Foraging Activity and Pollen Collection by Honey Bees and Bumble Bees in a Shared Landscape

By

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Thesis Abstract

Pollinators provide essential ecosystem services, and approximately 87% of flowering plants benefit from animal pollination. Bees are particularly important pollinators of both crops and wildflowers. However, bee populations are in decline. These declines are attributed to factors that include exposure to agrochemicals, pests and pathogens, and habitat loss. Social bees, such as honey bees and bumble bees must balance the dynamic nutritional needs of the hive with resource availability, that is, flowering plants that provide nectar and pollen resources. To do so, honey bees and bumble bees possess unique strategies to communicate colony needs and allocate foragers to the task of gathering resources. For example, honey bees have an elaborate dance communication system that allows bees to share information about the quality of a resource, and the distance and direction to that resource. This is thought to allocate cohorts of foragers to patches in the landscape that have the highest rate of reward production. On the other hand, bumble bees are not understood to possess such an elaborate system of communication, but instead use a trapline foraging strategy, whereby individual bees learn and remember routes among profitable resource patches that minimize distances traveled and maximize resource acquisition. For this research we sought to explore the relationship between these foraging strategies, and the foraging activity and pollen collection patterns of honey bees (*Apis mellifera)* and bumble bees (*Bombus impatiens)* in a shared landscape. The study took place at the West Madison Agricultural Research Station (WMARS) in southern Wisconsin. We began by observing the temporal foraging activity of honey bees and bumble bees using radio frequency identification (RFID). We concurrently collected pollen samples from bees returning from foraging bouts to determine the identity of pollen they were collecting, and the protein and amino acid content of collected pollen. Our findings are discussed in the context of the challenges bees face in acquiring resources, and the strategies they use to address them.

Chapter 1

Strong interspecific differences in foraging activity observed between honey bees and bumble bees using miniaturized radio frequency identification (RFID)

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Abstract Central place foragers depart from and return to a central location with enough resources for themselves, and in many cases, for the group. Honey bees and bumble bees are eusocial central place foragers. Honey bees have large perennial colonies while bumble bee colonies are annual and considerably smaller. Foraging range, body size and division of labor also vary between these two bee species. Honey bees use their unique dance language to recruit foragers to the most profitable patches. Bumble bees exploit patches individually and develop trapline foraging patterns. We expect such differences among bee species to engender differences in foraging activity. Moreover, variation in resource availability and in colony needs over the flowering season, can affect bee foraging activity. Finally, spatial variation in resource availability may impact bumble bees to a greater extent than honey bees due to their smaller foraging range. Using miniaturized radio frequency identification (RFID), we tracked the foraging activity of individual bees to and from hives at three sites and over five time periods. Pollen pellets were also collected from bees returning to the hive. We compared the European honey bee, *Apis mellifera*, and the common eastern bumble bee, *Bombus impatiens*. Linear mixed effect models determined the impact of bee species, time of season (period) and site, and

their interactions, on multiple foraging metrics calculated from the RFID data and on pollen dry weight. Relative to honey bees, individual bumble bees made more foraging trips each day, resulting in a greater time spent foraging. A greater proportion of RFID tagged bumble bees foraged each day and bumble bees brought heavier pollen sacs to the hive compared to honey bees. Foraging bout duration did not vary between bee species and none of the foraging metrics varied among time periods or among sites. Both bee species brought heavier pollen sacs back to the hive at the beginning and the end of the flowering season. These results are discussed in terms of species differences in foraging strategies, size of individuals and colonies, and temporal variation in colony needs and resource availability.

Keywords: radio-frequency identification, bumble bee, honey bee, foraging effort, pollen pellet, site, time period

Introduction

Approximately 87% of flowering plants around the globe (Ollerton 2011) and 35% of all crops grown for human consumption (Klein et al. 2007) benefit from animal pollination. Bees are important visitors to both crops and wildflowers, yet many bee species are in decline because of the combined effects of habitat loss, pesticide exposure, and pathogens (Naug 2009; Cameron et al. 2011; Goulson et al. 2015). Bees are limited by the area of habitat available which is essential for nesting and gathering of floral resources, and the negative impact of habitat loss on bees may be most pronounced in areas where natural habitat is already limited (Winfree et al. 2009). In addition, exposure to neonicotinoid pesticides negatively affects the ability of honey bees to navigate back to their hive following artificial displacement (Fischer et al. 2014) and

increases the foraging effort of bumble bees (Stanley et al. 2016). Importantly, these sublethal effects of neonicotinoid pesticide exposure can further exacerbate the negative impacts of pathogens such as Nosema and black queen cell virus on bees (Doublet et al. 2015). Given the numerous challenges facing bees, a better understanding of bee foraging over time and space, and for distinct species would facilitate the development of sound conservation strategies.

Bees are central place foragers, implying that they must depart a nesting site, locate and gather resources, and return with these resources to the hive or nesting area (Charnov 1976). Honey bees and bumble bees are both generalist eusocial foragers that collect resources from a broad spectrum of plant taxa (Waser et al. 1996). The identity and quality of flowering plant resources can vary through time and space, and, therefore, the strategies used by central place foragers to gather resources must be amenable to these fluctuations in resource availability (Goulson 1999). Honey bees will forage at distances generally less than 6 km (Visscher and Seeley 1982), but only a small fraction may forage within a 0.5 km radius around the hive (Beekman and Ratnieks 2000), as indicated by waggle dance decoding. In contrast, a foraging range of less than 800 m was identified for several bumble bee species (*Bombus terrestris*, *B. pratorum, B. pascuorum*, and *B. lapidarius*) using sister-sister pairing with microsatellites. (Knight et al. 2005). Wolf and Moritz (2008) obtained comparable results using distance from nest sampling in *B. terrestris.* In addition, the foraging range of bumble bees tends to decrease with increasing resource availability and decreased land fragmentation (Redhead et al. 2016), and high local resource availability can increase queen production (Herrman et al. 2017). These results suggest possible differences in foraging strategies of honey bees and bumble bees in response to available resources.

Honey bees are perennial with a queen actively laying eggs and the colony capable of surviving multiple years (Seeley 1978). In contrast, a bumble bee colony is annual, and the founding queen only lives for a single foraging season. New bumble bee queens are produced in the fall, disperse and hibernate through the winter, ultimately building a new hive the following foraging season (Michener 2000). In addition, while individual bumble bees are larger than honey bees, honey bee colonies are much larger than bumble bee colonies. While a honey bee colony can contain 60,000 workers, a bumble bee colony can have between 50 and 250 workers, depending on the bee species**.** Moreover, honey bees have a higher level of communication and eusociality with a more structured division of labor relative to bumble bees**.** A known mode of information transmission for honey bees is the dance language, which communicates the distance, direction, and quality of resources to prospective foragers (Von Frisch 1967). The dance language is thought to allocate foraging workers to the best available patches in the landscape to gather resources (Couvillon and Ratnieks 2015). While individual honey bees do not all follow dances before foraging, they are more likely to do so if they are novice foragers, have not foraged for a while, or their latest foraging trips were not rewarding (Biesmeijer and Seeley 2005). Bumble bees, in contrast, do not have a dance language to communicate resource location, but they do actively "run" around the inside of their hive following a return to the hive with resources, possibly to stimulate foraging activity (Dornhaus and Chittka 2001). In addition, activation of bumble bee foragers occurs following the addition of floral scent into a hive, and this behavior is especially pronounced when the scent is added to honey pots (Molet et al. 2009). Honey bees may therefore locate rewarding resources more efficiently relative than bumble bees.

While bumble bees are known to rely on trapline foraging strategies when visiting flowers, this strategy is not typically used by honey bees (Pasquaretta et al. 2017). Trapline foraging allows individuals to follow learned routes known to be profitable (Thomson et al. 1997; Ohashi and Thomson 2008). Trapline foraging is considered an optimal strategy, whereby individual bees learn the location of rewarding patches, and repeatedly visit these patches in a predictable route that develops over multiple independent foraging bouts (Keasar et al. 2013). Trapline foraging increases the overall rewards obtained per plant visit (Williams and Thompson 1998) and decreases overall search times **(**Saleh and Chittka 2007). Pasquaretta et al. (2017) used network analysis to investigate the development of trapline foraging patterns in bumble bees and honey bees and found that bumble bees tend to quickly develop optimal routes at smaller spatial scales, while optimal trapline routes do not develop in honey bees except, possibly, at larger spatial scales.

We expect these differences in life history, body and colony size, division of labor, communication, and foraging strategies to engender differences in foraging activity between these two groups of bees. Foraging activity metrics include foraging bout duration, number of foraging bouts per bee and proportion of foragers gathering resources on a given day. The quicker location of rewards generated with the waggle dance and the trapline foraging of bumble bees may both affect foraging bout duration. The smaller colony size of bumble bees could necessitate a greater proportion of the bumble bee workforce being allocated to foraging relative to honey bees, and at the individual bee level, greater foraging activity per bee. Temporal variation in resource availability over the flowering season could also affect bee foraging. If fewer resources are available early and late in the flowering season, bees may spend more time

foraging during these periods relative to the middle of the flowering season to bring sufficient resources to the hive. But colony needs will also affect foraging and are likely to change over the flowering season. Pollen needs are expected to be greater during brood production while, at least for honey bees, nectar needs may increase later in the season in order to make sufficient honey to survive the winter months. Lastly, variation in resource availability among sites may impact bumble bees to a greater extent than honey bees due to their smaller foraging range (Visscher and Seeley 1982; Pasquaretta et al. 2017). There are many reasons to expect foraging activity to vary over time, over space and among bee species.

The application of radio frequency identification (RFID) technology has increased enormously over the last ten years and miniaturization of the chips have permitted its use to determine movement of honey bees and bumble bees to and from their hives (Pahl et al. 2011; Schneider et al 2012). The use of radio frequency identification (RFID) provides a relatively novel and reliable tool to gather data on individual bees, as each microchip contains a unique identification number. Previous research using RFID demonstrated that honey bees can home in on their hive from 13km away (Pahl et al. 2011). However, neonicotinoid pesticides can decrease the overall homing success (return rate) of foraging honey bees (Henry et al. 2012) and lower the foraging activity and increase foraging time of honey bee individuals (Schneider et al. 2011). Likewise, bees exposed to Fibronil pesticide via treated feeding sites decreased the number of foraging bouts and increased foraging bout duration (Decourtye et al. 2011). Moreover, RFID data indicated strong diurnal foraging patterns of two bumble bee species at northern latitudes with 24-hour daylight sun (Stelzer and Chittka 2010). RFID technology therefore represents a powerful tool for gathering data on foraging patterns of bees.

In the current study, we used miniaturized radio frequency identification (RFID) techniques to measure and compare the foraging activity of two bee species, the European honey bee, *Apis mellifera*, and the common eastern bumble bee, *Bombus impatiens*. RFID data were collected in 2016 at three separate sites and throughout the flowering season. These data were used to quantify different foraging metrics at the colony, individual bee, and foraging bout levels. In addition, we assessed the weight of pollen pellets bees brought back to the hive following a foraging trip in an attempt to link foraging time to resource acquisition. The impact of bee species, site, time of flowering season, and their interactions on the different foraging metrics and on pollen dry weight were examined using linear mixed effect models. Results are discussed in the context of differences in life history, colony size, and foraging strategies between these two bee species, and with respect to variation in colony needs over time and resource availability over time and space. Identifying spatial, temporal and species differences in foraging metrics would help land managers improve conservation strategies for pollinator communities.

Materials and Methods

Study Area and Bee Species

This study was conducted at the West Madison Agricultural Research Station (WMARS) in Madison, WI. This area is in a suburban-agricultural landscape, with a high proportion of arable experimental crop lands, roadside habitats, and suburban gardens (Figure 1). Radio Frequency identification (RFID) data and pollen pellets were collected from three sites in the summer of 2016 (Figure 1). Each site was selected based on a qualitative estimate of plant species richness within a 0.5 km radius around the hives and suggested increasing species richness from sites 1, 3, and 2, respectively. One hive of the European honey bee, *Apis mellifera,* and one hive of the

common eastern bumble bee, *Bombus impatiens* were placed at each site and separated by 60m at site 2, and 100m at sites 1 and 3. Each honey bee hive consisted of 2- deep frames vertically stacked in a wooden observation hive, with approximately 2000 bees, each of which was housed in a 1.2 m^3 wooden box with an exit tunnel allowing access outside. At the beginning of the experiment the bottom frame of each honey bee hive consisted of approximately half the frame covered in a combination of capped and open brood, while the top frame consisted of at least half a frame of honey. Each hive was queen right. These initial conditions allowed the colony some room to grow, albeit highly limited by the small hive size. Each bumble bee hive (Koppert Biological Systems, Howell, MI, USA) was placed in a small wooden shelter located 0.5 m off the ground and contained ~ 75 worker bees at the start of the experiment. Among sites, the hive locations ranged from 700 to 1500 meters apart (sites 1-2: 700 m; sites 1-3 1500 m; sites 2-3 1400 m).

