

What are the bees' needs? Analyzing the diet breadth for three Rocky Mountain Bumble Bees

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Abstract

Bumble bees are crucial pollinators in both natural and agricultural ecosystems. For effective conservation of this important group of pollinators, it is essential to understand their basic ecology, such as their interactions with floral food resources. In this study, we used a long-term dataset (5 year) of season-long bumble bee floral resource use to investigate the floral diets of three common bumble bee species native to the Colorado Rocky Mountains (*Bombus bifarius*, *B. flavifrons*, and *B. appositus*). We compared the floral diet richness among the three species overall and also among the castes (queens, workers, males) within each species. Our analyses revealed that floral diet richness differs among the three focal bumble bee species, which we hypothesize is due to variation in species phenology and morphology. When comparing the floral diet richness within species and across the different bumble bee life stages (i.e. castes), we found a clear distinction between workers and reproductives (males and queens) in species that emerge earlier in the season, while the later emerging *B. appositus* had consistently low diet richness in all castes. We gained a better understanding of their individual diet breadths, and how different castes take advantage of floral resources throughout the summer. This study provides important information on the basic ecology and resource needs of an important group of pollinators, and suggests that focusing on what life stage of bumble bees overlooks important variation.

Key words: *Bombus*, *B. appositus*, *B. bifarius*, *B. flavifrons*, corolla length, diet breadth, phenology, physiology, plant-pollinator interactions, pollination

Introduction

Despite bumble bees being crucial pollinators in both ecological and agricultural ecosystems, there has been a continued decline in their populations over the past few decades (Goulson et al. 2015). There are numerous factors that contribute to this loss, including toxic pesticide use, habitat loss, climate change, and invasive species (Goulson et al. 2015). A major contributor to population health across species is floral abundance and availability (Roulston and Goodell, 2011). Bees rely on continuous floral availability throughout their active season in order to maintain a healthy colony (Ogilvie et al. 2017). In fact, many pollinators, including bumble bees, exhibit considerable flexibility in their resource use across the summer growing season

(CaraDonna and Waser 2020). It is important to protect native bees since pollinator diversity is required to enhance the reproduction in crops, wild plants, and maintain vegetation diversity (Roulston and Goodell, 2011). Therefore it is important to understand basic components of their ecology, such as their floral resources used across their entire activity periods.

To better understand the pollination ecology of wild bumble bees, we must look into specific interactions between individual plant and pollinator species (Connop et al. 2010). In this study, we focus on three subalpine bumble bee species (*Bombus bifarius*, *B. flavifrons*, and *B. appositus*) that are observed in long-running datasets collected in and around Gothic, Colorado (Ogilvie et al. 2017). These species are common and relatively abundant, with varying tongue lengths, body sizes, and activity periods making them ideal for observational study and for determining their diet range (Ogilvie et al. 2017). The bumble bee life cycle remains similar across species. The queens emerge in early spring before moving underground to produce workers, which then emerge in early summer. New males and queens emerge in late summer and reproduce in the fall. There is some correlation between bumble bee species' proboscis length and the flowers commonly visited by that species (Inouye, 1980). This means that species with a long proboscis such as *B. appositus* are capable of foraging on plants with long corolla tubes, whereas species with shorter tongues (such as *B. bifarius*) feed on flowers with shorter corollas.

Here we examine variation in the floral diet breadth of *Bombus bifarius*, *B. flavifrons*, and *B. appositus* to better understand their floral resource use and natural history. We ask: (1) do these three bumble bee species vary in diet breadth overall? (2) within each species, do the castes (queens, workers, males) vary in their diet breadth? (3) how do such patterns within species compare across species? Given variation in bee phenology and morphology, we hypothesize that *B. bifarius*, *B. flavifrons*, and *B. appositus* should have different floral diets breadths from one another, as well as across castes within the species.

Methods

This study uses previously collected observational data collected by *Dr. Jane Ogilvie* and *Dr. Paul CaraDonna* from 2015—2019 (and ongoing) of bumble bee-flower interactions throughout the summer growing season around the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado. Six permanent sites have been established, each with the three dominant habitat types where bumble bees are commonly observed: dry meadows, wet meadows, and aspen forests. During weekly bee observation days, bumble bee interactions with flowers are observed while walking through each habitat within a site for 20 minutes (1 hour at each site). During each observation period bumble bee species, caste, and behavior (foraging, nest-searching), and if foraging, what plant species the bees were foraging from, was recorded.

