

# Conservation Status and Population Structure Comparisons of Abundant and Declining Bumblebee Species

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## Final Report to the Audubon Society of Greater Denver, Lois Webster Fund

Bumblebee declines have been documented in many parts of the world (Williams et al. 2009, Williams and Osborne 2009). The importance of bumblebees as pollinators of native plants and crops has been well documented (Free 1993, Kearns and Thomson 2001, Goulson 2003, Velthuis et al. 2006) and their decline could have negative effects on pollination services (Kearns et al. 1998, Allen-Wardell et al. 1998, Potts et al. 2010). Declines appear to be related to a wide variety of factors including anthropogenic habitat change, nesting site availability, loss of overwintering habitat, and pesticide use (Kearns and Thomson 2001, Goulson et al. 2005, Evans et al. 2009). In North America, non-native parasites transmitted from commercially raised bumblebee pollinators have been implicated as well (Evans et al. 2009). Most studies have focused on environmental factors as the cause of declines. Only recently has population genetics been employed to detect genetic vulnerability of bumblebees (Goulson et al. 2008, UN FAO 2008, Zayed 2009).

Several species of North American bumblebees are considered to be in decline, including *Bombus affinis*, *B. terricola*, and *B. occidentalis* (Evans et al. 2009). *B. franklini* (with a restricted range in California and Oregon) has not been collected since 2006 (IUCN 2009). *B. pensylvanicus* and *B. sonorous*, with broad ranges, are also listed among species that are in decline (Committee on the Status of Pollinators in North America 2007). Baseline data have not been compiled for most regions of North America, making it difficult to assess the conservation status of bumblebee species or to plan their management (Committee on the Status of Pollinators in North America 2007). Although species ranges can be deduced from older collections, it is only recently that people have practiced systematic sampling to document the abundance of bumblebees (Williams and Osborne 2009). In regions where intensive monitoring has been conducted, negative trends have been demonstrated. The National Academy of Sciences Committee on the Status of Pollinators (2007) specifically calls for the long-term monitoring of North American pollinators and the use of genetic techniques to examine risks to pollinators.

In Boulder County, we have records as well as bumblebee specimens dating back to the late 1800s that provide information on which species occurred in the area. Based on location data, we can also estimate the elevation at which these species were collected. Only recently has my research team attempted to document abundance of these species through systematic sampling to evaluate the status of bumblebees at several elevations along the Front Range.

In addition to the monitoring work, we have begun to evaluate the role of genetic variation in bumblebee declines by using molecular techniques to compare declining species with stable, more abundant species. Conservation genetic research on bumblebees is in its early stages (Packer and Owen 2001, Zayed 2009), but two recent papers indicate that low genetic diversity can contribute to bumblebee declines (Cameron et al. 2011, Lozier and Cameron 2009). Low genetic diversity is associated with an inability to adapt to environmental change (Packer and Owen 2001, Schmitt and Hewitt 2004, Zayed 2009). Thus reduced genetic diversity would make bumblebees even more susceptible to environmental factors like pesticides, newly introduced parasites and habitat fragmentation. An understanding of genetic variability and population structure of bees will provide information relevant to the conservation and management of species (Zayed 2009).

## **Project Description**

### ***Field Work***

#### ***Methods***

Surveys of bumblebee diversity and abundance began during the summer of 2010 and resumed in May of 2011. The surveys will continue through 2014. Nine study sites have been established at three elevations in the Front Range of Colorado, to take advantage of the steep elevational gradient and diversity of plant communities in the Front Range. Three low elevation sites (about 1,700 m) are grasslands of the Great Plains, three mid elevation sites (about 2,600 m) are in meadows embedded in forests of mixed conifers and aspen, and three high elevation sites (about 3,350 m) are in the alpine zone immediately east of the Indian Peaks Wilderness Area, which forms the Continental Divide. Surveys of each one-hectare site were performed six or more times over the course of the summer of 2010 and three to nine times in 2011 (Table 1). Teams captured bees with hand nets and bumblebees were identified in the laboratory and preserved for genetic analyses. Additional sites on disjunct mountains and on the eastern plains will be surveyed in the next few years to provide more material for genetic comparisons.

In 2011, low elevation plots were first surveyed on May 26<sup>th</sup>, delayed by two weeks of rainy weather that kept bumblebees from flying. Dr. Diana Oliveras and I conducted the field work with the assistance of Summer Sugg, an undergraduate student assistant. High elevation plots remained under snow later than expected this summer, so sampling there was delayed (Table 1).

