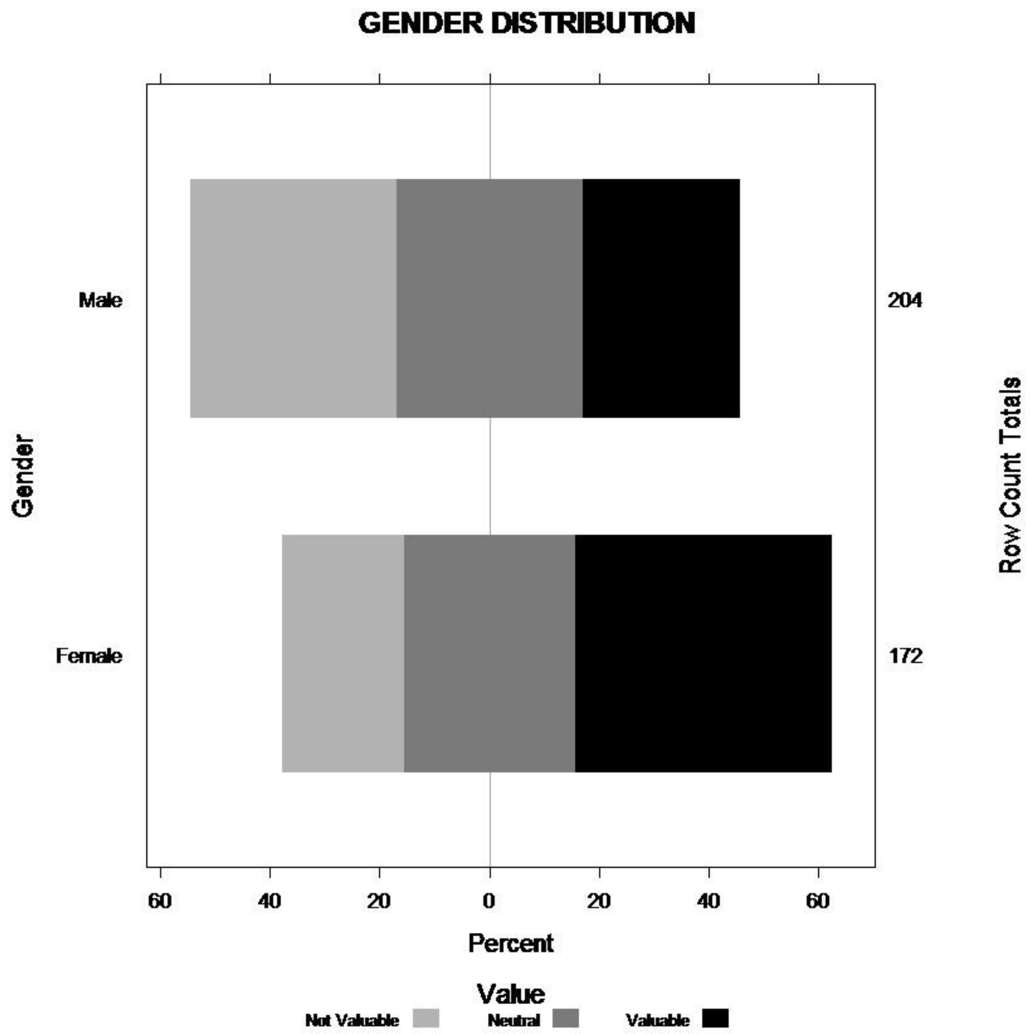

Figure 6.3 Likert plot of the proportions of survey respondents indicating culturally significant plants are not valuable, neutral, or valuable to themselves and their families, sorted by gender. Percentage of responses is provided on the x-axis, gender category on the left y-axis and row count totals (tabulations) on the right y-axis.