Data collection

Radio Frequency Identification (RFID) data and pollen pellets were collected from the three sites and over five time periods between mid-June and mid-September. Within each period, we collected data for a total of three days from each site, moving among sites each day to randomize data collection among sites. Data were collected simultaneously from the honey bee and bumble bee hives at an individual site, using RFID reader pairs specific for honey bees and bumble bees, respectively. This pattern of data collection resulted in9 data collection days within each period, with each site being visited every 4 to 5 days. Furthermore, the total duration of each period ranged from 10-17 days depending on weather (Table 1). The RFID data and pollen pellets were typically gathered on non-rainy days when the temperatures ranged between 21 and 35^{o}C.

Radio Frequency Identification

Prior to each of the five data collection periods, a uniquely coded passive RFID tag (mic3 – TAG 64-bit RO, iID2000, 13.56 MHz system, 1.0 mm x 1.6 mm x 0.5 mm; Microsensys GmbH, Erfurt, Germany) was glued onto the thorax of 70 honey bees and 20 bumble bees at each site as they were observed returning to their hive. We aimed at tagging bees returning with pollen sacs to ensure they were foragers. However, some tagged bees did not have pollen sacs and we assumed they were collecting another resource such as nectar or water. Honey bee foragers rarely return to working inside the hive (reviewed in Johnson 2010) but tend to remain foragers until their death, or until winter arrives. At each hive, bees traveled through a 1" diameter tube and through 2 RFID readers spaced 7.5cm apart. We used one reader pair that was explicitly designed to gather data from honey bees, and another reader pair that was designed for bumble bees (iID2000, 2k6 HEAD; Microsensys GmbH, Erfurt, Germany). Each reader of a pair had a unique identity, and the pair was used to ascertain the direction of travel by bees, i.e. whether a bee was moving in or out of the hive. A foraging bout was indicated when a bee passed through the inner reader, followed by the outer reader, and at least 5 minutes elapsed until the next encounter with the outer reader, following the method of Gill et al. (2012). The RFID data were collected for 24 hours each day and the readers were moved among sites each morning between 8:30 and 10:30 am depending on weather. In general, the readers were moved at 9am June-August, and then closer to 10am as the nights became cooler later in August and into September.

Pollen Collection

To gather pollen, up to twenty individual bumble bees and forty individual honey bees were caught as they returned to the hive with pollen pellets. These bees did not have RFID tags. Individual bees were collected into 2 dram plastic vials which were placed in a cooler filled with ice packs until a bee was no longer able to move (approximately 5-10 minutes for honey bees, 10-20 minutes for bumble bees). Both pollen pellets were removed from the bee, and each pellet was stored separately in a 1.5 mL microcentrifuge tube. Bees were subsequently released near the hive entrance. Following collection, pollen pellets were kept on ice and, upon return to the laboratory, were placed in a 20^{o}C freezer until ready for drying and weighing. One pollen pellet per bee was dried at 45^{o}C for 24 hours and subsequently weighed to the nearest tenth of a milligram.

Statistical Analyses

Radio Frequency Identification

We examined foraging activity metrics, calculated from the RFID data, across multiple levels. At the colony level, the dependent variable was the percentage of tagged bees foraging each day. We did not determine the proportion of the hive that were foragers as we did not want to disturb the hive during the collection of foraging data. At the individual bee level, we computed three dependent variables from the RFID data. For each day, we examined i) the average duration of a foraging bout per bee ii) the number of foraging bouts per bee, and iii) the total duration of foraging per bee (sum of all foraging bout durations per bee). Lastly, at the foraging bout level we used duration of a foraging bout as dependent variable.

We used linear mixed effect models (proc Mixed, SAS v. 9.4) to determine the impact of site, period, bee species and their interactions on the foraging activity metrics. While the fixed effects were similar in all models and included site, period, species and all two-way interactions, the level of replication and the random variables were different across each level of analysis, i.e. colony, individual bee, and foraging bout. For the proportion of tagged bees, the sole colony level foraging activity metric, day was the replicate in the model and the random effect was the three-way interaction, site*period*species (Table 2). For analyses examining foraging metrics at the individual bee level, an individual bee was the replicate and the random factors included the three-way interaction site*period*species together with an additional day(site*period*species) term (Table 2). Finally, at the foraging bout level, where a foraging bout itself was the replicate, the random factors included the two three-way interactions present for the individual bee level analyses, together with a bee(site*period*species*day) term (Table 2). At the foraging bout level, the duration of a foraging bout was log transformed prior to analysis, while at the individual bee level the foraging bout duration and the number of foraging bouts per bee were log transformed to improve the model residuals.

In all three models the fixed effect explanatory variables were tested against the site*period*species error. In the colony level model, the random effect variable site*period*species was tested against the residual error (Table 2). In the individual bee model, the site*period*species random effect was tested against the day(site*period*species) error, while the day(site*period*species) random effect was tested against the model residual error (Table 2). Finally, for the model at the foraging bout level, the site*period*species random effect was tested against the day(site*period*species) error, the day(site*period*species) random effect

was tested against the bee(site*period*day*bee) error and the bee(site*period*day*bee) was tested against the model residual error (Table 2).

Pollen Dry Weight

Pollen collection effort is represented by the dry weight of pollen pellets. We used a linear mixed effect model (proc Mixed, SAS v. 9.4) to determine the impact of site, period, bee species and their interactions on the dry weight of pollen pellets being returned to the hive. The fixed effects in the model included site, period, species and all two-way interactions. A pollen pellet was the unit of replication, and the random factors included the three-way interaction site*period*species together with a day(site*period*species) term (Table 3). While the fixed effect explanatory variables were tested against the site*period*species error, the site*period*species random effect was tested against the day(site*period*species) error, and the day(site*period*species) random effect was tested against the model residual error (Table 3). Pollen dry weights were square root transformed prior to analyses to improve the residuals of the model.

Results

Radio Frequency Identification

At the colony level, a greater proportion of tagged bumble bees foraged each day ((mean +/- SE) (0.28 ± 0.03) relative to the proportion of tagged honey bees (0.19 ± 0.02) (N = 76 days) (Table 4). While we collected RFID data over 9 days at each site, no foraging activity was recorded on some days. This was true for 4 out of 9 days in period 1 for bumble bees and 3 days in period 5. For honey bees, no foraging data were recorded on one day in period 1, 3 days in period 2 and 1 day in periods 3 and 4. Although it varied between bee species, the proportion of tagged bees

was not influenced by site or period or by any of the two-way interactions between bee species, site or period (species*site), (species*period) or (site*period) (Table 4). In other words, the proportion of bees foraging each day was similar among sites and time of year (period) and the pattern among sites or among periods was similar for the two bee species (Table 4). Moreover, the proportion of tagged bees foraging during the different periods was similar among sites (period*site) (Table 4).

At the level of the individual bee (N = 703 individual bees), none of the factors examined, bee species, site, period, or their two-way interactions affected the average duration of a foraging bout (Table 5). Foraging bout duration for a bee was similar among sites, among periods and among bee species (Table 5). However, on any given day, the number of foraging trips per bee and the total time a bee spent foraging differed among bee species (Table 5). Bumble bees made significantly more foraging trips in a day (5.9 ± 0.4) relative to honey bees (4.6 ± 0.2) and they spent more total time foraging each day (bumble bees: 346.9 min ± 16.2; honey bee: 222.4 min ± 6.6). Site, period and the two-way interactions did not influence either the number of foraging trips per bee or the total time a bee spent foraging each day (Table 5). Finally, at the level of a foraging bout (N= 3502 foraging bouts), none of the factors or their two-way interactions affected the average duration of a foraging bout (Table 4). An average foraging bout lasted 58.5 +/- 2.0 min for bumble bees, in contrast to 48.71 +/- 0.8 for honey bees. Although it was slightly longer for bumble bees the difference could not be explained by differences between bee species in our model.

Pollen Dry Weight

We obtained the pollen dry weights of 1598 pollen pellets. There was a statistically significant effect of species and period on the weight of pollen pellets brought back to the hive and a weaker site*species interaction (Table 6). Using multiple means comparisons to examine the interaction between site and species, the average pollen pellet weight was always greater for bumble bees than for honey bees (Fig. 2) and the difference between species was statistically significant at two sites and borderline at the third (Site 1: df = 1, 7; t = 4.00; P = 0.0052; Site 2: df =1, 7; t = 6.56; P = 0.0003; and Site 3: df = 1,7; t = 2.34, P = 0.052). We therefore considered the impact of the main factor of species on pollen pellet dry weights. For any foraging bout, bumble bees returned with significantly heavier pollen pellets relative to honey bees (bumble bee: 7.73 mg +/- 0.25; honey bee: 3.68 mg +/- 0.08) (Table 6). Moreover, the weight of pollen pellets brought back to the hive following a foraging bout varied among periods over the flowering season (Table 6). Bees returned to the hive with heavier pollen pellets during the first (mean +/- se) (6.81 mg +/- 0.24) and last (6.38 mg +/- 0.32) periods, relative to the second (4.09 mg +/- 0.16), third (3.34 mg +/- 0.19) and fourth (3.71 mg +/- 0.25) periods (Fig. 3). The weight of pollen pellets brought back to the hive by individual bees were similar between the first and last periods (Fig. 3). Such differences among periods were similar for bumble bees and honey bees as indicated by the lack of a statistically significant interaction between period and bee species (Table 6).

Discussion

The foraging activity of bumble bees was greater than that of honey bees. Relative to honey bees, an individual bumble bee embarked on more foraging bouts each day. In addition, a greater proportion of bumble bee foragers actually foraged on a given day. However, the duration of a

foraging bout did not differ between these two bee species. The average duration of a foraging bout by an individual bee each day lasted 58.5 +/- 2.0 min for bumble bees, in contrast to 48.71 +/- 0.8 for honey bees. But, because individual bumble bees did more foraging trips in a day, they spent more total time foraging each day.

Foraging bout duration can be affected by the time it takes for bees to reach rewarding resource patches and by the time a bee spends foraging at that resource, collecting either pollen or nectar. The waggle dance can facilitate the location of rewarding resources by honey bees, although not all individual bees observe the dance prior to foraging ((Biesmeijer and Seeley 2005). Moreover, honey bees tend to have a larger foraging range relative to bumble bees (Visscher and Seeley 1982; Knight et al. 2005). Many foraging models for flower visiting insects assume that the time traveling between patches is negligible relative to the time spend foraging within patches (Goulson 1999). In contrast to honey bees, bumble bees must learn, and remember profitable locations, and develop trapline foraging patterns among patches (Ohashi and Thomson 2008), while honey bees do not tend to develop optimal trapline routes (Pasquaretta et al. 2017). Results of the current study suggest that the foraging strategies of both bee species translate into similar foraging bout durations, from the time a bee leaves the hive to the time it returns to the hive. The more individualistic trapline foraging mode of bumble bees seems to permit them to gather resources in the same amount of time as the more direct method of information transmission for resource quality and location communicated by the waggle dance of honey bees (Thomson et al. 1997; Oshaki et al. 2007; Lihoreau et al. 2010; Couvillon et al. 2014; Ratnieks and Shackleton 2015). Future studies should determine the differences in foraging bout durations between individual honey bees that follow the waggle dance and those that do not to increase our

understanding of the impact of honey bee communication on foraging bout duration. Moreover, when comparing bumble bees and honey bees foraging within patches, Brunet (unpublished data) observed similar foraging bout duration within a patch for these two bee species. Future studies should examine in more details the reasons why, despite the various differences in their foraging strategies, foraging bout duration from the time a bee leaves the hive to the time it returns to a hive remains similar between these two bee species.

Individual bumble bees spent more time foraging each day and a greater proportion of the foragers were active each day relative to honey bees. Although we did not gather information on the proportion of the colony that were foragers, in order not to disturb the hive during collection of foraging data, the proportion of the foragers that were active each day is a strong descriptor of colony level foraging activity as new foragers were tagged at the start of each time period. The observed interspecific differences were consistent over the flowering season and among sites, as indicated by the lack of significant interactions between bee species and period or site in our mixed model. This pattern supports consistent and stable interspecific differences in the activity levels of honey bees and bumble bees to gather resources. Differences in life history and in colony sizes between these two bee species may help explain observed differences in foraging activity. Bumble bee colonies are annual and small in contrast to the perennial and large honey bee colonies. Given such differences, each bumble bee worker may need to put forth more effort to build up and sustain the colony relative to a honey bee worker. But, if activity level relates to colony size, with an increase in colony size over the season, we would also expect the foraging effort per bee and percent of colony foraging to decrease, which we did not observe. We therefore suspect other factors, besides colony size and life history, help explain observed

differences in foraging activities between these two bee species. The more complex division of labor of honey bee colonies could represent such a factor and may facilitate a lower foraging activity per individual and at the colony level. Furthermore, considering the honey bees in this experiment were restricted to a 2-frame observation hive with limited space for the colony to grow, and therefore limiting growth associated changes in colony needs, these results may differ from experiments conducted under more typical circumstances, and further studies should be undertaken on larger hives. Finally, more research is needed to elucidate whether and how differences in life history, colony size and social structure contribute to interspecific differences in bee foraging activity.