In addition to sampling the nine regular plots, we also surveyed the following locations:

Kodak State Wildlife Area, Weld County

Fred State Wildlife Area, Weld County

Elk Meadows, University of Colorado Mountain Research Station, Boulder County

Long Lake perimeter, Boulder County

Brainard Lake Road, Boulder County

## **Results**

Eighteen species of bumblebees were collected in 2011 (Table 2; identifications will be confirmed by CU entomology collection manager).

Two species of concern to conservation biologists are *Bombus occidentalis* and *B. pensylvanicus*. Both species have shown declines in recent years in other parts of the United States. According to entomologists, both species were once common in Boulder County. We did not collect a single specimen of *B. occidentalis*, and saw only one individual *B. pensylvanicus* in 2011.

The number of bumblebees caught per unit time was compared among sites, elevation and years (2010 and 2011) using analysis of variance (ANOVA; SAS 9.3). There was no significant difference between years or between the sites within each elevation ( $p = 0.19$ ,  $df = 6$ ). However, there was a significant difference in the rate of capture among elevations ( $p = 0.02$ ,  $df = 2$ ). The mean capture rate at the low elevation was significantly lower than either the high or mid-elevation (Bonferroni post-hoc test; mean capture/hour = 6.19 at the high elevation, 6.15 at the mid-elevation, and 2.31 at the low elevation). Capture rates remained high throughout August in the mid and high elevation plots (Figure 1).

All the bees collected from the nine sites in 2010 have been identified, labeled and databased, and identifications have been checked by Virginia Scott, a bee expert at the CU Museum's (UCMC) entomology collection. The specimens from 2011 are currently being processed, and all specimens are being incorporated into the UCMC collection.

## **Lab Work**

### **Methods**

In many species, there are robust associations between molecular variability and population fitness (Reed and Frankham 2003). I hypothesized that genetic variation would be greater for *Bombus appositus* than for *B. occidentalis* and *B. pensylvanicus*, two less abundant species. Both *B. appositus* and *B. occidentalis* are known to have occurred across the elevational distribution of the study. While *B. appositus* populations appear to be stable, *B. occidentalis* populations have declined precipitously over the last few years in many parts of western North America (Williams and Osborne

2009). *B. pensylvanicus* lacks the elevational distribution of the other two species but has a broad distribution throughout much of the US and southern Canada. It occurs only at our low elevation sites. Lozier and Cameron (2009) have characterized its genetic diversity in Illinois, where the species is in decline.

I have extracted DNA from 46 *B. appositus*, 47 *B. occidentalis* and 42 *B. pensylvanicus* specimens using Qiagen Tissue Extraction kits (Qiagen Sample and Assay Technologies, Valencia, CA, US office). DNA quality of a subsample (30 specimens) was checked using a Thermo Scientific Nanodrop Spectrophotometer. These specimens included fresh material collected for this project, as well as historical museum specimens. Comparisons of genetic variation of the modern specimens and old specimens provide insight into changing levels of genetic variation over time. Museum specimens came from both the UCMC and from the entomology collection of Colorado State University, and date from 1902 to 2004. Extraction of DNA from museum specimens involves only the removal of one leg from the specimen, preserving the specimen's value (Thomsen et al. 2009, Strange et al. 2009).

Polymerase Chain Reaction (PCR) technology was used to amplify segments of mitochondrial DNA (mtDNA) extracted from these bees. Two primer pairs were chosen to isolate (1) a section of mtDNA of the cytochrome b gene, (Primer BGL; Collins and Gardner 2001) and (2) mtDNA of the 16S region (Primer HG; Hines et al. 2006) from each specimen. Differences in both genes have been used to detect recent evolutionary changes among insect populations. Polymorphisms in cytochrome b are reflected in amino acid substitutions in a cytochrome within the cellular respiration electron transport chain. The 16S mtDNA codes for ribosomal RNA within the mitochondria. Thus both genes are essential to proper functioning of the mitochondria.

The primers isolate base pair segments ranging from 450 to 950 base pairs. Results using these primers were good for bee specimens collected in 2010. However, the results were not always consistent with the older museum specimens. Dr. Jessica Metcalfe (Ecology and Evolutionary Biology, University of Colorado), who works with ancient DNA, suggested that the older DNA had degraded into small fragments. Since DNA naturally deteriorates as specimens age, and can be negatively affected by storage and processing of museum specimens, degradation varied among specimens. Therefore, new primers were designed (Mitton, University of Colorado) to isolate smaller fragments of genetic material, ranging from 150 to 400 base pairs. Using these new primers, DNA was successfully amplified from older museum specimens as well as fresh material.