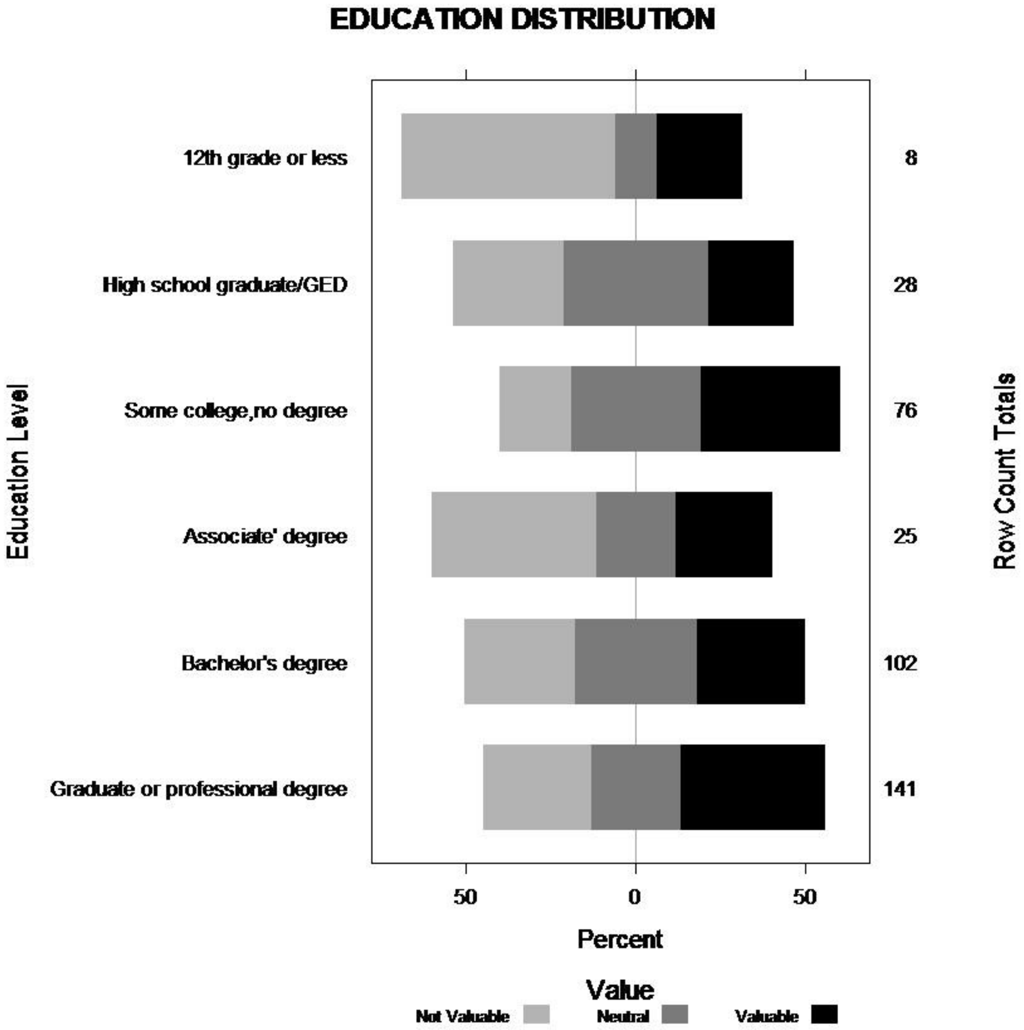

Figure 6.4 Likert plot of the proportions of survey respondents indicating culturally significant plants are not valuable, neutral, or valuable to themselves and their families, sorted by education level. Percentage of responses is provided on the x-axis, education level on the left y-axis and row count totals (tabulations) on the right y-axis.