Bumble bees brought heavier pollen pellets back to the hive relative to honey bees, even though both bee species had similar foraging bout durations. Because most bees tend to forage either for pollen or for nectar during a foraging bout (Brunet, unpublished data), this result suggests that bumble bees are more efficient than honey bees at retrieving pollen from the plants they visit. The observed interspecific difference in pollen pellet size may result from honey bees being smaller than bumble bees. Within a bee species, larger pollen and nectar loads are correlated with increased body size of foragers (Goulson et al. 2002). However, it is unclear how this pattern might translate among bee species. Individual bumble bees made more foraging trips in a day and brought back more pollen to the hive each time, relative to honey bees. Moreover, a greater proportion of the bumble bee foragers were active each day relative to honey bees. Such patterns should translate into a greater amount of pollen available per capita for bumble bees relative to honey bees. Bumble bees, due to their larger size, may have greater pollen requirements than honey bees, and indeed bee body size is correlated to the amount of protein

received by developing larvae (Roulston and Cane 2002). Differences in body size among bee species may therefore help explain differences in foraging patterns that optimize the amount of pollen brought back to the hive to meet a colony's need.

Across all levels, the foraging activity of these two bee species did not vary over the flowering season (period) or among sites. The time a bee spent foraging per bout or per day and the proportion of foragers active each day did not change over the flowering season and did not differ among sites. Moreover, this pattern was true for both bee species. The lack of variation in foraging activity over the season or among sites was surprising. We expected greater foraging activity early and late in the flowering season because of the lower expected resource availability. Moreover, the known differences in foraging ranges between these two bee species suggested among site variation in foraging activity for bumble bees but not for honey bees (Visscher and Seeley, 1982; Pasquaretta et al. 2017). But colony needs may also change over the flowering season. We expect greater pollen needs earlier in the season for both bee species as more brood may be produced relative to later in the season. Later in the season, we expect greater nectar needs for honey bees as they are building honey reserves for the colony to survive the winter months. Bumble bee colonies, however, are producing new queens and may still have high pollen needs. Interestingly, we gathered some data for bumble bees on the proportion of bees returning to the hive with and without pollen pellets. Although the sample sizes were uneven among periods, the trend suggested that most foragers returned to the hive with pollen pellets during the first (95%) and last (96%) periods, while a greater proportion of the foragers returned with nectar in the three middle periods (47, 15 and 33%, respectively).

We did not gather such data for honey bees although it should be determined in future studies. However, because foraging bout duration did not change over the flowering season, these data suggest no apparent differences in the time a bee spends foraging for pollen vs. nectar. The larger pollen production for bumble bees early and late in the flowering season suggest a greater pollen need, possibly for growing larvae early in the season and queen development in late summer/early fall. Interestingly, colony needs can influence the proportion of foragers collecting pollen or nectar but it did not influence foraging activity in general (time spent foraging and proportion of foragers active each day). Moreover, any variation in resource availability over time and space did not significantly influence foraging activity for these two bee species in the current study.

Both bee species brought more pollen back to the hive per foraging bout at the beginning and end relative to the middle periods of the flowering season, even though the time spent foraging remained constant throughout the flowering season. The temporal differences in the amount of pollen gathered suggest that it took longer to collect resources from flowers in mid-summer relative to early or late summer. Couvillon et al. (2014) proposed that summer is the most challenging season for honey bees because bees foraged at greater distances in mid-summer relative to spring and fall. Danner et al. (2018) found honey bees returned the greatest amount of pollen to the hive early in the season, in April and May. Taken together, these results support the notion that summer may be the most challenging season for bees, at least for eusocial bees. One potential explanation for this pattern is that, although resources may increase in mid-summer, the density of bees also increases and, thus, the level of competition for shared resources.

The use of miniaturized RFID allows for continual tracking of individual bees and provides a real-time view of the activity of foraging bees in both field and laboratory contexts and furthers our ability to test hypotheses of optimal foraging in bees and other invertebrates. Future research using RFID could contrast the foraging activity of these two bee species over different landscapes to determine whether the similarities and differences observed in this study are consistent over variable landscapes. Future research could also further elucidate the role of bee size, colony size, communication strategies, and division of labor on their impact to bee foraging behavior. In addition, relating bee foraging activity more directly to available resources, and to energy intake and expenditure would provide crucial understanding as to the optimal foraging behavior of social bees. Future useful technological developments for the study of bees could include designing miniature RFID tags that can be read from a further distance and to permit their use in solitary bees. In addition, the development of affordable and small-scaled technology that could be used to track bees as they move over the landscape, both among flowers and among plants within patches and among patches would represent a breakthrough in the study of bee foraging and bee movement.

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Figure captions

Figure 1. Aerial photograph of the site locations. One honey bee and one bumble bee hives were located in the middle of each circle depicted as a 0.5 km radius at each location.

Figure 2. The mean +/- dry weight (mg) of individual pollen pellets collected by honey bees (solid line) and bumble bees (dashed line) at sites 1, 2, and 3.

Figure 3. The mean +/- dry weight (mg) of individual pollen pellets collected by bees over the five time periods. Time periods with different letters are statistically different from one another as indicated by multiple means tests.

Figures:

Figure 1

Figure 2

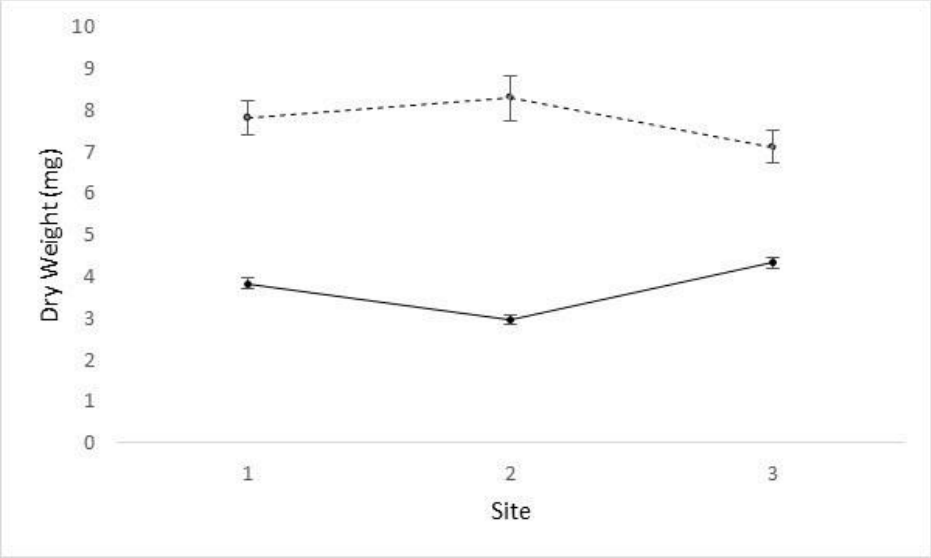
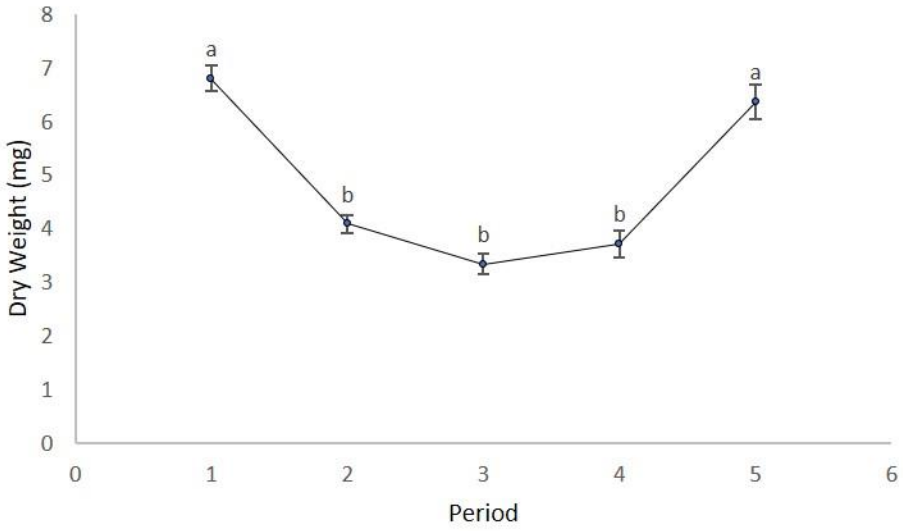


Figure 3



Tables

Table 1. Range of dates for collection of the Radio Frequency Identification (RFID) data and pollen pellets for each of the five periods over the three sites.

Period	Date Range
1	June 14- 27
2	July 1- 13
3	July 23- August 2
4	August 8- 25
5	August 30- September 13

Table 2. The linear mixed effect models used to examine the impact of site, time period, bee species and their interactions on the different metrics of bee foraging effort, at the colony, individual bee or foraging bout levels.

Replicate	Fixed Effect Variables	Random Effect Variables	Foraging Effort
		Colony Level	
Day	Site Period Species Site*Species Period*Species Site * Period	Site * Period * Species	% Tagged bees
		Individual bee level	
Bee	Site Period Species Site*Species Period*Species Site * Period	Site * Period * Species Day(Site*Period*Species)	Average duration of a foraging bout per bee each day Number of foraging bouts per bee each day Total foraging bout duration per bee per day

Table 2. The linear mixed effect models used to examine the impact of site, time period, bee species and their interactions on the different metrics of bee foraging effort, at the colony, individual bee or foraging bout levels.

Replicate	Fixed Effect Variables	Random Effect Variables	Foraging Effort
		Foraging bout level	
Foraging bout	Site Period Species Site*Species Period*Species Site * Period	Site * Period * Species Day(Site*Period*Species) Bee(Site*Period*Species*Day)	Foraging bout Duration

Table 3. Linear mixed effect model used to examine the impact of site, time period, bee species and their interactions on the weight of individual pollen pellets being returned to the hive by foragers.

Replicate	Fixed Effect Variables	Random Effect Variables
Pollen pellet	Site	Site * Period * Species
	Period	Day(Site*Period*Species)
	Species	
	Site*Species	
	Period*Species	
	Site * Period	

Table 4. The impact of site, time period, bee species and their two-way interactions on different metrics of bee foraging effort at the colony and foraging bout levels. The mixed effect linear models used for analyses at each level of the foraging effort metrics are summarized in Table 2. The variables df stands for degrees of freedom, F for the F statistics and P-value is the probability value for the specific factor or interaction in the model.

	Colony Level			Foraging Bout Level		
Fixed Effect Variable	Proportion Tagged Bees Foraging			Foraging Bout Duration		
	df	F	P-value	df	F	P-value
Site	2, 8	0.78	0.491	2, 8	0.25	0.788
Period	4, 8	1.11	0.417	4, 8	2.29	0.148
Species	1, 8	5.37	**0.049**	1, 8	1.16	0.313
Period x Species	4, 8	0.23	0.913	4, 8	0.32	0.854
Site x Species	2, 8	1.10	0.378	2, 8	2.81	0.119
Site x Period	8, 8	0.62	0.746	8, 8	1.47	0.299

Table 5. The impact of site, time period, bee species and their two-way interactions on different metrics of bee foraging effort at the individual bee level. The mixed effect linear models used for analyses of the foraging effort metrics are summarized in Table 2. The variables df stands for degrees of freedom, F for the F statistics and P-value is the probability value for the specific factor or interaction in the model.

	Individual Bee Level								
Fixed Effect Variable	Average Foraging Bout Duration			Total Duration of Foraging			Number of Foraging Bouts		
	df	F	P-value	df	F	P-value	df	F	P-value
Site	2,8	0.13	0.880	2, 8	0.14	0.873	2, 8	0.35	0.714
Period	4,8	1.59	0.266	4, 8	1.08	0.427	4, 8	2.91	0.093
Species	1,8	1.93	0.203	1, 8	13.33	**0.007**	1, 8	9.38	**0.016**
Period x Species	4,8	0.55	0.703	4, 8	0.83	0.541	4, 8	1.73	0.237
Site x Species	2,8	3.13	0.099	2, 8	0.73	0.511	2, 8	0.78	0.491
Site x Period	8,8	1.49	0.293	8, 8	1.51	0.288	8, 8	2.69	0.092

Table 6. The impact of site, time period, bee species and their two-way interactions on the average weight of a pollen pellet returned to the hive by foragers. The mixed effect linear model used for the analysis is summarized in Table 3. The variables df stands for degrees of freedom, F for the F statistics and P-value is the probability value for the specific factor or interaction in the model.

Fixed effect Variable	df	F	P-value
Site	2, 7	1.97	0.210
Period	4, 7	14.29	**0.002**
Species	1, 7	44.05	**0.0003**
Period x Species	4, 7	1.24	0.376
Site x Species	2, 7	4.88	**0.047**
Site x Period	8, 7	2.46	0.126

Chapter 2

Contrasting the patterns of pollen collection and resource preferences between honey bees and bumble bees in a shared landscape

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Abstract: Honey bees and bumble bees are generalist foragers that provide valuable ecosystem services. To gather resources honey bees have an elaborate communication system to recruit foragers to profitable resource patches. Bumble bees on the other hand are known to quickly develop trapline foraging routes that minimize distances traveled and maximize reward intake, the premise behind optimal foraging theory. These strategies are thought to facilitate the acquisition of pollen and nectar when the identity and nutritional quality of these resources varies both spatially and temporally. In this study, we take a comparative approach to examine the pollen foraging patterns of the honey bee (*Apis mellifera*) and common eastern bumble bee (*Bombus impatiens*) in a shared landscape, and test predictions stemming from their known foraging strategies. To do so, we collected pollen pellets from individual bees and identified the pollen contained therein to plant family and morphotype (below family) level. We also surveyed resources available to bees within 500m of each hive location to test for resource preferences. We predict that honey bees will be more frequently flower constant (collect only one pollen morphotype or family on a foraging trip) and collect a lower diversity of pollen than bumble bees, and that both species will preferentially forage on some plant species in our study area.