Amplified PCR products from these two primers were sent out for sequencing (Sequencing Lab, Molecular, Cellular and Developmental Biology, University of Colorado, and Functional Biosciences, Madison, WI). Sequences from different specimens were compared using Sequencher® software

(Gene Codes Corporation, Ann Arbor, MI). Forward and reverse sequences from each specimen were compared for accuracy. Some of the specimens did not sequence well and these were omitted from the results. These specimens will be re-amplified and sequenced again in the near future.

The cytochrome b mtDNA fragment was identical for all specimens of *B. occidentalis* tested (Table 3). There were only two disagreements among the 38 individuals of *B. appositus*. Far more variation was present for *B. pensylvanicus*, but all of the variation was among two individuals collected in 2010. All other specimens were all identical for this gene fragment.

For the 16S gene, variation was greatest for *B. occidentalis*, but all the variation was due to three individuals. Variation was high among *B. pensylvanicus*, with many individuals contributing to the differences. Notably, the same two individuals that were significantly different than the rest for the cytochrome b fragment were also different for this locus. Variation was the least for *B. appositus*.

## **Discussion**

### ***Field surveys***

Insect populations tend to show large variations in spatial and temporal variability (Herrera 1988; Minckley et al. 1999; Cane and Tepedino 2001; Roubik 2001; Williams et al. 2001). Surveying bumblebees for two summers may not be adequate to produce a complete species list for this region, and I intend to continue surveying for at least three more summers. However, it is interesting to note that the two species of concern in other parts of the US, *B. occidentalis* and *B. pensylvanicus*, both once common along the Front Range of Colorado, were largely absent from our study. In fact, only a single individual of *B. pensylvanicus* was collected during the two-year survey. This finding suggests that these species are following a similar trend of decline along the Front Range. *B. occidentalis* was a species cultivated for commercial pollination until commercial populations were decimated by pathogens in the 1990s (USDA 2011). Entomologists suggest that diseases and parasites may have transferred to wild colonies (Committee on the Status of Pollinators in North America 2007, Otterstatter and Thomson 2008) leading to rapid declines.

According to records at the UCMC and the Bee Biology and Systematics Laboratory at Utah State, *B. occidentalis* was once widespread and common across the state of Colorado, found in thirty-five counties. In 2008 and 2009, there were some reports (sometimes a single specimen) of this species in Boulder, Chaffee, Gunnison, Jefferson, Larimer, Mesa, Montrose and Summit Counties. The species has been added to the Xerces Society Red List of endangered insects and classified as “imperiled.” As an example of the extent to which the bee populations have declined, in the early 1980s *B. occidentalis* made up 27% and 55% of the bumblebees collected in berry fields and natural

vegetation, and in cranberry fields respectively (Colla and Ratti 2010). In 2003, *B. occidentalis* made up less than 1% and 0.1% in the same areas.

In both years of our survey, bumblebees were captured at significantly lower rates at low elevation plots. Due to the lack of abundance data from the past, it is unclear whether this is a natural phenomenon. Low elevation plots tend to dry out as the season progresses, leading to decreases in floral resources, while higher elevation sites continue to flower. Early in the season of 2011, there were many queens flying at low elevation, leading me to believe that populations would be high this summer. This was not the case. Although natural differences in climate could cause the differences among elevations, there are many other factors that could negatively affect bumblebees at the low elevation sites. Compared to high elevation sites, low elevation sites have much higher human populations and far more development in the form of roads, buildings and other types of habitat alterations. In addition, there is no commercial agriculture near the mid and high elevation sites. Beekeepers at lower elevations in Boulder County have indicated that 2011 was a very bad year for honeybees, and some have implicated the increasing use of systemic pesticides on crops in contributing to their declines (Theobald, Boulder County Beekeepers Association, personal communication). Factors affecting honeybees would most likely show effects on bumblebees as well.