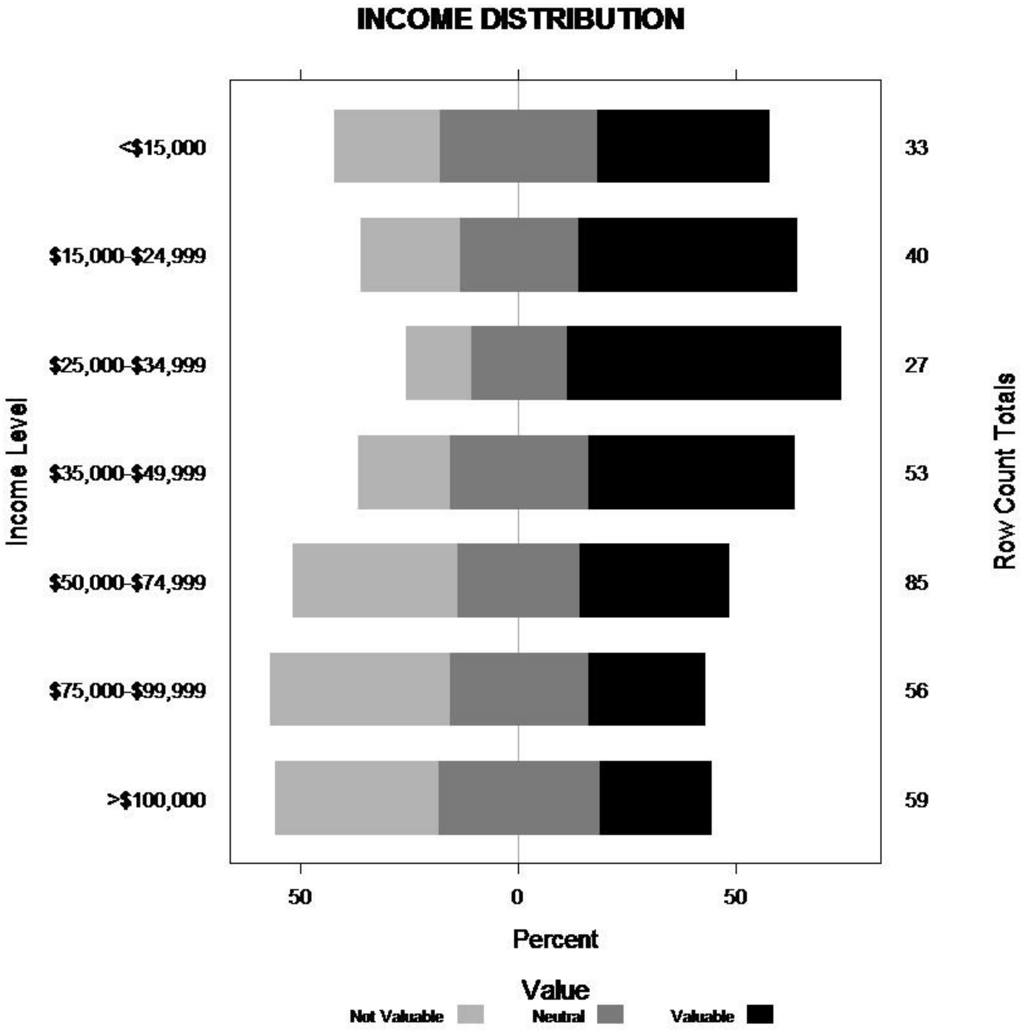

Figure 6.5 Likert plot of the proportions of survey respondents indicating culturally significant plants are not valuable, neutral, or valuable to themselves and their families, sorted by income level. Percentage of responses is provided on the x-axis, income level on the left y-axis and row count totals (tabulations) on the right y-axis.