During five time periods, from June until September 2016, we monitored three bumble bee and three honey bee hives, collecting pollen samples concurrently from both species at one site each day. Bumble bees were generally less flower constant than honey bees on individual foraging bouts, and they collected a greater diversity of pollen relative to honey bees. Furthermore, both bee species showed preferences for some plant groups, with the highest preference for Fabaceae pollen. Bumble bees also showed a preference for Asteraceae pollen. These results are discussed in the context of bee foraging strategies, with specific reference to pollen foraging by honey bees and bumble bees in developed habitats.

Keywords: honey bee, bumble bee, pollen, foraging, resource, preference

Introduction

Bees are important pollinators of many crops and wildflowers, with their ecosystem services being valued at €153 billion (Gallai et al. 2009). Honey bees are an introduced pollinator around the globe and contribute to approximately 19% of all crop plant visits in regions which they occur (Hung et al. 2018). However, both managed and wild bees increase crop production (Garibaldi et al. 2013), and similar factors likely contribute positively to both honey bee and wild bee health (Evans et al. 2018).

Bees are central place foragers, in that they must depart from their nest, locate and gather resources, and return with enough resources for themselves and to provision their offspring, and in the case of social bees, other adults in the colony. As central place foragers the resources available to bees are directly related to their foraging range, or how far from the nest they can

travel to collect resources. Moreover, the costs associated with foraging such as increased predation risk (Visscher and Dukas, 1997), and exposure to pathogens and pesticides (Oldroyd, 2007) preclude foraging large distances unnecessarily. For example, honey bees have the potential to forage at distances greater than 10km (Beekman and Ratnieks 2019), however these distances are not frequently observed while bees are collecting resources. Instead, in the northeastern United States, using decoded waggle dances, Visscher and Seeley (1982) determined the median foraging distance of honey bees was 1.7km. Similarly, *Bombus terrestris* workers in Germany were commonly observed foraging 1.75km from the nest (Walther-Hellwig and Frankl 2000). Finally, *Bombus pascorum* and *Bombus lapidarius* foragers in England had average foraging distances of 512m and 631m, respectively, and foraging distances were negatively correlated with the proportion of suitable resources surrounding the nests (Carvell et al. 2012). These studies demonstrate that bees will not forage at large distances unnecessarily, and emphasize the importance of maintaining suitable forage throughout a landscape.

Bees forage for a variety of resources, including nectar and pollen (Goulson 1999), resins (Simone-Finstrom and Spivak 2010), and water (Kühnholz and Seeley 1997). However, nectar and pollen are the major food resources for bees, with nectar being the primary source of sugars, and pollen being the primary source of protein and other macronutrients and minerals (Roulston and Cane 2000). Patterns of resource acquisition are linked to colony needs but this relationship still needs to be more clearly elucidated. For example, the distances traveled by honey bees while foraging can vary temporally, likely as a result of overall decreases in resource availability (Couvillon et al. 2014a), while the diversity and total abundance of pollen resources collected by honey bees increases later in the foraging season (Danner et al. 2017). Furthermore, the distances

traveled by foragers also seem to vary depending on whether bees are collecting nectar vs. pollen (Couvillon et al. 2014b). Finally, their may be a positive effect of resource diversity on colony growth, as was observed for *Bombus impatiens* colonies (Lanterman and Goodell 2018). These results highlight the need to study resource collection as it relates to resource availability, and to investigate this throughout the foraging season.

Plant taxa vary in how accessible their resources are to specific bee species and also in the quantity and nutritional complement of resources presented to bees (Roulston et al. 2000). We may therefore expect bees to forage on a variety of plant species to fulfill the requirements of the colony. Moreover, differences in communication levels and foraging strategies of distinct bee species may lead to unique patterns of pollen collection. For example, honey bees have the dance communication to recruit foragers to particularly profitable patches as measured by the rate of reward production (Von Frisch, 1967; Seeley et al. 1991). In contrast, individual bumble bees monitor the environment for changes in resource availability through 'majoring' and 'minoring' behavior (Heinrich 1979), while developing and following 'trapline' foraging routes (Pasquaretta et al. 2017; Woodgate et al. 2017). These differences in strategy may have direct effects on the foraging dynamics of bees. For example, because of the waggle dance, cohorts of honey bee foragers may be divided up among a few large resource patches, whereby all bees in a cohort are collecting solely from one plant species. We would therefore expect most honey bees to have one plant morphotype in their pollen sacs, what we call flower constant, and that variation in pollen types being returned to the colony occurs as a result of different groups of bees being recruited to distinct plant species to forage. In contrast, we expect individual bumble bees to visit more than one floral resource within a foraging trip, as trapline foraging may result in bees visiting a

greater diversity of smaller resource patches. Therefore, individual bumble bees are expected to be less flower constant relative to honey bees, and we expect greater species richness in pollen collection within a foraging bout for bumble bees. Moreover, we expect individual bumble bees to collect pollen from different plant species, and together with lower rates of flower constancy, to result in an overall greater diversity of resources being returned to bumble bee colonies relative to honey bees.

As a result of the variation in resource availability among plant taxa, bees may demonstrate a preference toward some plant groups. In fact, bumble bees chose native plants over exotic plants in invaded grassland ecosystems more frequently than would be indicated by the availability of each type (Harmon-Threatt and Kremen 2015). In contrast, generalist social bees, including honey bees, may also collect pollen from non-native plants when they are most prevalent, suggesting the use of resources is based on availability rather than preferences (Wood et al. 2018). It is likely that bees balance availability and preferences. For example, bees may collect pollen or nectar from an agricultural resource pulse, but forage on available weedy species before and after, and this behavior may help diversify the pollen diet of generalist bees (Nicholls and Altieri 2013; Requier et al. 2018).

In this study, we examined and contrasted the patterns of pollen collection between honey bees (*Apis mellifera*) and bumble bees (*Bombus impatiens*) in a shared landscape, and throughout a foraging season. We determined the frequency of flower constant foragers between the two bee species and quantified the richness of plants represented in their pollen loads. Furthermore, we

examined the richness and diversity of all pollen being returned to bee colonies each day. In addition, we determined whether honey bees and bumble bees showed preference towards certain plants and how such preferences changed between bee species and for each bee species through time. The null hypothesis of random foraging was derived from the frequency of the different plant taxa in the study area. To address these objectives, we examined the plant composition of pollen pellets brought back to the hive by individual bees each day and determined rates of flower constancy by individual foragers, along with the richness and diversity of pollen brought back by all bees in a colony. Statistical models determined whether the proportion of flower constant foragers and the richness and diversity of collected pollen varied among bee species, through time, and among sites. To examine preferences by bees, we first quantified the plant resources available within a 500 m radius of the hives at each site and throughout the flowering season. We then contrasted resource availability to the composition of the pollen pellets to detect floral preferences. Based on the known differences in foraging strategies between these two bee species we predict that i) fewer individual bumble bees will be flower constant relative to honey bees, ii) the diversity and richness of pollen brought back to the hives will be greater for bumble bees relative to honey bees, and iii) both honey bees and bumble bees will preferentially forage from a few preferred plant taxa over others. These results are discussed in the context of bee foraging strategies, and how these relate to dynamic colony needs.

Materials and Methods

Study Area and Bee Species

This study was conducted between June and September 2016 at the West Madison Agricultural Research Station (WMARS) in southern Wisconsin. This area consists of experimental agriculture and suburban development. We selected three sites at WMARS based on personal observations of variable plant species diversity. For example, site one was in an alfalfa hay production field, site two was located adjacent to an ornamental garden and a patch of native prairie plants, and site three was in a first-year fallow alfalfa hay production field. At each site we placed one honey bee, *Apis mellifera*, and one bumble bee, *Bombus impatiens*, hive. Individual honey bee and bumble bee hives within a site were separated by 60-100m (site 2: 60m; at sites 1 and 3: 100m). The distance between hive pairs among sites ranged from 700-1500m (sites 1-2: 700m; sites 1-3: 1500m; and sites 2-3: 1400m). Wooden observation hives were used for honey bees, with approximately 2000 workers and an actively laying queen arranged on two vertically stacked deep frames. The bottom frame of each honey bee hive consisted of open and closed brood cells covering approximately half the frame, while the top frame was at least half covered in honey stores. The remaining cells were a mixture of empty cells or bee bread. To guard against weather, the observation hive was placed in a $1.2m^3$ wooden box with an exit tunnel providing access outside. Bumble bee colonies (Koppert Biological Systems, Howell, MI, USA) consisted of approximately 75 workers at the beginning of the experiment, and each hive was placed in a small wooden shelter located 0.5m off the ground.

Pollen Collection from Individual Bees

Pollen pellets were collected from individual honey bees and bumble bees upon their return to the hives. At each of the three sites, pollen pellets were collected over five time periods between mid-June and mid-September in 2016 (Table 1). Within each period, we aimed to collect pollen

pellets over three days per site, moving among sites each day to randomize data collection. Pollen pellets were collected on non-rainy days when the outside temperature ranged between 21 and 35ºC. We began looking for returning foragers between 9 and 10 am on collection days. Individual bees were caught into 2-dram plastic vials as they returned to the hive with pollen pellets and placed in a cooler filled with ice packs until a bee was no longer moving (approximately 5-10 minutes for honey bees, 5-20 minutes for bumble bees). Both pollen pellets were removed from the bee, and each pellet was stored separately in a 1.5 mL microcentrifuge tube. Each tube was labeled with the bee species, site, and time period, plus sample number to indicate an individual bee, and the letters "a" or "b" to distinguish pollen pellets from each corbicula of an individual bee. Following pollen pellet collection, bees were released near the hive entrance.

We continued this process until 40 honey bees and 20 bumble bees were captured on each collection day. More honey bees were collected relative to bumble bees each day to reflect the larger size of the honey bee colonies. This pattern of pollen collection resulted in 9 collection days per period, with each site being visited every 4 to 5 days and the total duration of each period ranging from 10-17 days depending on weather. Our goal was to collect 60 pollen pellets from bumble bees and 120 from honey bees from each site during each of the five time periods for a total of 900 pollen pellets for bumble bees and 1800 for honey bees. However, on some days the pollen foraging frequency was low, and we could not collect the required number of pollen pellets (Table 2). This was particularly pronounced at the end of July during period three. Ultimately, we collected 486 pollen pellets from bumble bees over 34 days, and 1048 pollen pellets from honey bees over 33 days (Table 2). Pollen pellets were kept on ice and, upon return

to the laboratory, were placed in a -20ºC freezer until ready for drying and weighing. One pollen pellet per bee was used to identify the plant morphotypes present in pollen loads as detailed below. This pollen pellet was dried at 45ºC for 24 hours and subsequently weighed to the nearest tenth of a milligram prior to analysis.

Plant identity in pollen pellets

Each individual pollen pellet was broken apart with a small pestle in a 1.5mL microcentrifuge tube, followed by the addition of a pollen staining solution. The stain consisted of a 1:1 ratio of deionized (DI) water to glycerin solution, with ~10 drops of Safarin-O added per 200mL of solution. Depending on pellet size, between 200 and 500 µL of stain solution was added to each pollen pellet in the microcentrifuge tube, and the solution was homogenized by vortexing for 30 seconds. Ten µL of homogenate was then placed onto a microscope slide, topped with a coverslip and sealed along the edges using clear nail polish (Jones 2012). Using light microscopy, we counted and identified 500 pollen grains in each sample at 400x magnification. We made a library of visually distinct pollen morphotypes, and each pollen grain was identified to the lowest possible plant taxonomic level using reference samples collected from the study site, and online atlases (https://www.flickr.com/photos/161453633@N02/collections; paldat.org; http://blogs.cornell.edu/pollengrains; https://globalpollenproject.org/). We were able to identify each pollen grain to plant family, and in a few cases to plant genus and species. Generally, plant species was only inferred when we had reference samples, or the morphotype had highly unique defining features (e.g. *Tilia americana*). Several characteristics were used to identify each pollen grain, including shape, the number of apertures and pores, ornamentation, texture, and relative size.

Following pollen identification, the proportional abundance of each plant taxon within a pollen pellet was calculated. We did these calculations at both the plant family and morphotype levels. The morphotype classification included all the various identifications below family including genus (e.g. *Hemerocallis spp.*) or species level classification (e.g. *Lotus corniculatus*) together with unknown morphotypes that are labeled according to the known plant family (e.g. Fabaceae_Other) or family plus defining features (e.g. Asteraceae_Spines, Asteraceae_Lophate). This approach facilitated an estimate of the total richness of pollen types that have distinct morphologies (21 total morphotypes), which is greater than the number of families represented in pollen pellets (16 total families).

