

Does bumble bee (*Bombus spp.*) diet breadth vary with differences in floral resource abundance?

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Abstract

In the Rocky Mountains of Colorado, changes in the availability of floral resources through the growing season may affect the ability of bumble bee colonies to successfully complete their life cycle and produce reproductive individuals in the season's end. A way bees could potentially deal with this challenge is by broadening their diets—visiting a wider variety of flower species for pollen and nectar. I investigated the diet breadths of three bumble bee species in that region at the species and individual levels, asking whether bee diet breadths varied with floral resource abundance. I found that at both the species and individual level, bees' diet breadth does not seem to vary with floral resource abundance. Bees may have another strategy for dealing with a lack of floral resources, or their diet breadth may be constrained by other factors such as competition.

Introduction

Plant-pollinator interactions are fundamental parts of natural communities. Many flowering plant species rely upon pollinators to reproduce, and pollinators rely upon plants for most to all of their nutrition. Around the Rocky Mountain Biological Laboratory (RMBL), and in other alpine environments, bumble bees are abundant and effective pollinators (Bingham & Orthner 1998), in part because they can tolerate the extreme environmental conditions (Heinrich 1981). Like most systems, bumble bees and their floral partners are experiencing stresses due to climate change, including changes in floral season timing and abundance (Kerr *et al.* 2015, Miller-Struttman *et al.* 2015, Forrest 2015). For bumble bees, these changes could be quite problematic, as they need to be able to fit their entire life cycle into the brief time between snowmelt and the end of the flowering season. Their essential function as pollinators in subalpine meadows and probable sensitivity to climate change-driven floral shifts makes it important to understand how bumble bees might respond to climate change-driven stress.

In the spring soon after snow melts, bumble bee queens emerge from their overwintering locations and begin searching for a suitable place to establish a nest. Once a nest site is chosen, the young queens lay their first brood. Over the course of the season, a queen will lay a series of broods, most of which will develop into female workers who help their mother collect nectar and pollen for the colony and care for the developing eggs and larvae. The final brood, however, is different—they hatch into a mix of males and reproductive females who will be the next year's queens (Goulson 2010, Heinrich 1981). Because the queen is the only reproductive member of the colony, the entire colony's reproductive success ultimately hinges on the size and success of this final brood. As one can imagine, the more workers the queen is able to produce throughout the season, the larger this final brood can be, as there are more individuals available to collect food and care for their developing reproductive siblings. However, the number of workers a queen can produce is limited by the amount of food at her disposal—it takes a lot of pollen and nectar to build baby bees. The new queens' chances of survival through the winter are also limited by flower availability—if they are unable to build up sufficient fat stores, they will likely not survive the winter. For these reasons, it is essential that the colony have access to flowers throughout the entire growing season, from the queen's initial emergence to the new queens' entrance into overwintering sites.

Unfortunately, climate change is changing the availability of flowers throughout the season. In the RMBL area, climate change over the last 30-40 years has appeared in the form of earlier dates of snowmelt in the spring, warmer temperatures during the growing season, and more frequent damage to flower buds from spring frosts (Inouye 2008, Anderson *et al.* 2012, Iler *et al.* 2013). These changes can all affect flowering phenology and abundance of wildflower species (Forrest 2015). For example, flowering seasons are getting longer but the total number of flowers produced across the season is staying approximately constant (CaraDonna *et al.* 2014), resulting in a mid-season shortage of floral resources (Aldridge *et al.* 2011). Such changes in floral resources could have significant effects on bumblebee populations and

communities, as they rely entirely on continuous availability of pollen and nectar throughout the season to complete their lifecycle (Heinrich 1981).

As floral resource availability shifts, it is important to know if and how pollinators like bumble bees might be able to deal with those changes. One way that bumble bees might be able to deal with decreased resource availability is by visiting multiple flower species, rather than specializing on a single resource. This would give them a sort of backup plan—if their favored flower is unavailable or in short supply, they have other foods to fall back on. At the species level, it is known that bumble bees tend to partition resources based on tongue length and flower corolla length: species with longer tongues forage on flowers species with longer corollas, and short-tongued bees on shorter flowers (Inouye 1980). However, we know very little about how generalized or specialized individual bees are in their foraging habits. There is limited research suggesting that individual bumble bees tend to specialize on a particular flower species for foraging (Heinrich 1976), and that the dominantly preferred flower species is often supplemented by a secondary “minor” preference (Heinrich 1979). These two studies comprise the majority of our knowledge of individual foraging behavior in the field, and further information about individual foraging flexibility in different floral resource contexts is unknown.