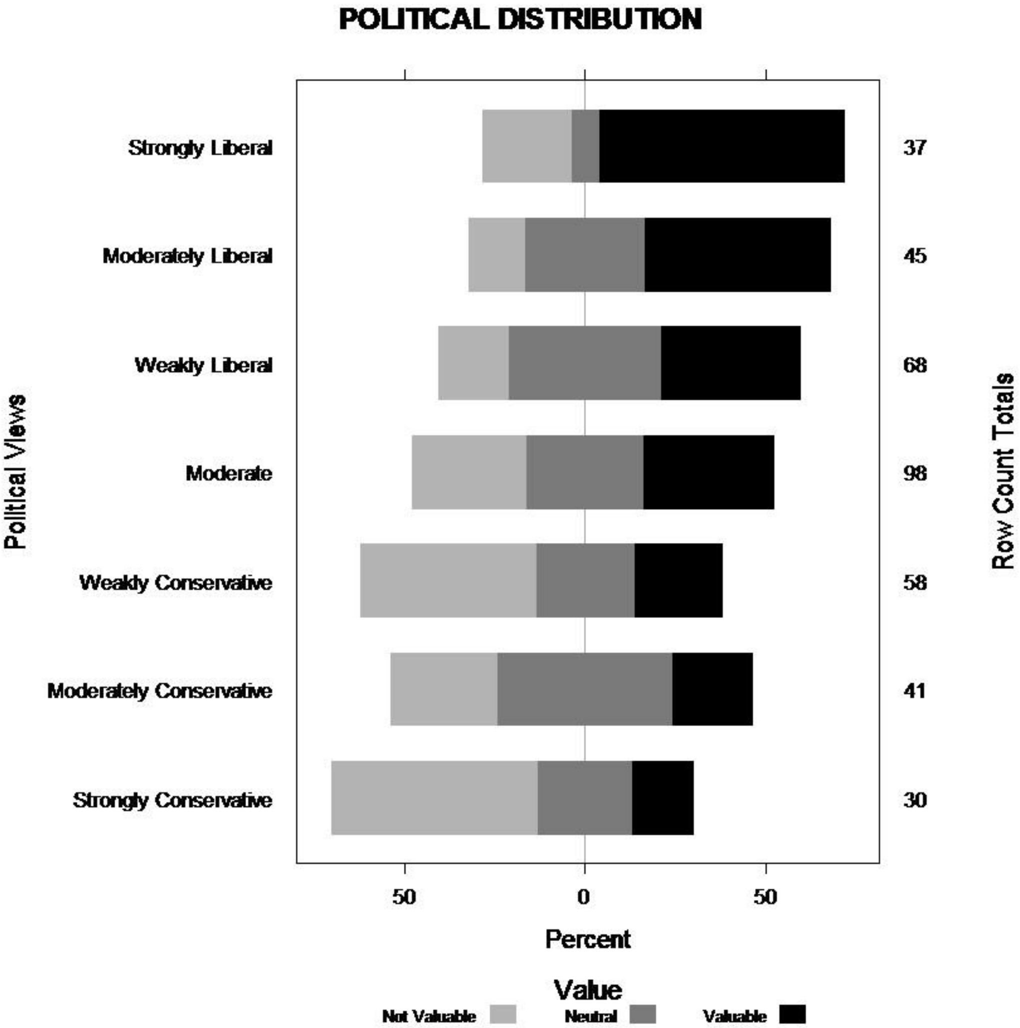

Figure 6.6 Likert plot of the proportions of survey respondents indicating culturally significant plants are not valuable, neutral, or valuable to themselves and their families, sorted by political views. Percentage of responses is provided on the x-axis, political views on the left y-axis and row count totals (tabulations) on the right y-axis.