Because we did not find set standards for the minimum abundance necessary to consider a pollen taxon as 'collected' by foraging bees, in contrast to 'contamination,' we selected three threshold values encountered in the literature, 1%, 3% and 5%. These corresponded, respectively, to counting 5, 15, or 25 pollen grains in a sample of 500 total grains. Furthermore, for the different variables examined below, flower constancy of individual bees and the richness and diversity of pollen collected by a colony, comparing the results obtained using the different threshold levels permitted us to determine if and how threshold values affected the results. We could also examine the impact of the threshold criterion on the results obtained for the plant family and the plant morphotype level classifications. Finally, we verified that our sample size of 500 pollen grains was large enough to accurately quantify all morphotypes present in individual pollen pellets by making rarefaction curves using the function 'rarecurve' in the 'vegan' (Oksanen et al. 2019) package in R (R Core Team 2013). This analysis indicated that 200 pollen grains captured all pollen morphotypes and families (Fig. 1).

Flower constancy

We called an individual bee flower constant if the pollen pellet brought back to the hive contained pollen grains from only one plant morphotype (morphotype constancy) or family (family constancy). For each classification level, morphotype or family, we determined constancy for each of the three threshold levels. We calculated the proportion of foragers that were morphotype or family constant each day for a bee species, time period, and site. Linear mixed models (proc mixed in SAS v. 9.4) were used to determine the impact of bee species, time period, site, and their two-way interactions on the proportion of foragers that were morphotype or family constant. Day was the replicate in the model and bee species, period, site and the two-way interactions were fixed effects. The three-way interaction term, site*period*species, was included as a random effect in the model, and provided the error term against which each fixed effect was tested.

Pollen richness and diversity

To examine the foraging patterns of colonies, we calculated the richness and diversity of pollen brought back to the hive each day at both morphotype and family classification levels. Richness and diversity values were calculated using the pooled bumble bee or honey bee pollen pellets collected on a given day at a site and time period. As described earlier, threshold levels of 1,3, or 5% at the level of individual pollen pellets were used for inclusion of a plant taxon, and each threshold value produced a unique data set for further analyses. Pollen richness values were calculated by summing the total number of plant morphotypes (species, genus or type) or plant families present in the daily collection of pollen pellets at a given site and time period. Pollen diversity was calculated using the Simpsons D Index (1-D):

$$\sum_{i=1}^{S} p_i^2$$

where p_i is the proportion of morphotype or family i, and S is the total number of morphotypes or families, both calculated over the pooled pollen pellets collected each day. Pollen diversity was calculated using the 'diversity' function in the 'vegan' package (Oksanen et al. 2019) in R (R Core Team 2013). Diversity values ranged from 0, indicating no diversity, i.e. all pollen grains represented one plant morphotype or family, to 1, indicating infinite diversity, where pollen grains were collected in equal abundance from multiple plant morphotypes or families. To standardize among pollen pellets, given pollen grain counts were not always exactly 500, Simpsons D was calculated by summing the proportions, instead of absolute counts, of pollen morphotypes or families over all pollen pellets collected by a hive on a given day. The impact of bee species, period, site, and their two-way interactions on pollen richness or pollen diversity were examined using similar linear mixed models to those previously described in the flower constancy section.

Plant composition of pollen pellets

While the richness and diversity metrics summarized the number and relative abundance of plant morphotypes or families present in a pollen pellet, it did not provide information about variation in the identity of plant morphotypes collected at the different sites and time periods by the two bee species. For example, while a similar value for species richness indicated that bees collected pollen from a given number of plant types, it did not determine whether bees collected from the same or from different plant types. To examine the plant composition of pollen pellets, we

describe the relative abundance of plant families and morphotypes represented in pollen samples collected by honey bees and bumble bees through time and among sites.

Plant resource availability

While pollen pellets provided information on the richness, diversity, and identity of plants that bees were foraging on for pollen, knowledge of resource availability, or the plant species that were flowering throughout the season, would help determine whether honey bees and bumble bees selected plants randomly, i.e. relative to their abundance, or preferred certain plant morphotypes. To quantify resource availability, we performed four field surveys (survey periods) at each site within the study area once per month beginning in mid-June and continuing through mid-September (Table 1). The exception to this was site one, for which the July survey was missed. These were treated as missing observations in our analyses. For each survey, we established five 100 x 2 meter transects at each site, for a total of 15 transects throughout the study area. One exception to this distance was one transect at site three which, due to initial measurement error was placed 600 meters from the hives. Transects were placed in areas adjacent to crop plantings. Each transect was subsampled using ten 1-meter diameter circular quadrats. We counted the number of species present and the relative abundance in each quadrat. To estimate floral abundance, we counted the number flower clusters for plants with racemes (e.g. Fabaceae) or composite flowers (e.g. Asteraceae), or the number of individual flowers (e.g. Caryophllaceae).

As we had too many zero counts at the quadrat level, richness and diversity metrics for resources were estimated at the transect level, which resulted in five replicates at each site during each

survey period. Species richness represented the number of plant species in a transect, and family richness the number of plant families represented in a transect. Furthermore, we grouped observed plant species in the resource surveys into pollen morphotype groups to facilitate comparison with pollen types collected by bees. Specifically, plants in the Asteraceae family known to possess pollen with a clear 'spine-like' ornamentation were grouped into the 'Asteraceae_Spines' category to match the pollen morphotype data. This category included *Tagetes spp., Erigeron annuus, Rudbeckia hirta, Cirsium arvense, Cirsium vulgare,* and *Solidago canadense.* Plants with lophate pollen were grouped in the 'Asteraceae_Lophate' group and included *Cichorium intybus, Taraxicum officinale,* and *Hieracium canadense.* The plant species *Daucus carota* and *Pastinaca sativa* were grouped as the 'Apiaceae_Type', *Potentilla spp.* was classified as 'Rosaceae_Type', *Phleum pratense* as 'Poaceae_Type', and both *Stellaria media* and *Silene latifolia* were classified as 'Caryophyllaceae_Type'. Finally, *Medicago sativa, Trifolium repens,* and *Securigera varia* were pooled into the 'Fabaceae_Other' category.

Diversity metrics were calculated based on relative floral abundance, using the Simpson D index at the species, family, and morphotype levels as described previously. We determined the impact of site and survey period on plant richness or plant diversity using a linear mixed model (proc mixed, SAS v. 9.4) with site and survey period as fixed effects, and their interaction as a random effect. A transect was the replicate in the model, and the fixed effects were tested against the two-way interaction error term.

Bee preference for floral resources

If bees foraged randomly, we would not expect a difference between the plant morphotypes they foraged on and the abundance of these plant morphotypes in the plant surveys. To determine whether bees showed preferences for floral resources or foraged randomly, we compared the relative abundance of flowering plants in the field to the plants identified in pollen samples collected by bees. To facilitate comparisons between the plant resources available in the field and the types of pollen collected by bees, we used the pollen morphotype categories described in the plant resource availability section. Furthermore, for floral resources, we calculated the relative abundance estimates of plant morphotypes as a proportion of total flower abundance. These relative floral abundance estimates were calculated for the entire summer, and for each survey period. To estimate the occurrence of plant morphotypes in the pollen pellets, we counted the number of occurrences for which a plant morphotype was observed in at least 3% of a pollen pellet. One occurrence would indicate that an individual bee collected pollen from plants within a morphotype category.

We used the Chi-square goodness of fit test to determine whether bees showed a preference for specific plant morphotypes or foraged randomly, i.e. based on the abundance of the plant morphotypes in the surveys. To perform this analysis, we first determined the expected values for plant morphotypes in pollen pellets based on plant resource availability, based on the null hypothesis of random foraging. In order to do this, we calculated the proportional abundance of each plant morphotype identified in resource availability surveys. We also compiled and ranked the number of observations of each plant morphotype found in bee collected pollen samples. The total number of pollen samples in each analysis was then multiplied by the proportional abundance of each plant morphotype in resource surveys to generate the expected number of

observations for each morphotype in bee collected pollen pellets under random foraging. This procedure was performed separately for honey bees and bumble bees, first using all data collected throughout the summer, and then for each period separately. This resulted in six tests for each bee species. When the number of observations for a plant morphotype in bee collected pollen was less than 5, that morphotype was pooled into an 'other' category to meet the minimum number of counts per cell requirement of the Chi-square test. If the number of observations in pollen samples for 'other' was still below 5 it was omitted from the analysis. Plant morphotype categories that were solely identified in pollen samples, but not identified in resource surveys were omitted from these analyses, because we were unable to estimate expected values from resource surveys. However, we reported these values to determine how often bees foraged on plant morphotypes not recorded in our plant resource surveys.

We ran Chi-square tests with and without the 'other' category to better understand what effect, if any these pooled categories had on the overall results. A statistically significant Chi-square test would indicate that bees do not forage randomly, but instead provide evidence that bees show a preference towards some plants that are represented by specific pollen morphotypes. To determine which plant types were preferred by bees, we looked at Pearson residual values from significant Chi-square tests, which indicated the contribution of each morphotype group to the overall Chi-square. A positive residual value would indicate a preference, or more frequent collection than would be predicted based on availability, while a negative value indicates that bees collect this resource in a lesser amount than would be predicted by availability, and a possible avoidance.

Results

Flower constancy

The percentage of morphotype constant foragers varied over the flowering season for both honey bees and bumble bees, but the patterns of temporal change were different between the two bee species (Table 3; Fig. 2). To illustrate this point, at the 3% threshold for morphotypes bumble bees were less morphotype constant than honey bees during periods one ($df = 7$; $t = 3.23$; $P = 0.014$), two ($df = 7$; $t = 5.60$; $P = 0.001$), four ($df = 7$; $t = 3.38$; $P = 0.012$), and five ($df = 7$; $t = 3.22$; $P = 0.015$) (Fig. 2). In period three, honey bees had lower rates of morphotype constancy than bumble bees, however this difference was not statistically significant ($df = 7$; $t = 2.19$; $P = 0.065$) (Fig. 2). Furthermore, within bumble bees, the frequency of flower constancy was lower during period two than periods one ($df = 7$; $t = 3.79$; $P = 0.007$), three ($df = 7$; $t = 3.71$; $P = 0.008$), four ($df = 7$; $t = 2.41$; $P = 0.047$), and five ($df = 7$; $t = 3.06$; $P = 0.018$) (Fig. 2). In contrast, within honey bees, a lower proportion were morphotype constant in period three than periods one ($df = 7$; $t = 4.15$; $P = 0.004$), two ($df = 7$; $t = 2.84$; $P = 0.025$), four ($df = 7$; $t = 3.33$; $P = 0.013$), and five ($df = 7$; $t = 3.5$; $P = 001$) (Fig. 2). The proportion of flower constant foragers did not vary among sites for either the morphotype or family analyses at any of the threshold levels. Finally, there were no statistically significant interactions between site and either species or period on the proportion of flower constant foragers (Table 3).

We also observed differences among threshold levels and among classification levels (morphotype vs. family) in the frequency of flower constant foraging bouts by honey bee and bumble bee foragers (Table 3). For example, when flower constancy was calculated based on plant families, as opposed to plant morphotypes, we did not detect a statistically significant

period effect, and the interaction between period and bee species was borderline at the 5% threshold level (Table 3). For either classification level, the interaction between period and bee species was not statistically significant at the 1% level (Table 3). Finally, the proportion of flower constant foragers did not vary among sites for either the morphotype or family analyses at any of the threshold levels, and there were no statistically significant interactions between site and either species or period (Table 3).

Pollen richness and diversity

None of the factors included in our model affected the morphotype richness of pollen collected by honey bees and bumble bees (Table 4). However, there was a statistically significant two-way interaction between period and bee species on the number of pollen families collected by bees when analyzed at the 3% threshold level (Table 4). Specifically, bumble bees collected pollen from a greater number of plant families relative to honey bees in period one ($df = 7$; $t = 3.10$; $P = 0.017$), with a marginally significant effect in period two ($df = 7$; $t = 2.28$; $P = 0.057$), but collected from fewer plant families in period three ($df = 7$; $t = 2.48$; $P = 0.042$) (Fig. 3). There were no differences between bee species in period four ($df = 7$; $t = 1.71$; $P = 0.132$), or period five ($df = 7$; $t = 0.06$; $P = 0.957$) (Fig. 3). Finally, while there were no differences among periods for bumble bees, honey bees collected pollen from a greater number of plant families during period three than period one ($df = 7$; $t = 3.32$; $P = 0.013$) or period two ($df = 7$; $t = 2.65$; $P = 0.033$) (Fig. 3). Overall, bumble bees collected pollen from 5.38 ± 0.35 plant morphotypes and 4.26 ± 0.27 plant families in a given day, while honey bees foraged for pollen on 4.36 ± 0.33 plant morphotypes, representing 3.39 ± 0.27 plant families.