However, the degree of individual foraging flexibility is an important aspect of the ability of bumble bees to cope with climate change: in situations with scarce resources, the more flexible an individual is, the more likely she is to be able to obtain sufficient food for herself and the colony; and the more individuals differ in what resources they exploit or how flexible they can be, the better the species as a whole can cope with changing resource availability.

While species foraging habits are simply the sum of individual foraging habits, the math could play out in a variety of ways. Imagine a species that as a whole is willing to visit five flower species. This could mean that each individual specializes in one of those five species, or that each individual is a generalist that visits all five, or anywhere in between. In this scenario, if a colony is established in a meadow that has very few flowers of each food species, colonies at

either end of that spectrum would have very different success: colonies comprised of individuals who specialize may have limited food because those individuals are less efficient foragers, spending a lot of their time searching for flowers instead of collecting resources. On the other hand, colonies comprised of generalists will have more resources, as those individuals spend a larger portion of their time collecting resources.

My research addresses two questions related to bumble bee diet breadth: (1) Does the diet breadth of bumble bee *populations* vary with meadow floral abundance, and does this effect differ among bee species? (2) Does the diet breadth of *individual* bumble bees vary with meadow floral abundance, and does this effect differ among bee species? I hypothesize that both individuals and populations will use more flower species (have broader diet breadths) in low abundance meadows, in order to fulfill colony resource needs. I also expect different bumble bee species to vary in their individual and population diet breadth because of differences in tongue lengths, which partly determine what range of flowers they can exploit (Inouye 1980). For example, bees with short tongues may be limited in their diet breadth if a meadow has few short-corolla flowers. This research will add to our knowledge of individual bumble bee foraging behavior, which is fundamental to bumble bee ecology, and to help understand how bumble bees may respond to floral resource changes that accompany global climate change.

Methods:

Study Site

This research was conducted at the Rocky Mountain Biological Laboratory (RMBL) in Gothic, CO, USA (elevation 2900 m), and the neighboring Gunnison National Forest. Six study sites, spaced by approximately one kilometer, were chosen in subalpine meadows in the East River Valley with both wet and dry habitats. For my question addressing population-level diet breadth, I also included observations taken in the aspen forests adjacent to the meadows at each site.

The Gothic area is highly limited by snow—flowers cannot bloom at a site until the winter's snowpack has melted out, and the season ends when the snows return. Thus, this area has a shorter growing season than nearby lower elevation sites.

Question 1: Does the diet breadth of bumble bee populations vary with meadow floral abundance, and does this effect differ among bee species?

Data collection. For this question, I used an existing three-year (2015-2017) dataset collected by Jane Ogilvie and her research assistants at the RMBL. These data were collected at six sites (Figure 1) ranging from Rosy Point (southernmost) to Rustler's Gulch (northernmost). Sites were spaced by approximately 1 km to be sure that the same bees would not be observed at multiple sites (based on foraging distance data from Elliott 2009). Each site is delineated into three habitat types: aspen-dominated forest, wet meadow, and dry meadow. Within each habitat type, there are five 20 x 0.5 m belt transects (50 m² per habitat, 150 m² per site). Once a week through the entire growing season, Jane or her research assistant visited each site and counted all bumble bee-visited flowers blooming within the transect according to a standardized protocol. This gave us our measure of floral availability. In addition, Jane visited each site once a week and spent twenty minutes in each habitat type (60 min per site per sampling date), counting every bumble bee she saw and noting its behavior (nest-searching, flying through, or foraging). If foraging, she also noted the flower species it was visiting, whether it was collecting pollen or nectar, and whether it had corbiculae pollen loads. These observations gave us information about bumble bee diet breadth. For my analysis, I focused on three species of bumble bees found around the RMBL: *Bombus appositus*, *B. flavifrons*, and *B. bifarius* (long-, medium-, and short-tongued species, respectively, Pyke 1982).