Additionally, each week floral resource abundance was quantified in a series of five permanent transects (20 x 0.5m) in each habitat at each site (15 total transects at each site).

We quantified the richness of floral resources used by each species at each site in each year. First, we tested the normality of each data-set using a Shapiro-Wilk's normality test. We then compared richness across and within species, using ANOVA if data were normally distributed, and Kruskal-Wallis test when data were non-normal. For both questions, we tested for differences between species or castes using Tukey HSD and Dunn multiple comparison tests. For all of these statistical tests we used R (version 4.0.2). When creating the boxplots we filtered out plant species with rare visitation (less than 2 visits) because they did not make up a significant portion of the *Bombus* diets.

Results

Our first question asked whether floral diet breadth varied across the three *Bombus* species. We found that the diet richness of each species was distinct, with significant gaps between the three means (Fig. 1: ANOVA: $F = 48.93$, $P = 6.45e-15$ ($P < 0.05$)). *Bombus flavifrons*' diet richness had the greatest variability of the three species with a noticeably higher mean diet richness than *B. bifarius* and *B. appositus*.

The second hypothesis focused on whether there was a difference in diet richness across the castes of an individual species. For *B. bifarius*, the relationship between caste and diet richness was variable, with individual caste comparisons showing queens and males to have similarly low diet richness compared to workers (Fig. 2a). The diet richness across *B. flavifrons* castes was also irregular, with workers' diet breadth being noticeably higher in relation to their reproductive counterparts (Fig. 2b). *Bombus appositus* (Fig. 2c) was an outlier among the three species, as the relationship between its castes and diet richness was consistent.

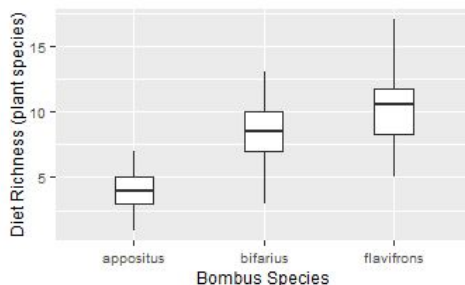


Fig. 1. This boxplot is showing the diet richness of the three *Bombus* species by comparing diet richness across the three *Bombus* species. Tukey HSD pairwise comparisons showed all species had significant differences ($P < 0.05$).

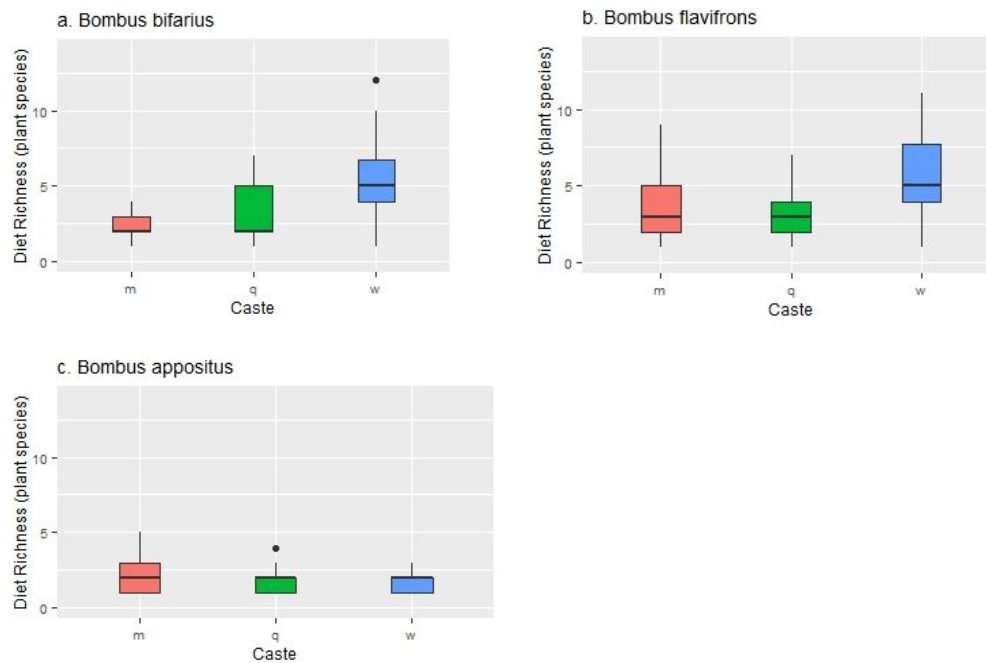


Fig. 2. These boxplots are showing the diet richness of each species' castes (male: *m*, queen: *q*, worker: *w*): (a) comparing diet richness across *B. bifarius* castes (*m-w* and *q-w*), (b) *B. flavifrons* castes (*m-w* and *q-w*), and (c) *B. appositus* castes. Letters *m*, *q*, and *w* indicate Dunn comparisons; hyphenated letters indicate significant differences at $P < 0.05$.