Concerning pollen diversity, at the morphotype level there were no effects of bee species, time period, site, or their interactions on the diversity of pollen collected by bees across all threshold levels (Table 5). When pollen diversity was classified at the plant family level, however, we observed statistically significant differences between the two bee species and among time periods (Table 5). These results were consistent across threshold levels. Using the 3% threshold we found that bumble bees collected more diverse pollen (mean ± se) (0.54 ± 0.03) relative to honey bees (0.33 ± 0.04) (df = 7; t = 3.33; P = 0.013) (Fig. 4a). Among periods, bees collected pollen from a lower diversity of plant families in period one (0.24 ±0.07) than periods three (0.57 ± 0.04) (df = 7; t = 3.44; P = 0.011), four (0.53 ± 0.05) (df = 7; t = 3.48; P = 0010), or five (0.51 ± 0.05) (df = 7; t = 3.11; P = 0.017) (Fig. 4b). The diversity of pollen collected during period two (0.42 ± 0.05) was greater than period one, but this effect was marginally significant (df = 7; t = 2.31; P = 0.055) (Fig. 4b). There were no differences between period two and periods three, four, or five (Fig. 4b). Finally, there were no effects of site or any of the two-way interactions on the diversity of plant families represented in bee collected pollen samples (Table 5).

Plant composition of pollen pellets

Considering the congruency between the 3% and 5% threshold levels in the proportion of flower constant foragers, we will report pollen composition using the 3% threshold as a minimum for inclusion as 'collected' pollen. Overall, 65% of pollen brought back to hives by honey bee foragers over the flowering season was from the Fabaceae plant family (Fig. 4a). In contrast, Fabaceae was represented in 38%, and Brassicaceae was represented in 34% of all bumble bee collected pollen (Fig. 4a). Among periods, Fabaceae pollen was the most abundant during all periods except for period five (Fig. 4b). Specifically, Fabaceae pollen made up 82.6% of the

pollen collected in period one, 59% in period two, 40.8% in period three, 42.8% in period four, but only 25.6% in period five (Fig. 4b). In period five, Asteraceae became the most dominant (31.3%) plant family represented in all pollen samples along with Brassicaceae (26.2%) (Fig. 4b).

Plant families over period and site

Fabaceae was the most dominant plant family represented in pollen samples collected by bumble bees during period one (43.1%), period two (40.8%), and period four (33.9%) (Fig. 5a). Asteraceae was the most common plant family represented in pollen samples during period three (40.9%), and Brassicaceae was the most common in period five (44.2%) (Fig. 5a). Furthermore, Brassicaceae was the second most abundant plant family represented in pollen samples collected by bumble bees during period one (37.9%) and period two (28.1%), and during period four was in near equal abundance (33.7%) with Fabaceae (Fig. 5a). In contrast, for honey bees, Fabaceae made up 97% of all pollen collected during period one and was still dominant in period two (69%), period three (42%), and period four (49%) (Fig. 5a). In period five, Asteraceae was the most dominant group, with 46% of the pollen collected by honey bees, while Fabaceae only made up 19% of the pollen collected during period five (Fig. 5a).

For bumble bees, pollen collection patterns were remarkably consistent among sites, with the most dominant pollen types represented by the Fabaceae and Brassicaceae families, followed by Asteraceae (Fig. 5b). A similar consistency among sites was observed for honey bees, except that, at each site, honey bees collected most of their pollen from plants in the family Fabaceae

(Fig. 5b). At site two, Geraniaceae was the second most collected pollen family (9.1%), whereby at sites one and three it was present in 0.3% and 3.6% respectively (Fig. 5b). Honey bees at site three collected a greater proportion of their pollen from the Apiaceae family, which represented 25.1% of all pollen collected, relative to 6.2% at site two and 1% at site one (Fig.5b).

Plant morphotypes over period and site

Bumble bees commonly collected Brassicaceae_Type (B) and Fabaceae_Other (F) pollen during period one (38% B, and F), two (28% B, 36% F), four (33% B, 28% F), and five (44% B and 31% F) (Fig. 6a). In period three, Asteraceae_Spines was the most dominant morphotype (35%) collected by bumble bees (Fig. 6a). Apiaceae_Type pollen was more common in period three (19%), while pollen from *Tilia americana* (Malvaceae) was only present in period one (8%) and period two (12%) (Fig. 6a). For honey bees, period one was dominated with Fabaceae_Other pollen (75%) (Fig. 6a). Fabaceous plants remained relatively abundant in all periods. Notably *Lotus corniculatus* was the most abundant morphotype in period two (45%) and period four (27%), while Fabaceae_Other was the most prevalent (30%) pollen type during period three. In contrast Asteraceae_Spines became the most dominant (42%) during period five.

Bumble bee pollen collection patterns were consistent among sites, with Fabaceae_Other and Brassicaceae_Type pollen most common (Fig. 6b). There was less consistency among sites for honey bees (Fig. 6b). Specifically, Fabaceae_Other pollen made up 36%, 38%, and 42% of pollen morphotypes collected by honey bees at sites one, two, and three respectively. Furthermore, *Lotus corniculatus* was the second most abundant pollen morphotype at sites one

(32%) and two (28%), but only made up 8% of pollen morphotypes collected by honey bees at site three (Fig. 6b). Moreover, Apiaceae_Type pollen was the second most abundant (25%) of all pollen collected by honey bees at site three (Fig. 6b).

Plant resource availability

There was a statistically significant effect of site on the richness of flowering plant families available to bees (Table 6). Specifically, site one had more plant families, or greater family richness (mean ± se) (2.47 ± 0.31) than site two (1.47 ± 0.15) (df = 5; t = 3.34; P = 0.021) (Fig. 7a). There were no statistically significant differences in plant family richness between sites one and three (site 3: 2.1 ± 0.14) (df = 5; t = 1.27; P = 0.259), or sites two and three (df = 5; t = 2.39; P = 0.062) (Fig. 7a). Finally, there were no differences among sites or periods in the richness of plant species or morphotypes (Table 6; Fig. 8), or in the diversity of families (Fig. 7), species or morphotypes available to bees (Table 6; Fig. 8).

Plant families available to bees

Fabaceae, Asteraceae and Apiaceae were the three most common plant families at all sites (Fig. 6a). Asteraceae was the most common plant family at site one (52%), while Fabaceae was the most common at site two (49%) and site three (63%) (Fig. 7a). Asteraceae was the second most common plant family at site two (40%) and site three (19%), while Apiaceae was third most prevalent plant family represented at site one (16%), site two (9%), and site three (16%) (Fig. 7a). Among resource surveys, Fabaceae was the most abundant in June (53%) and July (70%) but decreased in abundance through August (48%) and September (15%) (Fig. 7b). Furthermore,

Asteraceae was the second most abundant plant family in June (44%) before decreasing in abundance in July (3%), but then increasing in abundance into August (24%), and ultimately becoming the most prevalent plant family in September (62%) (Fig. 7b). In contrast, Apiaceae was only present in 0.6% of observations in June, but increased to 23% in July, 26% in August, and 14% in September (Fig. 7b).

Plant morphotypes available to bees

The most common flowering plant morphotype at site one was Asteraceae_Spines (52%), and Apiaceae_Type (16%) (Fig 8a). At site two Fabaceae_Other was the most prevalent (21%), followed by *Trifolium pratense* (18%), and Asteraceae_Lophate (17%) (Fig. 8a). At site three Fabaceae_Other was most prevalent (31%), followed by *Lotus corniculatus* (24%), and Apiaceae_Type (16%) (Fig. 8a). Among resource surveys, in June Asteraceae_Spines was the most prevalent plant morphotype (43%), followed by Fabaceae_Other (25%), and *Lotus corniculatus* (24%) (Fig. 8b). In July, Fabaceae_Other became the most prevalent (30%), followed by *Trifolium pratense* (30%) and Apiaceae_Type (23%) (Fig. 8b). In August, Apiaceae_Type became the most abundant (26%), followed by *Lotus corniculatus* (19%), Fabaceae_Other (18%), and Asteraceae_Spines (13%) (Fig. 8b). Finally, in September Asteraceae_Lophate was the most common plant morphotype (23%), followed by Asteraceae_Unknown1 (21%), Apiaceae_Type (14%), and Fabaceae_Other (10%) (Fig. 8b).

Bee preferences for floral resources

Bumble bee preferences

Bumble bees did not forage randomly within our study area overall (χ^2 = 286; df = 5; P < 0.0001) or at any of the time periods (period 1: (χ^2 = 95.47; df = 2; P < 0.0001); period 2: (χ^2 = 2048; df = 4; P < 0.0001); period 3: (χ^2 = 90.1; df = 1; P < 0.0001); period 4: (χ^2 = 89.1; df = 4; P < 0.0001) and period 5: (χ^2 = 179.9; df = 2; P < 0.0001)). Over the flowering season, bumble bees demonstrated an overall preference for Fabaceae_Other, and to a lesser extent Asteraceae_Lophate and Asteraceae_Spines morphotypes (Table 7). They tended to avoid Apiaceae_Type, *Trifolium pratense* and *Lotus corniculatus* plants (Table 7). Their preferences varied among periods (Table 7). In period one, they preferred Fabaceae_Other while in period two, they preferentially foraged on Asteraceae_Lophate, and Asteraceae_Spines, and to a lesser extent Fabaceae Type. In period three, bumble bees showed a preference for Asteraceae_Spines and Fabaceae_Other morphotypes. These results were consistent whether 'other' category was included or excluded in the analyses. Chi-squares for periods 4 and 5 were performed without the 'other' category, as it had less than five observations. During period four, bumble bees showed a preference for Asteraceae_Spines, Fabaceae_Other, and Asteraceae_Lophate. Finally, in period five bumble bees showed a preference for Fabaceae_Other, and to a lesser extent Asteraceae_Spines (Table 7).

Honey bee preferences

Honey bees showed preferences for plant morphotypes within our study area (χ^2 = 464; df = 5; P < 0.0001). Specifically, over the entire season they exhibited a preference for Fabaceae_Other and *Lotus corniculatus*, and to a lesser extent Apiaceae_Type pollen (Table 8). Honey bees also exhibited preferences during each of the five periods during the flowering season (period 1: χ^2 =

512.5; df = 3; P < 0.0001); period 2: (χ^2 = 455.9 df = 4; P < 0.0001); period 3: (χ^2 = 45.8; df = 3; P < 0.0001); period 4: (χ^2 = 46.8; df = 3; P < 0.0001); period 5: (χ^2 = 302.4; df = 2; P < 0.0001). However, their preferred plant morphotypes varied over the flowering season (Table 8). During period one honey bees demonstrated a preference for Fabaceae_Other. In period two honey bees switched their preference to *Lotus corniculatus*, and to a lesser extent Apiaceae_Type. Honey bees in period three preferentially foraged on Asteraceae_Spines, *Lotus corniculatus*, Fabaceae_Other, and Apiaceae_Type. During period four honey bees showed a preference towards plants with the Asteraceae_Spines morphotype, along with *Lotus corniculatus* and Fabaceae_Other. Finally, in period five honey bees demonstrated a preference towards Asteraceae_Spines, and to a lesser extent Fabaceae_Other (Table 8).

Discussion

Honey bees are more flower constant during individual foraging bouts relative to bumble bees at both the morphotype and family level classifications. In addition, at the colony level, honey bees collected pollen from a lower diversity of plant families than bumble bees. These patterns support our predictions based on differences between species in their modes of communication and foraging strategies. The dance communication of honey bees for example, serves to allocate cohorts of foragers among resource patches that produce high rates of reward (Visscher and Seeley 1982), while bumble bees in contrast, learn and memorize trapline foraging routes that result in minimizing distances traveled while maximizing reward intake (Lihoreau et al. 2012). Furthermore, trapline foraging is the result of an individualistic learning process, by which bumble bees may visit multiple plant types within a foraging bout, especially during their early

foraging bouts, and when there is turnover in the identity and abundance of resources in the environment (Heinrich 1979).

Trapline foraging and the sharing of information about resource quality is not exclusive to either species. For example, honey bees have been observed following trapline routes in lab and field settings at small spatial scales, possibly as a strategy for optimally foraging among smaller nearby patches of multiple flower types (Buatois and Lihoreau 2016). In addition, while it is currently understood that bumble bees don't communicate distance and direction information, they do actively recruit workers to the task of foraging via the distribution of recruitment pheromone (Dornhaus et al. 2003), and the rapid movement of returning foragers throughout the nest (Dornhaus and Chittka 2001). Furthermore, newly recruited foragers learn the odor of resources being returned to the hive, which may facilitate more rapid exploitation of flowers (Molet et al. 2009).

Both bee species had a shared preference for Fabaceae_Other pollen morphotype. However, bumble bees also demonstrated a preference towards both Asteraceae pollen morphotypes (Asteraceae_Spines and Asteraceae_Lophate), while honey bees preferred Apiaceae_Type. These results demonstrate that both honey bees and bumble bees in this study system may rely on weedy plants for their nutritional needs. The prevalence of weeds in bee collected pollen has been similarly documented for honey bees in intensive agriculture systems (Requier et al. 2018), along with bumble bees and other wild bees (reviewed in Bretagnolle and Gaba 2015).