Statistical analysis. I compared population diet breadth of the three *Bombus* species among meadows that vary in total floral abundance. I pooled all worker-flower observations of a species at each of the six sites and summed the number of flower species the bees were

foraging on to create the population diet breadth. The measure of floral abundance was the summed number of bumble bee flowers per m² counted during the worker active period for each site and year. I then used a linear model and an ANOVA to test for differences in population diet breadth among the six meadows over three years (18 data points) and bumble bee species (with bee species population diet breadth as the response variable and meadow floral abundance and bee species as the fixed effects. I carried out all analyses in R version 3.3.2 (R Core Team 2016).

Question 2: Does the diet breadth of individual bumble bees vary with meadow floral abundance, and does this effect differ among bee species?

For this question, I focused on three species of bumble bees found around the RMBL: *Bombus appositus*, *B. flavifrons*, and *B. bifarius* (long-, medium-, and short-tongued species, respectively, Pyke 1982). Jane determined normal total floral abundance for the study period by analyzing the previous two years' of data (described above) for each site. We then selected the meadows with lowest and highest floral resources to be our focal study sites (Figure 2).

Data collection. To quantify individual bee diet breadth I caught and tagged bees, and then searched the meadow for marked bees, observing their foraging habits. I visited each meadow approximately every other day from June 30- July 30, 2017. To tag bees, I walked a fixed route through the meadow searching for unmarked individuals and trapped them in an insect net. Once trapped, the bees were immobilized using a "bee squeezer," which traps the bee between a foam pad and mesh. I then used gel superglue to attach a plastic tag painted with a unique pattern of one to three colors to the bee's thorax, between its wings (Ogilvie & Thomson 2016). At the time of capture, I recorded bee species, tag pattern, and caste (worker or queen), what flower species it was caught from and resource it was collecting, the presence of a corbiculae pollen load, and took a photo of the tagged bee.

Each day, I started searching on a different section of my route to ensure I would visit all areas of the meadow at multiple times of day throughout the season. Initially, I caught and tagged any bee I saw. As the season progressed and I began to re-sight marked bees, I followed any marked individual I saw for up to five minutes, recording the tag, sequence of flowers visited, resource (nectar or pollen) collected at each flower, and whether the bee had pollen loads. After I lost the bee, or five minutes passed, I returned to my search route and continued along. In the case that I saw both a marked and an unmarked bee of the focal species, I followed the known bee rather than catching and tagging the new bee. To quantify each site's floral resources, I used the data from the belt transects described above during the timeframe of my individual bee observations.

Statistical analysis. I compared individual bee diet breadth between meadows with low and high floral abundance. My data points were individual bees that I saw at least two times in a meadow. I used a generalized linear model with a Poisson distribution to model my data and a Chi-squared test to test for differences in individual diet breadth between low and high resource meadows and among bumble bee species (individual diet breadth as the response variable and meadow resources, bee species, and the interaction between those terms as the fixed effects). I did the same for *B. flavifrons* individuals that I saw at least four times, to see if my estimate of diet breadth's response to resource availability changed with the number of times I saw a bee. I carried out all analyses in R version 3.3.2 (R Core Team 2016).

Results

Question 1: Does the diet breadth of bumble bee populations vary with meadow floral abundance, and does this effect differ among bee species?

Across sites and years, *B. bifarius*, *B. flavifrons*, and *B. appositus* visited a range of 5-15, 4-14, and 1-6 flower species, respectively. During this three-year period, floral resource availability ranged from 39-132 flowers/m² across all sites. At the population level, bumble bee

diet breadth did not differ with floral resource availability, and that effect did not differ among species. (Figure 3). Analysis via linear model yielded nonsignificant results (ANOVA: floral abundance effect $F = 1.9039$, $P = 0.1740$; floral abundance*bee species interaction $F = 2.9058$, $P = 0.1341$). It is interesting to note that although diet breadth for all three species did not differ with resource abundance, diet breadth of *B. appositus* was significantly different (ANOVA, $F = 44.19$, $P = 1.3 \times 10^{-11}$) from those of *B. flavifrons* and *B. bifarius* (independent of resource availability).

Question 2: Does the diet breadth of individual bumble bees vary with meadow floral abundance, and does this effect differ among bee species?