### **Genetic studies**

I hypothesized that *B. occidentalis* would have the lowest genetic diversity among the three species since it is the species suffering the most severe decreases in the western US and Canada. In fact, for one of the two gene loci, there was no variation among all the bees sampled. However, at the other locus *B. occidentalis* showed the most genetic variation due to three unusual individuals. The collection data indicates that the 3 unusual individuals were collected between 1987 and 1993 from locations that are represented by other individuals used in the study, so location alone can not be responsible for the differences.

Because I was unable to collect any specimens of *B. occidentalis* in 2010 and 2011, all of the specimens used for genetic testing were museum specimens dating from 1902 through 1993. The majority of specimens came from the 1960s and 1980s, before the *B. occidentalis* declines. I will keep trying to attain specimens of *B. occidentalis* collected since 2000 for genetic testing.

Most *B. pensylvanicus* individuals showed the same genotypes, but a few specimens showed significant amounts of variation from the rest. Two individuals were particularly distinct. Both of these were collected in 2010 at the Beech Open Space property in North Boulder.

The striking variation among a few individual specimens was unexpected. As many as 14 polymorphisms at one gene locus were detected. These kinds of major differences often reflect population differentiation due to geographic isolation, or can occur in response to environmental variation in different geographic locales (elevational gradients, separation by a divide, etc.). It will be necessary to examine more individuals to determine what factors may be involved with these specimens. My original expectation was that each species would differ at the two loci, and there would be different numbers of polymorphisms among species. Although this is not what I have found for these two gene fragments, the polymorphisms that did appear are striking. The dramatic variation among these specimens hints at an interesting underlying phenomenon that is well worth exploring.

I have been working with Dr. Jeffrey Mitton to identify other gene segments that will provide variation and will sequence well. We have been experimenting with seven additional primers, several of which isolate genes within the control region of mitochondrial DNA. These primers have been used successfully to estimate genetic variation in honeybees. Once the procedures for using these primers have been perfected, I will sequence the same bees used in the cytochrome b and 16S studies. The results will yield a more powerful data set to analyze for differences in genetic diversity.

### **Summary**

Bumblebees are crucial pollinators of both crops and wildflowers. Declines in bumblebees in those areas where they have been extensively monitored are a cause for concern and reflect a bigger trend of pollinator declines in North America. Since there are minimal data on bumblebee abundance on the Front Range, this project is important in assessing the local conservation status of these important pollinators.

Declines in genetic variation among bumblebees are just starting to appear in the literature. This project will add to this new dimension in the understanding of small populations, the genetic effects of and the genetic consequences of declines.

### **Use of Funds**

All funds have been allocated to supplies and equipment for this study and none have been used for salary.

**Acknowledgements:** Funding for this project was provided by the Lois Webster Fund of the Audubon Society of the Greater Denver (2011) and the Boulder County Nature Association (2010).

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Table 1 - Field work 2011

SITE	Elevation	Number of visits	Number of species found at site
Sans Souci Beech Wonderland Lake	Low	8	5
	Low	8	4
	Low	8	4
Quartz Ridge Meadow Minnick Mud Lake	Mid	8	9
	Mid	9	7
	Mid	8	8
Mount Audobon East Knoll, Niwot Ridge Fourth of July Mine	High	1*	0
	High	3	4
	High	2	4

\* - 3 failed attempts due to snow depth

Table 2 - *Bombus* species at each set of elevations

Species	Low Elevation Sites	Mid-Elevation Sites	High Elevation Sites
	1700 m	2600 m	3350 m
<i>appositus</i>	✓	✓	
<i>auricomus</i>			
<i>balteatus</i>			✓
<i>bifarius</i>			✓
<i>californicus</i>		✓	
<i>centralis</i>		✓	
<i>fervidus</i>	✓		
<i>flavifrons</i>			
<i>fraternus</i>			
<i>frigidus</i>			✓
<i>griseocollis</i>	✓		
<i>huntii</i>		✓	
<i>kirbyellii</i>		✱	✓
<i>melanopygus</i>			
<i>mixtus</i>			
<i>morrisoni</i>			
<i>nevadensis</i>	✓	✓	
<i>occidentalis</i>			
<i>pensylvanicus</i>			
<i>rufocinctus</i>	✓	✓	✓
<i>sylvicola</i>			✓
<b>subspecies <i>Psithyrus</i></b> <i>(cuckoo bumblebees)</i>			
<i>insularis</i>		✓	
<i>fernaldae</i>	✓		
<i>suckleyi</i>	✓	✓	

**Table 3. Disagreements in mtDNA sequences among individuals\* of the same *Bombus* species, a measure of genetic variation.**