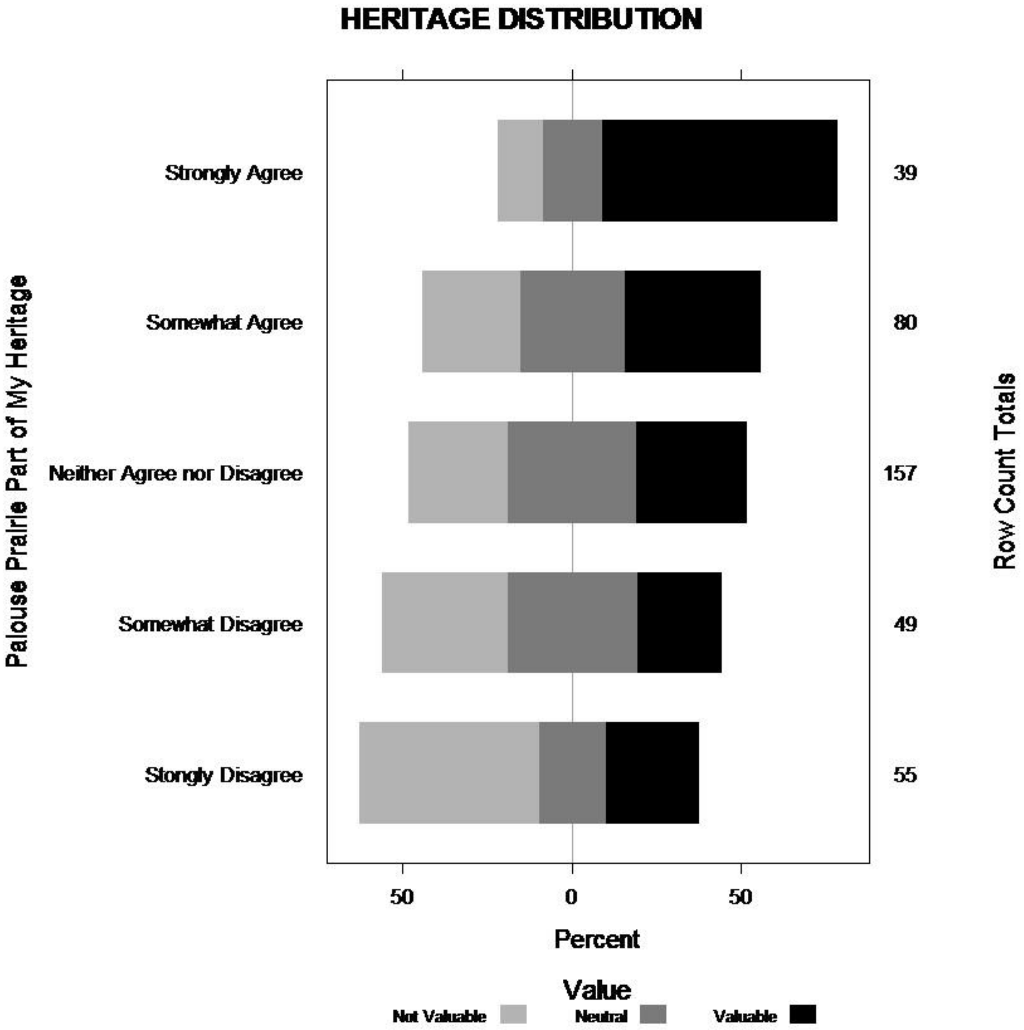

Figure 6.7 Likert plots show rating scale by heritage value choices of culturally significant plants. Row count totals (tabulations) by category are provided on the right y-axis label of the Likert plots. Percentage of respondents' choice by value rating is provided on the x-axis

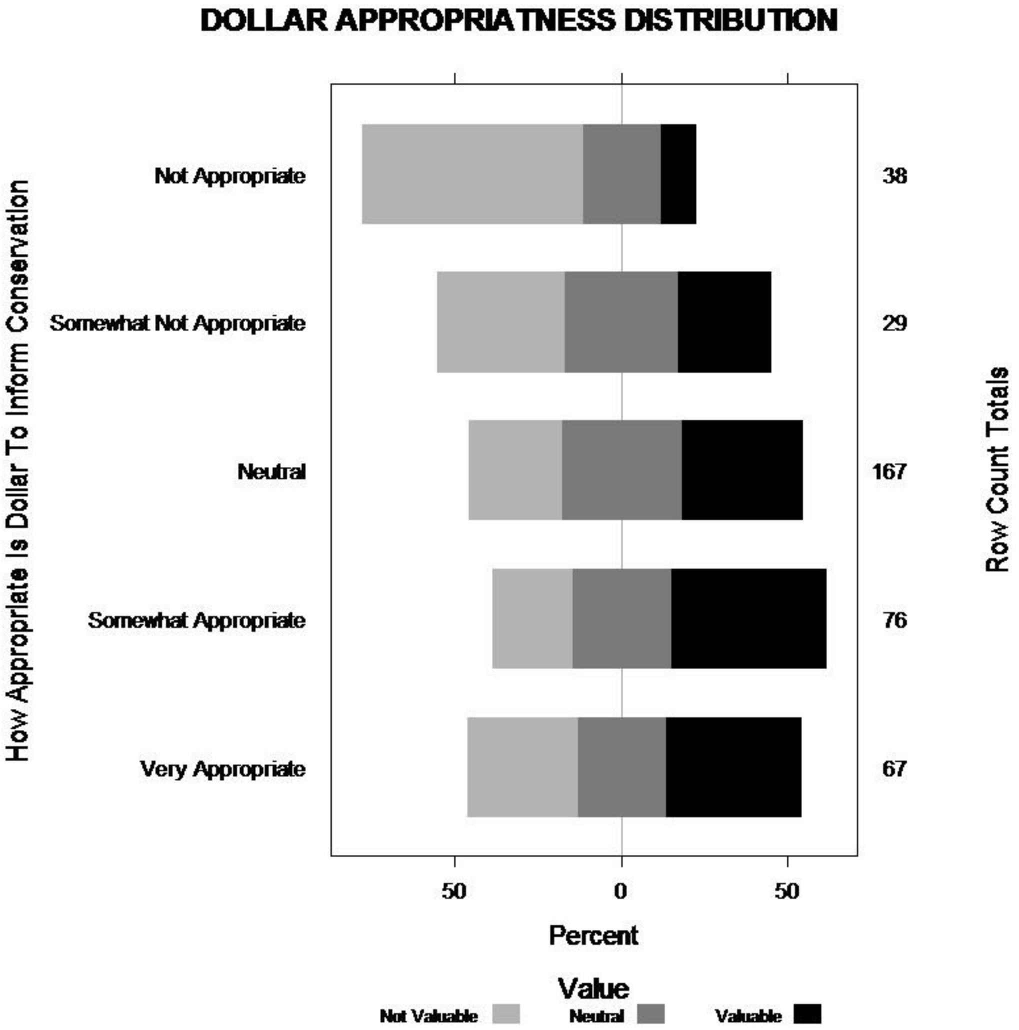

Figure 6.8 Likert plots show rating scale by dollar appropriateness value choices of culturally significant plants. Row count totals (tabulations) by category are provided on the right y-axis label of the Likert plots. Percentage of respondents' choice by value rating is provided on the x-axis.