At the resource-poor site I tagged 153 bees and saw 119 of them again (on 1-8 additional foraging bouts). *B. bifarius* individuals were observed on 1-4 flower species, and most visits overall were to *Potentilla pulcherrima*. Individuals of *B. flavifrons* were observed on 1-3 flower species each, with most visits overall to *Aconitum columbianum*. *B. appositus* individuals were observed mostly on *Delphinium barbeyi* and *Aconitum columbianum*, and they visited up to three flower species overall.

At the resource-abundant site, I tagged 131 bees and saw 118 of them again, on up to 11 additional foraging bouts. *B. bifarius* individuals were observed on 1-2 flower species, and most visits overall were to *Heliomeris multiflora*. Individuals of *B. flavifrons* were observed on 1-4 flower species each, with most visits overall to *Mertensia ciliata*. *B. appositus* individuals were observed mostly on *Delphinium barbeyi*, and they visited up to two flower species overall.

At the individual level, bumble bee diet breadth did not differ between the resource-poor and resource-abundant sites (Figure 4). Analysis via generalized linear model (Poisson) yielded nonsignificant results (Chi-squared: resource availability effect $P = 0.8080$; species effect: $P = 0.6034$; resource*species effect $P = 0.8774$). Analysis of *B. flavifrons* individuals seen at least four times also produced nonsignificant results (Figure 5; Chi-squared, $P = 0.5858$). It is worth

noting that there was a great deal of individual variation in diet breadth, with individual bees visiting anywhere from one to four flowers.

Discussion

In this study I examined whether diet breadth of three bumble bee species varied with floral resource availability at the individual and population levels. In both cases, I found no strong correlation between diet breadth and floral resource availability. This was a rather interesting and unexpected result—bumble bees appear to be able to deal with a deficit in floral resources without changing the flowers they are willing to visit.

There are a few potential reasons for this. For my individual level question, in order to maintain a sufficient sample size I had to restrict my analysis to bees observed on at least two foraging bouts (rather than restricting analysis to bees observed on a minimum of four or five bouts). It is possible that I simply did not resight bees on enough foraging bouts to observe their full diet breadth. I was able to observe individual *B. flavifrons* more frequently, so I reproduced my analysis on *flavifrons* individuals that I observed during at least four bouts. This analysis still produced nonsignificant results (Figure 5), leading me to two possible conclusions. First, perhaps it requires observation of more than four foraging bouts to detect any pattern that is present. Or, it is possible that there is indeed no relationship between individual diet breadth and floral resource abundance.

This second option is more likely. At the individual level, my sites weren't actually that different in floral resources (86 and 106 flowers per square meter, as opposed to 90 and 131 flowers per meter squared in a previous year), so that may have limited the difference in bees' response to floral resources. However, at the population level my sites varied a good deal in floral resources. Because I still detected no pattern in those data, there is likely no relationship between bee diet breadth and floral resource abundance, at least in terms of how I quantified floral resource abundance. However, it is quite possible that our methods of quantifying floral

abundance did not accurately reflect resource availability from a bee's perspective. We simply used raw flower counts to give us the number of flowers of each species per square meter, treating all flowers as equal. However, flower species vary in their quality of reward (volume of nectar and quality of pollen). To more accurately capture resource availability from a bee's perspective, it could be useful to weigh flower species according to their reward, with more rewarding flowers more heavily weighted relative to their raw abundance, and less rewarding flowers having a lower weight.

If we assume there is indeed no relationship between individual diet breadth and floral resource abundance, we are left with a big question—why don't bees seem to respond to resource availability, a factor that seems like it should be very important to colony success? The first, and most unpleasant, option is that they do not respond at all. Instead, colonies in resource-poor areas simply fare poorly and produce few or no reproductives at the end of the season. This may be the case, as bumble bee abundance across the six sites and years is positively related to floral abundance (J. E. Ogilvie, *unpublished data*). That is, bumble bee abundance does seem limited by floral resource availability in our study. However, there are many ways that bees could compensate for or otherwise deal with limited resources in a small patch.