Discussion

Bumble bees need a continuous supply of nectar and pollen throughout the season in order to maintain their colonies. When floral abundance is irregular throughout the summer, it negatively impacts the wellbeing of the colony and their population (Macfarlane and Patten, 1997). The tongue size of the *Bombus* can influence their diet breadth since their proboscis can determine the floral resources available to visit, and the time they emerge also impacts what floral sources they choose and what flowers are available to them (Inyoue, 1980, Connop et al. 2010). We found that the three *Bombus* species and their castes took advantage of different floral resources throughout the summer.

One factor that distinguishes the three species is their proboscis length. *B. flavifrons* may have had the greatest diet richness (Fig.1) due to its intermediate tongue length, which gives it access to flowers with both short and long corollas. *B. flavifrons* had more options compared to the other *Bombus*, whose tongues are more specialized. *B. bifarius* can easily access flowers with short corolla tubes, which were relatively abundant in this study. This allowed for a greater diet breadth than *B. appositus*, whose tongue is specialized for the less abundant long corolla flowers (Wright, 1988). Studies suggest that in order to create a colony, later emerging bumble bees,

such as *B. appositus*, need these specialized proboscises to access high quality flowers (Connop et al. 2010).

Phenology also plays an important role in the diet richness of individual species. *B. bifarius* emerges early in the summer. This allows it to interact with a greater number of unique flower species than *B. appositus*, which has the latest emergence of the three *Bombus*. *B. flavifrons* also emerge early enough to access most of the flower species available during the season.

Queens and males across all three species had a low diet richness in part due to their phenology (Fig. 2). Once a colony is formed in early summer, the queen no longer forages for food and stays below ground producing workers (Williams et al. 2014). This means that most queens only have access to flowers early in the season, and are limited in their choices. Males emerge later in the summer when flowers are less abundant, and only forage for themselves while they search for new queens (Williams et al. 2014). Workers are out for most of the season, regardless of species. This means that competition plays a greater role in their diet breadth than their species' phenology. *B. appositus* is the largest of the study species, especially when comparing workers, and can monopolize the long corolla, higher quality flowers they prefer (Inouye, 1978). Both *B. bifarius* and *B. flavifrons* are smaller and must generalize in order to obtain enough food to maintain their colonies.

It is also worth noting the correlation between the abundance of a specific *Bombus* species and its diet breadth, as the more abundant a particular species is, the less selective they are when foraging for pollen (Goulson and Darvill, 2004). *B. flavifrons* and *B. bifarius* were both observed frequently across the five year interval (Table S1), and their high diet richness may have been necessary in order to support large populations. However, *B. appositus* was observed less than half as often as the other two species, and this smaller observed population may have allowed it to be more selective in its foraging habits (Goulson and Darvill 2004).

In conclusion, we found that the diet richness was most clearly associated with the physiology and phenology of the individual species. This information is crucial for understanding how floral diversity may impact wild bumble bee populations, especially in light of continued pressure from various global changes. Future research into this topic could look into how corolla length correlates with nectar and pollen production, as well as bumble bee visitation rates across species. Another direction might be to look at different bumble bees' dependence on individual flower species by analyzing their floral diet distribution.

Supporting Information:

	<i>Bombus bifarius</i>	<i>Bombus flavifrons</i>	<i>Bombus appositus</i>
Total obs.	4538	5918	1867
2015	836	1178	531
2016	792	656	285
2017	665	851	374
2018	1006	645	140
2019	1239	2588	537

Table S1: Individual observations (both total and per year) for the three *Bombus* species. These values were used as a proxy for relative population sizes of the three species.

	Male (m)	Queen (q)	Worker (w)
<i>Bombus bifarius</i>	1610	625	2302
<i>Bombus flavifrons</i>	2877	835	2206
<i>Bombus appositus</i>	567	415	885

Table S2: The individual observation count for the three castes of each species. These values were used as a proxy for relative caste sizes within the three species.

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