Regarding pollen morphotypes, the most likely plant species contributing to the Fabaceae_Other pollen morphotype was *Trifolium repens*, as honey bee and bumble bee foragers with pollen pellets were often observed foraging on this plant throughout the season (personal observation). Furthermore, *Trifolium repens* is frequently documented as being in the top five most abundantly collected pollen types by honey bees (Keller et al. 2005). We also observed *Daucus carota* throughout the experimental area, which is likely to be the primary contributor to the Apiaceae_Type pollen morphotype. On the other hand, Brassicaceae was not observed in any of our resource availability surveys. While it is possible that small patches of Brassicaceae were missed in our surveys, the prevalence of the Brassicaceae_Type pollen morphotype, particularly in bumble bee pollen samples indicates that bees had located this resource in patches outside our survey area.

Considering the foraging strategies of bees, it is thought that honey bees recruit foragers to large monofloral patches, while bumble bees are more evenly distributed in the landscape. For example, honey bees have been observed strongly associating with large mass-flowering crops when in bloom, while bumble bees were observed at low abundances in the same large crop bloom, but also in low abundance in nearby semi-natural habitats (Rollin et al. 2013). To further illustrate this point, among the lesser collected pollen sources in this experiment, we observed a pulse of pollen collection by bumble bees from *Tilia americana* at the end of period one and into period two. Interestingly, we observed 46 bumble bee individuals collecting pollen from this tree species, 30 of which had collected *T. americana* as the primary pollen type. In contrast only five honey bee foragers collected *T. americana* pollen, and in amounts no more than 5% of a pollen pellet. The reason for this disparity is not clear but could relate to the foraging strategies of bees.

For example, if there are a small number of *T. americana* trees in the area, this might not be enough to affect a successful recruitment effort by honey bee scouts (Seeley et al. 1991). Bumble bees on the other hand could include some visits to this species on a trapline route. Future studies could explicitly test these hypotheses.

We observed overlap in pollen collection between honey bees and bumble bees, notably in the use of weedy plants. However, we also observed some disparities, such the use of *Tilia americana* pollen by bumble bees, and preferences for Apiaceae pollen by honey bees, and Asteraceae pollen by bumble bees. While we surveyed resources available to bees within 500 meters of the hive, bees are going beyond this distance, as some plant species that were collected (e.g. Brassicaceae, Geraniaceae, and *Tilia americana*) were not observed in our surveys. For example, suburban home gardens were also prevalent in the surrounding landscape, which could increase the diversity of resources available to, and collected by bees (Goulson et al. 2002).

Both honey bees and bumble bees are generalist foragers, and in this study collected pollen from several plant families and morphotypes. Many of these pollen morphotypes represent weedy species, possibly collected from the surrounding edges of crop plantings. However, it is also likely that pollen was collected from nearby suburban gardens, and unknown locations outside of the 500 m survey area of each site. Pollen provides an important source of nutrients for bees, such as proteins, lipids, and other macromolecules (Roulston and Cane, 2000), with important consequences for the size and overall health of adult bees (Roulston and Cane 2002).

Furthermore, the quality of pollen, measured by the presence and abundance of these nutritional factors is variable among plant taxa (Roulston et al. 2000).

We observed a moderate diversity of pollen resources collected by both honey bees and bumble bees throughout the summer. This implies that the foraging strategies implemented by both bee species result in the collection of diverse resources when available. However, there were differences between honey bees and bumble bees in the diversity of pollen families collected, but not morphotypes, along with differences in overall pollen resource preferences. This could result from preferences by bees for flowers with different morphologies, whereby each bee species is roughly collecting from the same number of resources, but one bee species, in our case bumble bees are expanding out to additional plant families (e.g. Asteraceae). Indeed, honey bees preferred multiple Fabaceae pollen types, such as *Lotus corniculatus* which was not preferred by bumble bees. Alternatively, plant families may vary in their nutrition profile, more so than among sub-familial taxonomic levels, and bumble bees may be collecting a greater diversity of resources to support their colony needs compared to honey bees. Further studies need to be undertaken to elucidate the differences between bee species in their preference for specific flower morphology, and in the nutritional complement of resources visited.

We also observed somewhat different results between threshold values for inclusion of pollen types as being collected vs contamination at both the morphotype and family levels. This has important implications for interpretations of flower constancy, and the richness and diversity of pollen collected by bee colonies. Applying these thresholds at the level of individual pollen

pellets we found the results from including pollen morphotypes or families present in 3% and 5% of a pollen pellet aligned in their outcomes, and with our data more generally. Specifically, our data seemed to indicate a differential species response through time in the rates of flower constancy by individual bees. However, at the 1% threshold this pattern did not show up statistically, while it did in both the 3% and 5% thresholds. Considering these results, we suggest that future studies examine the congruency between various thresholds for inclusion and what their data look like before deciding on which threshold is most applicable to a given study. We chose the 3% threshold here, as it is the minimum value that struck a balance between aligning with what we observed in our data, and the observed congruency with the next higher threshold value of five percent.

To further investigate the reason for differences in preferences between honey bees and bumble bees, and to further understand the role of analyzing pollen diversity metrics at the morphotype or family level, we would like to determine the relationship between the nutritional complement of collected pollen, specifically protein and amino acid profiles. Finally, we recommend stakeholders in agriculture, city planning, and landscaping, among others, consider the importance of maintaining diverse plant communities to in turn support diverse bee communities in developed landscapes.

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Figure Captions:

Figure 1: Rarefaction curve to determine a suitable sample size for number of grains. We counted 500 pollen grains per pollen pellet. A minimum of 200 pollen grains should be counted to identify all pollen morphotypes in pollen pellets.

Figure 2: The proportion (mean ± se) of collected bumble bees (red) and honey bees (blue) that are family (top) and morphotype (bottom) constant foragers during each period, and at each threshold value (1%, left; 3%; middle; 5%, right).

Figure 3: The richness of plant families represented in pollen samples collected by honey bees (blue) and bumble bees (red) among periods.

Figure 4: Diversity of plant families represented in pollen samples. There were no differences in the results between threshold values, therefore, the figures represent all pollen types represented in at the 3% threshold level. A) The diversity of pollen families collected by bumble bees and honey bees. All pollen from bumble bees and all pollen for honey bees across periods and sites were pooled. B) The diversity of pollen families collected by all bees during each period. All pollen was pooled from both species at all sites for each period. Different letters represent significant differences between periods. Simpsons D is indicated in gray.

Figure 5: The relative proportion of pollen families collected by bumble bees and honey bees among periods (A) and among sites (B). Taxon abundance from each site and species was pooled for each period (A). Taxon abundance from each period and species were pooled for each site (B).

Figure 6: The relative proportion of pollen morphotypes collected by bumble bees and honey bees among periods (A) and among sites (B). Taxon abundance from each site and species was pooled for each period (A). Taxon abundance from each period and species were pooled for each site (B).

Figure 7: Plant families observed during resource availability surveys within 500m of the hive locations at each site (A), and during each period (B). Of note, observations at site one were missed during the July survey period.

Figure 8: Relative proportion of morphotypes observed within 500 meters of the hive locations at each site (A) and at each time period throughout the summer (B). Note, there were no observation at Site 1 during July.

Figures

Figure 1

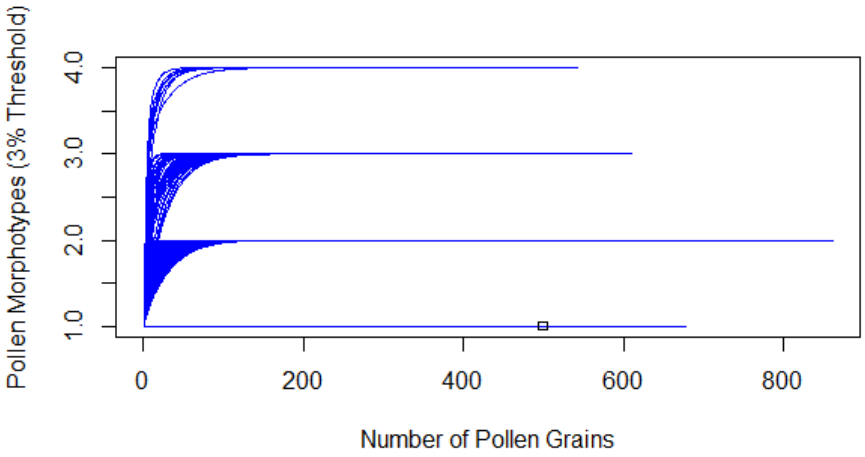
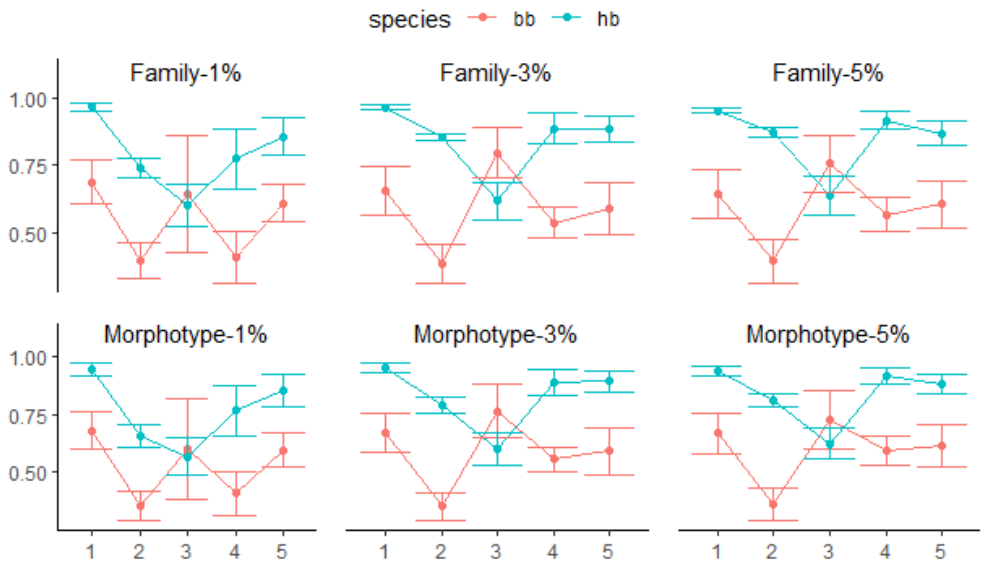


Figure 2


species
bb
hb
Family-1%
Family-3%
Family-5%
Morphotype-1%
Morphotype-3%
Morphotype-5%
1.00
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1
2
3
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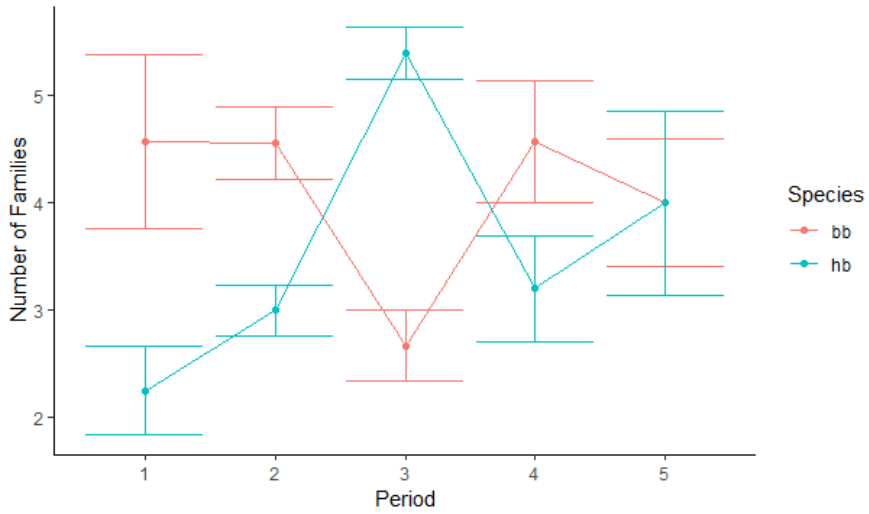
5