Perhaps those individuals with restricted diets can compensate for their diet inflexibility by foraging farther without incurring an energy loss. In the past, a negative relationship has been found between average foraging distance of workers in a colony and the availability of floral resources nearby that colony (Carvell *et al.* 2012), supporting the idea that bees in resource-poor areas forage farther to obtain food resources. It is also possible that the bees' normal foraging ranges are enough to compensate for resource patchiness. Osborne *et al.* (2008) found this to be true for *B. terrestris* in patchy landscapes of the UK and speculated that bees with similar foraging distances would likely be able to compensate in the same way. Because foraging distance is highly correlated with body size (Greenleaf *et al.* 2007), and my

focal species were similar in size to *B. terrestris*, the species in this study may simply respond to resource availability on a much larger scale than that at which I worked. However, working in the same area as I did, Elliott (2009) found that bees including my focal species *B. appositus* foraged no farther than 1 km, and generally spent their time within 100m of their nest. If this is indeed the case, my study sites were large enough to cover the majority of the bees' main foraging grounds, though the more extreme end of the spectrum exceeds my sites' bounds.

It is also possible that the bees' diet breadth is more strongly affected by some unmeasured factor than by meadow floral abundance. One likely candidate is competition. It has been found in the study area that bumblebees are subject to interspecific exploitative competition. David Inouye observed this phenomenon with *Bombus appositus* and *B. flavifrons* (two of the focal species of the present study), with the removal of *B. appositus* from an area resulting in *B. flavifrons* broadening its diet to include *Delphinium barbeyi* (Inouye 1978). Another study from around RMBL found that when the most abundant bumble bee species was removed from a site, the remaining bees broadened their diets (Brosi & Briggs 2013). These lines of evidence suggest that diet breadth may be restricted by interspecific competition.

In the future it could be productive to examine these hypotheses either by extending the present study (to cover more sites, larger areas, more bee species, and a larger proportion of the bumble bee season) or by performing manipulative experiments, excluding some species from an area to examine the effects of competition on diet breadth.

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Figure Legends

Figure 1. Map of the six study sites used to address the population-level question. The Rocky Mountain Biological Laboratory campus is labeled for reference.

Figure 2. Map of the two study sites used to address the individual-level question.

Figure 3. Species-level diet breadth of three species of bumble bee, measured in total number of flower species bees were observed visiting during the worker active season, as compared to floral resource abundance. Each point is an annual sum for one site in one year. (A) Species-level diet breadth of *Bombus flavifrons*, (B) *B. bifarius*, and (C) *B. appositus*. All $P > 0.05$

Figure 4. Individual-level diet breadth of three species of bumble bee, at high and low-resource sites. Analysis included all bees observed at least twice. All $P > 0.05$. Legend includes sample size of high | low abundance sites.

Figure 5. Individual-level diet breadth of *B. flavifrons* at a resource-poor and resource-abundant site. Analysis includes all individuals observed at least four times. $P > 0.05$.