<i>Bombus</i> species	Number of disagreements (number of specimens)	
	cytochrome b fragment	16S fragment
<i>appositus</i>	38 (2)	32 (1)
<i>occidentalis</i>	37 (0)	34 (34)
<i>pensylvanicus</i>	26 (30)	34 (16)

\* two sequences per individual

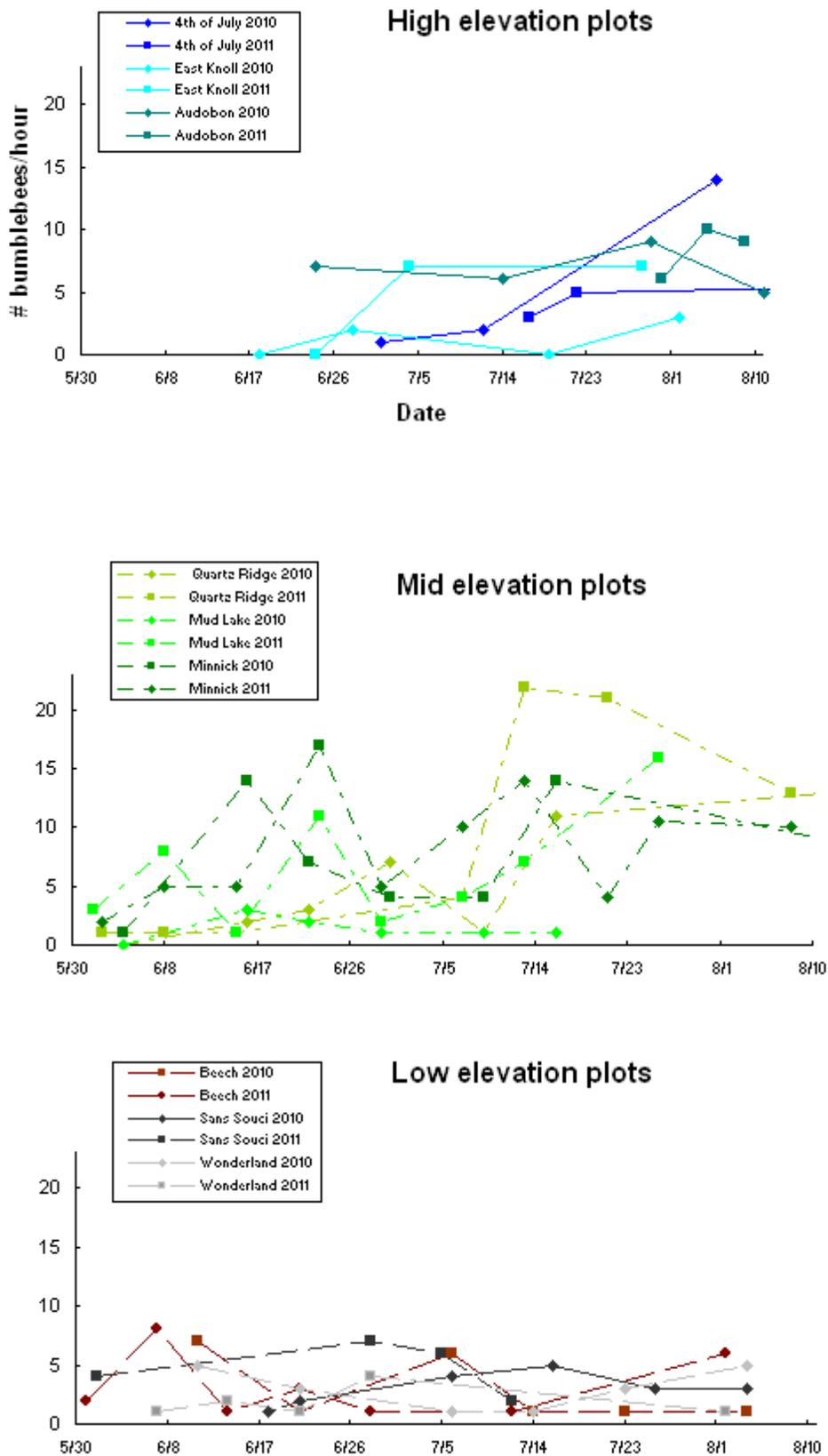


Figure 1. Number of bumblebees collected per hour across the sampling seasons 2010 and 2011 at different elevations.