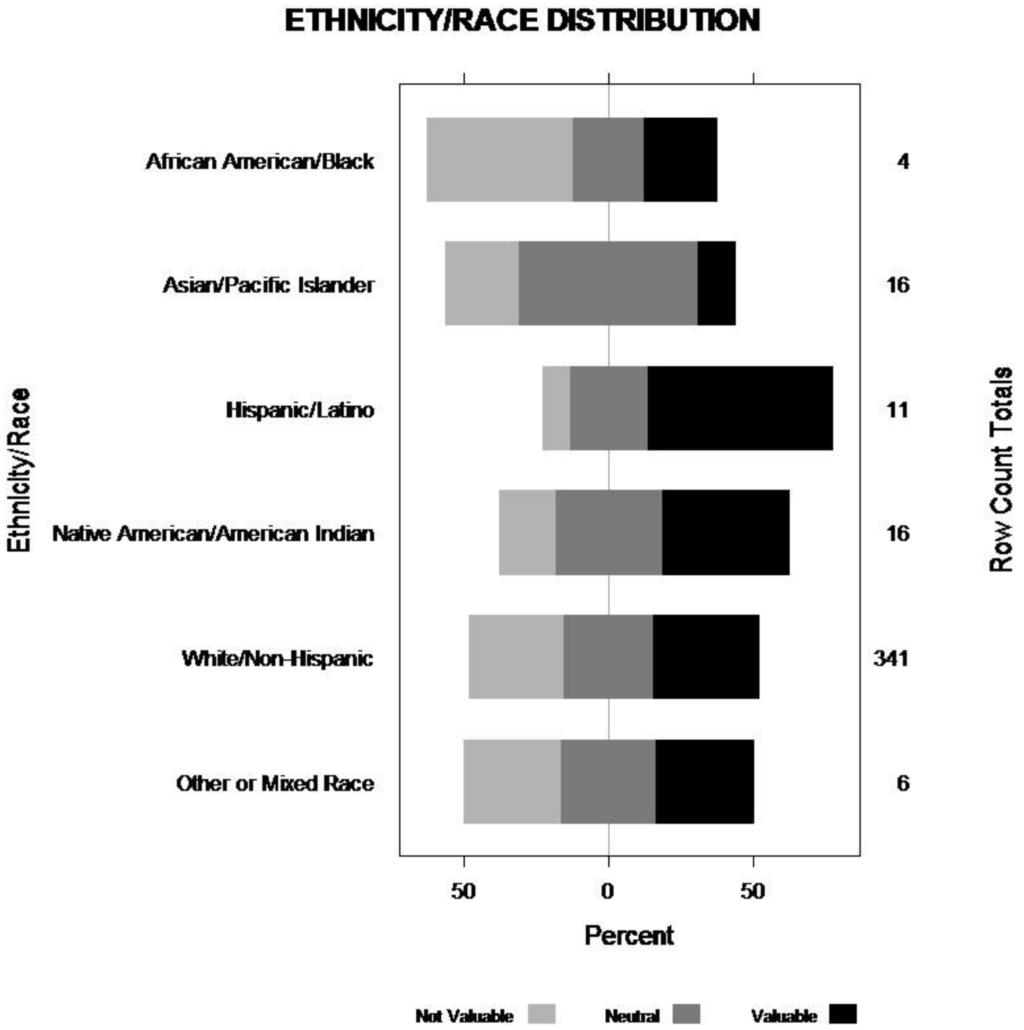

Figure 6.9 Likert plots show rating scale by ethnicity/race value choices of culturally significant plants. Row count totals (tabulations) by category are provided on the right y-axis label of the Likert plots. Percentage of respondents' choice by value rating is provided on the x-axis