Tables and Figures

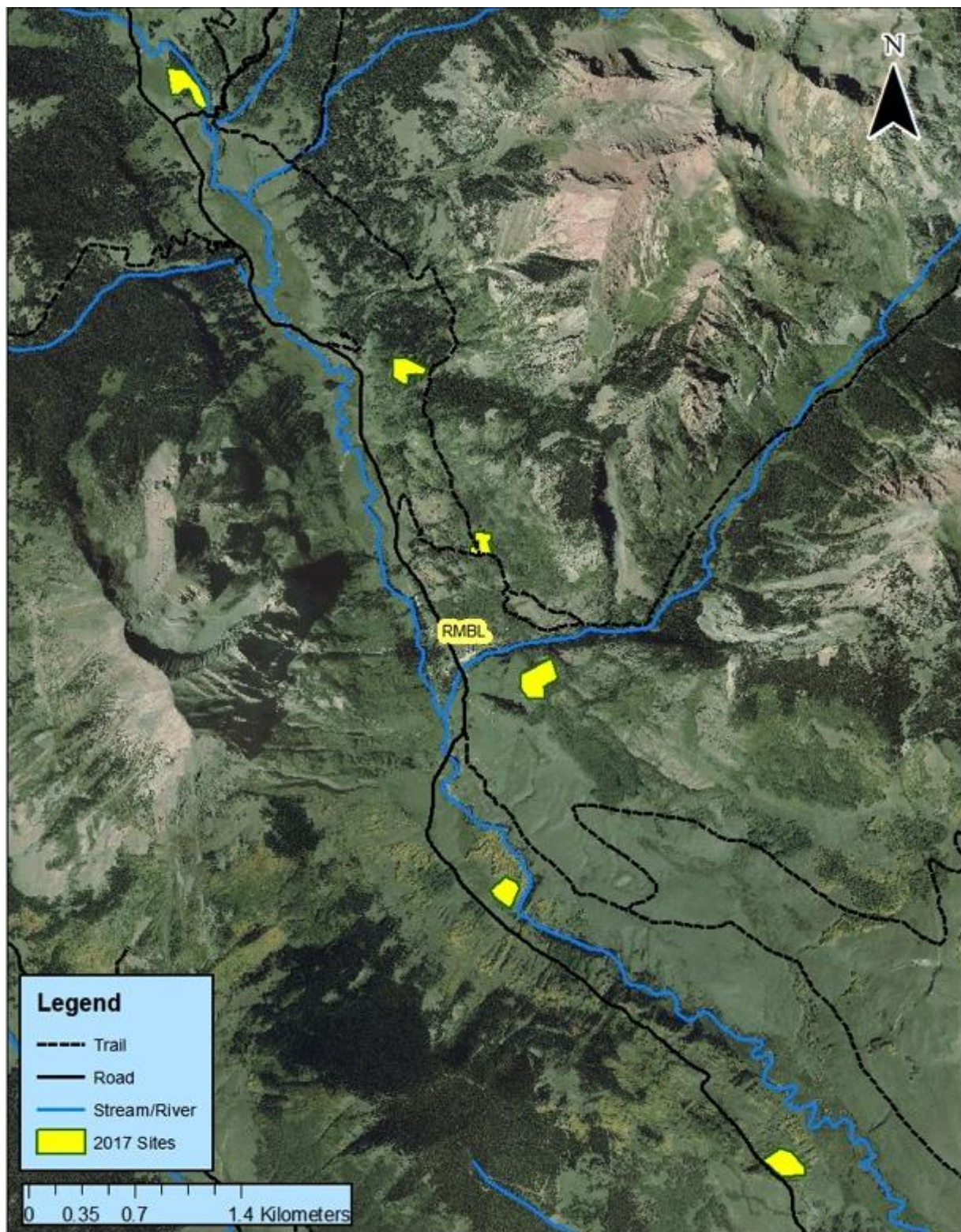


Figure 1

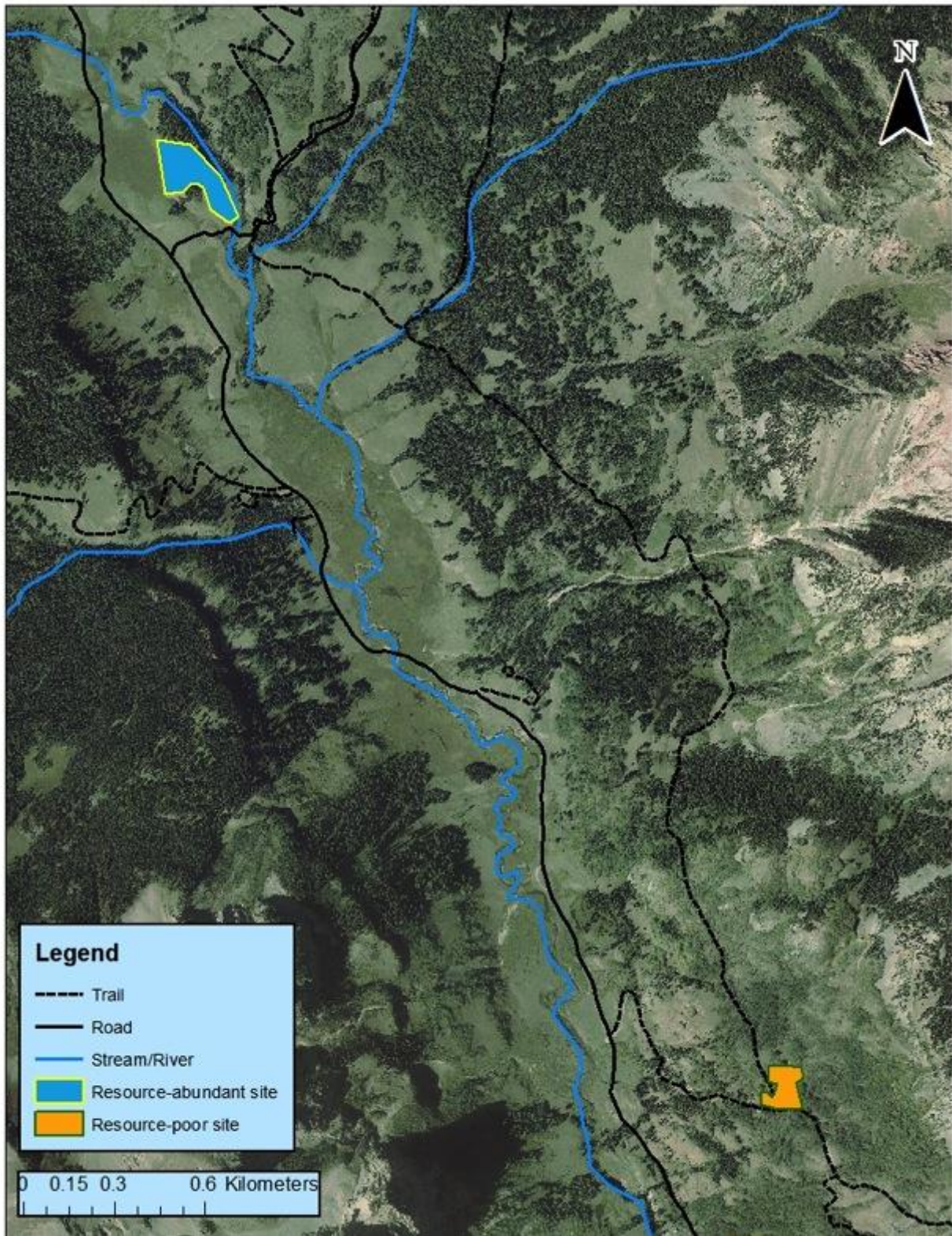


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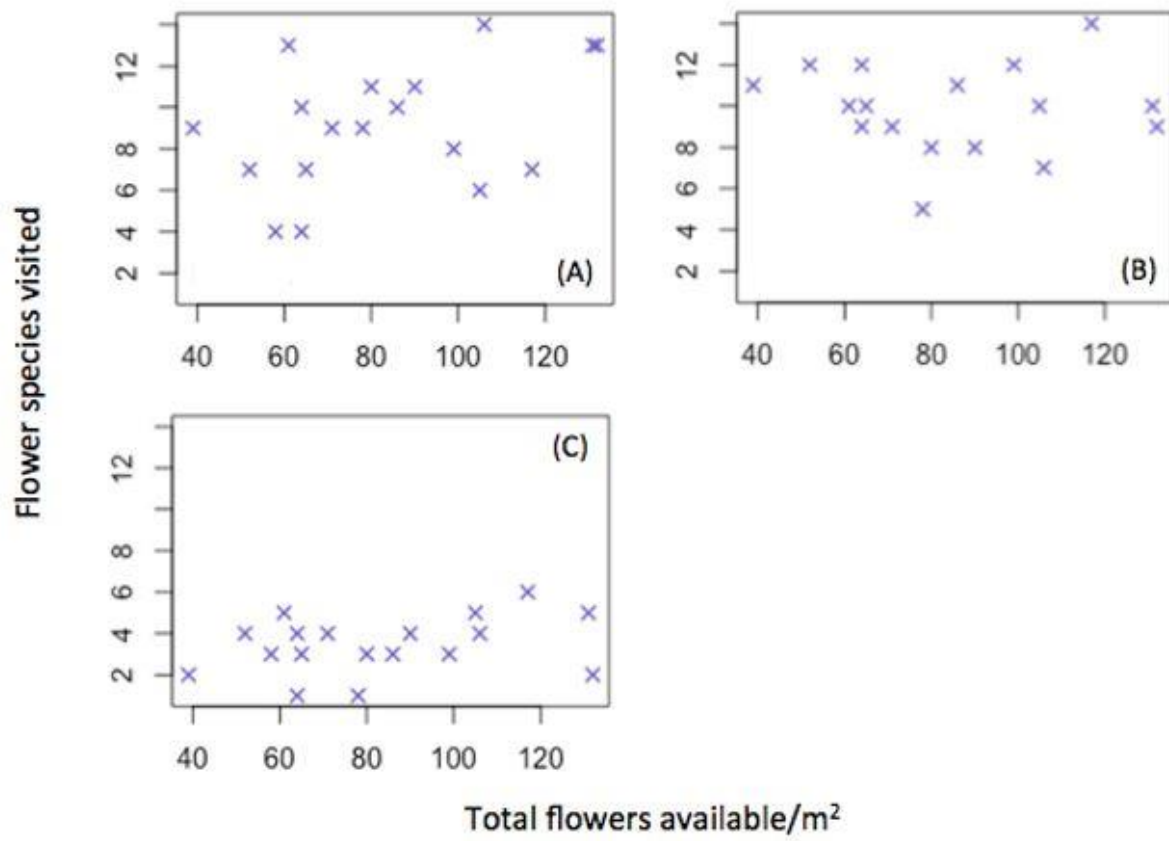


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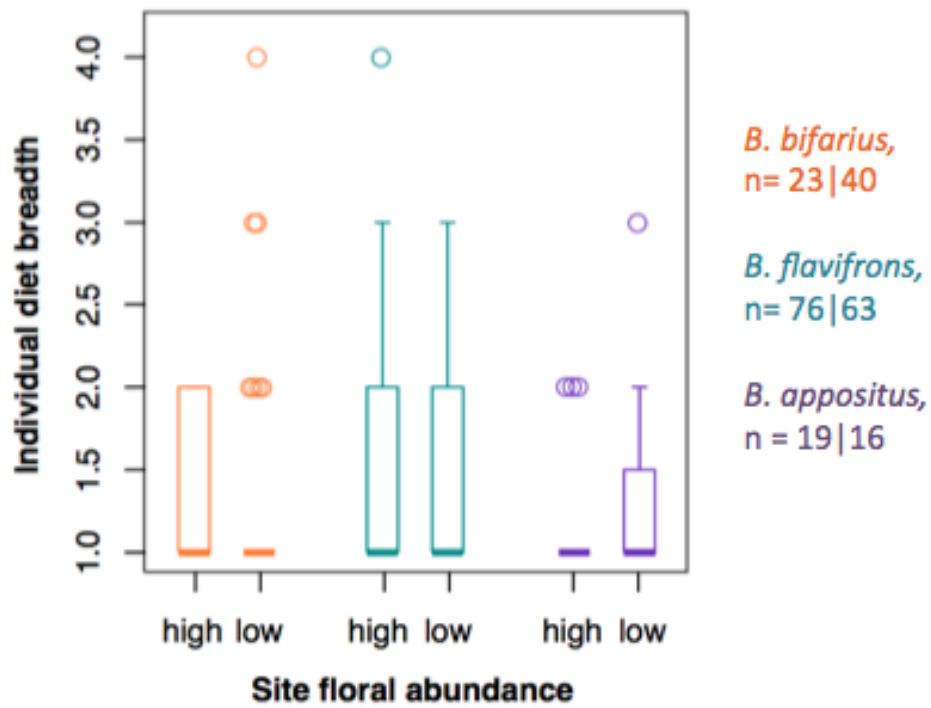


Figure 4

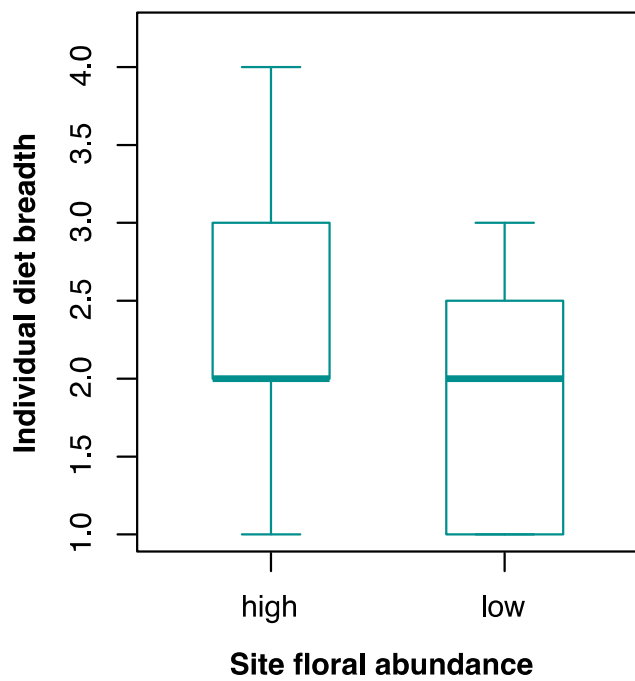


Figure 5