Figure 3



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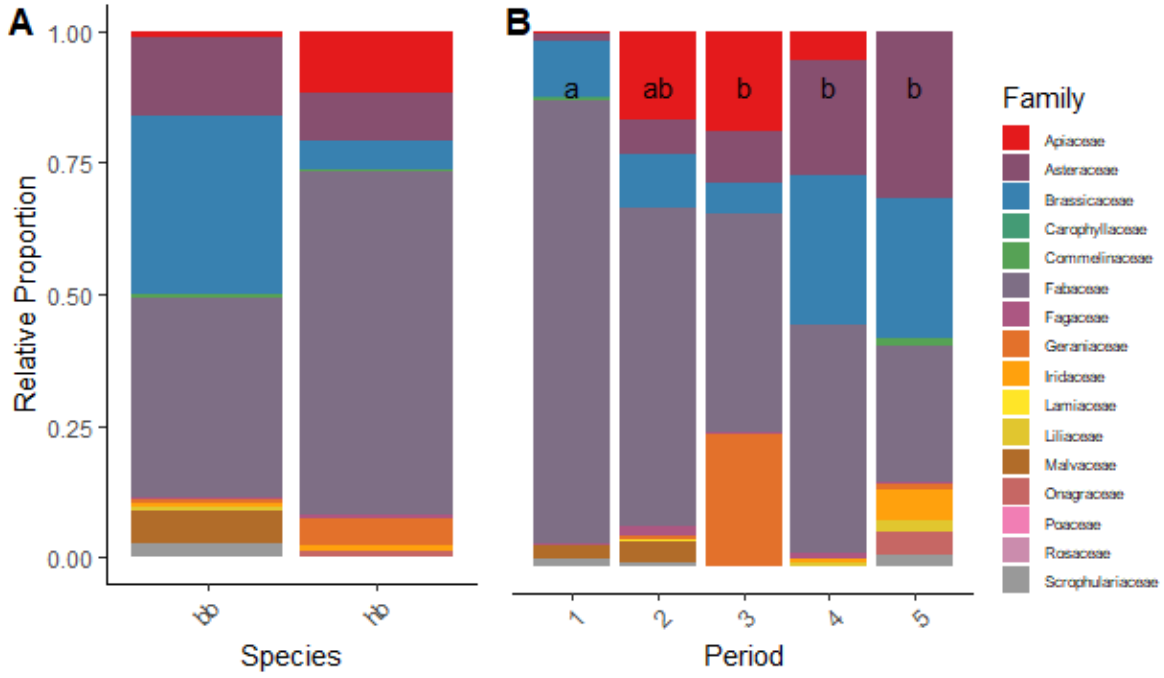
A
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Relative Proportion
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b
b
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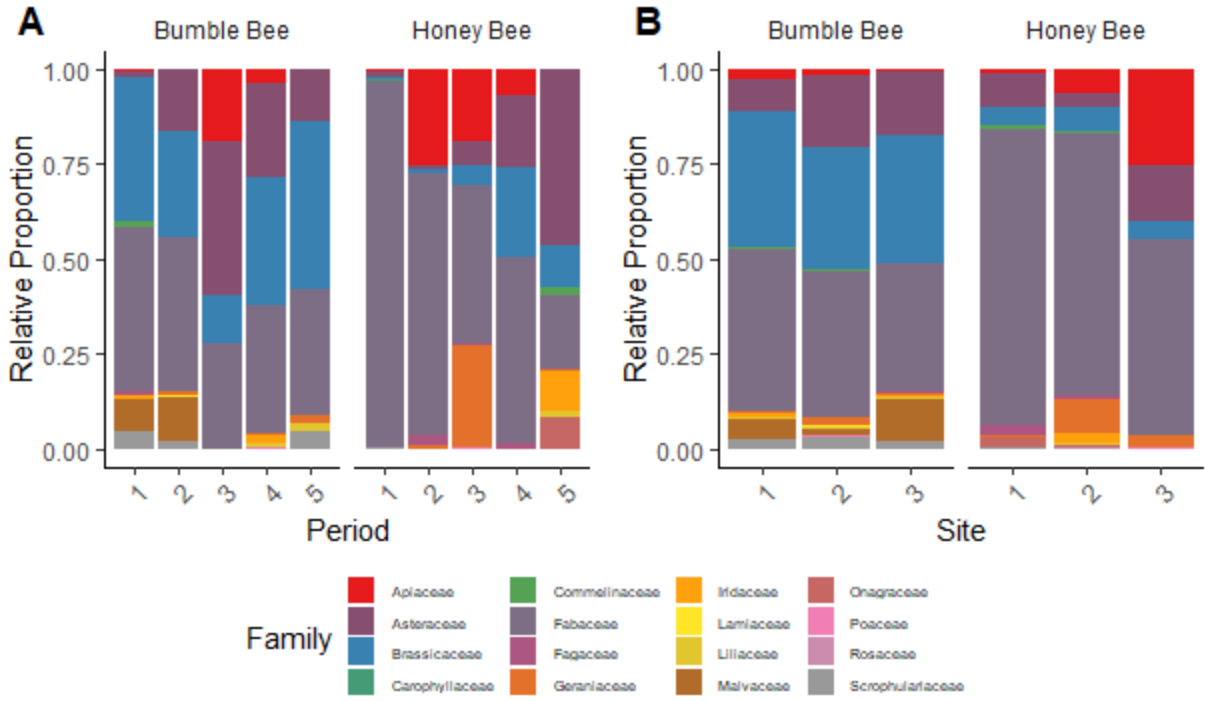
2
3
4
5
Family
Apiaceae
Asteraceae
Brassicaceae
Caryophyllaceae
Commelinaceae
Fabaceae
Fagaceae
Geraniaceae
Iridaceae
Lamiaceae
Liliaceae
Malvaceae
Onagraceae
Poaceae
Rosaceae
Scrophulariaceae

Figure 5:


A
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Honey Bee
B
Bumble Bee
Honey Bee
Relative Proportion
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0.75
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0.25
0.00
1
2
3
4
5
Period
Site
Family
Apiaceae
Asteraceae
Brassicaceae
Caryophyllaceae
Commelinaceae
Fabaceae
Fagaceae
Geraniaceae
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Malvaceae
Onagraceae
Poaceae
Rosaceae
Scrophulariaceae

Figure 6:

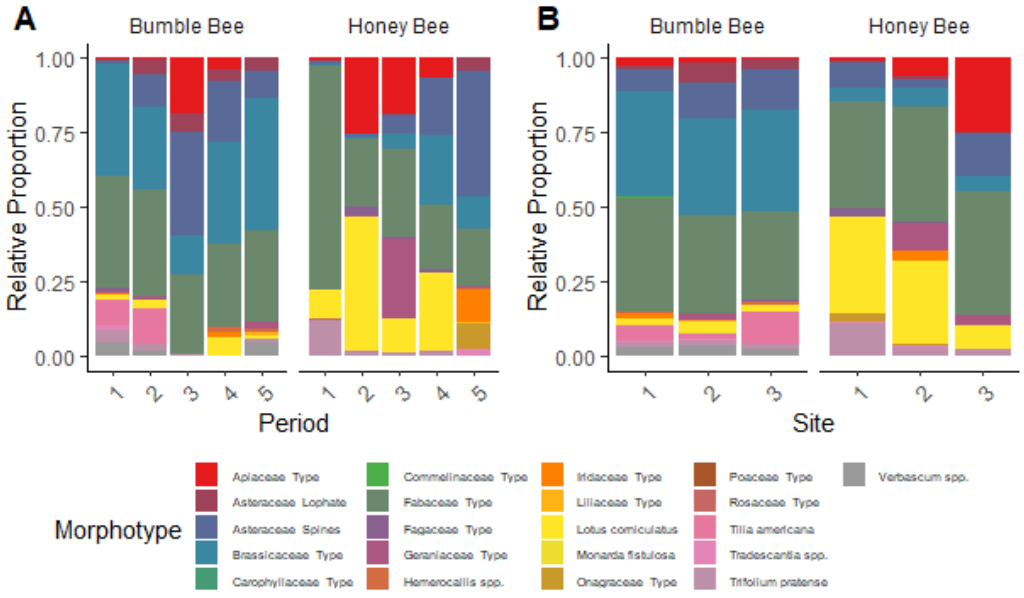
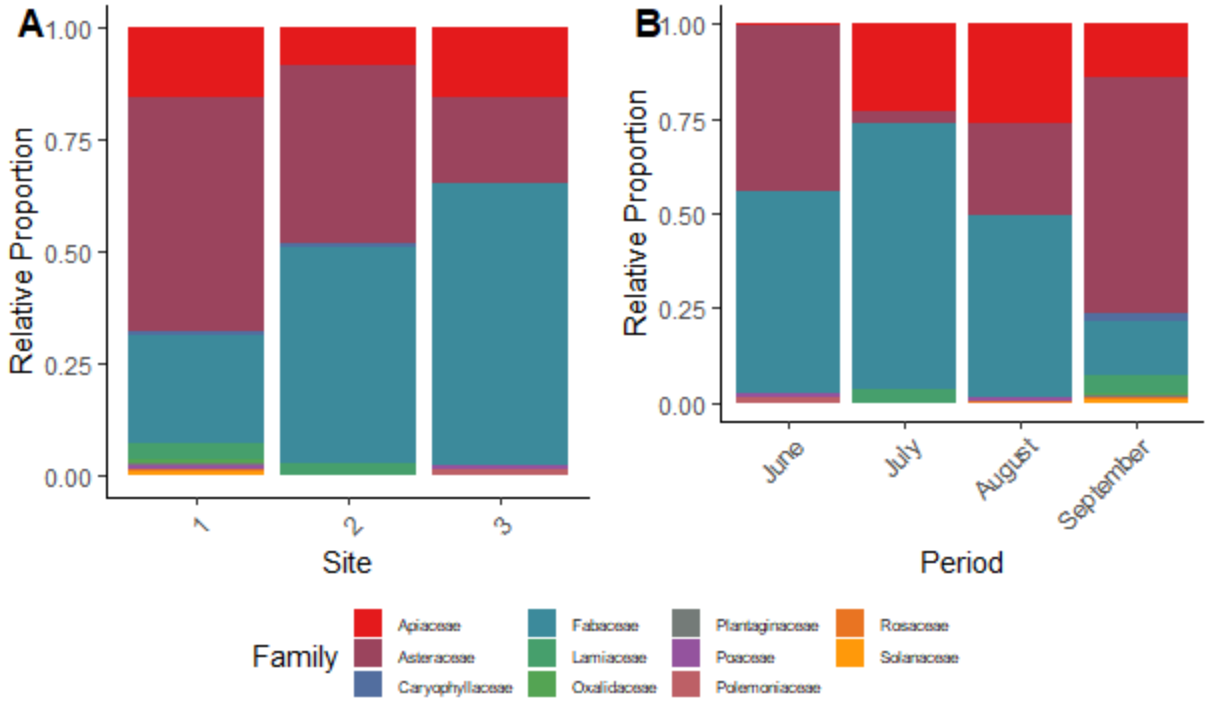

A
Bumble Bee
Honey Bee
B
Bumble Bee
Honey Bee
Relative Proportion
1.00
0.75
0.50
0.25
0.00
Period
Site
Morphotype
Apiaceae Type
Asteraceae Lophate
Asteraceae Spines
Brassicaceae Type
Caryophyllaceae Type
Commelinaceae Type
Fabaceae Type
Fagaceae Type
Geraniaceae Type
Hemerocallis spp.
Iridaceae Type
Liliaceae Type
Lotus corniculatus
Monarda fistulosa
Onagraceae Type
Poaceae Type
Rosaceae Type
Tilia americana
Tradescantia spp.
Trifolium pratense
Verbascum spp.

Figure 7: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1
2
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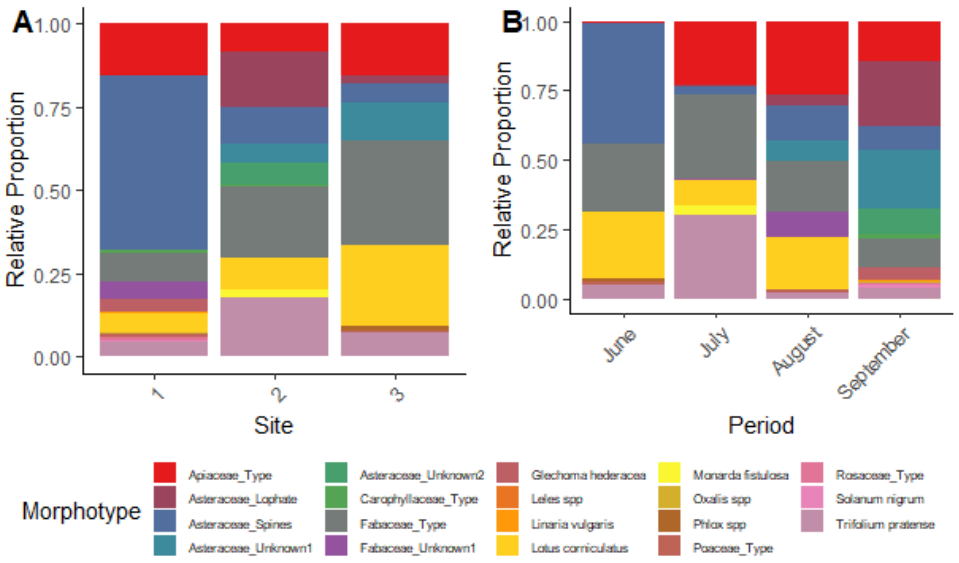
Site
June
July
August
September
Period
Family
Apiaceae
Asteraceae
Caryophyllaceae
Fabaceae
Lamiaceae
Oxalidaceae
Plantaginaceae
Poaceae
Polemoniaceae
Rosaceae
Solanaceae

Figure 8:


A
B
Relative Proportion
1.00
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0.50
0.25
0.00
1
2
3
Site
June
July
August
September
Period
Morphotype
Apiaceae_Type
Asteraceae_Lophate
Asteraceae_Spines
Asteraceae_Unknown1
Asteraceae_Unknown2
Caryophyllaceae_Type
Fabaceae_Type
Fabaceae_Unknown1
Glechoma hederacea
Leles spp
Linaria vulgaris
Lotus corniculatus
Monarda fistulosa
Oxalis spp
Phlox spp
Poaceae_Type
Rosaceae_Type
Solanum nigrum
Trifolium pratense

Tables

Table 1. Range of dates for collection of the pollen pellets for each of the five periods over the three sites.

Period	Pollen Collection Dates	Comparable Resource Period
1	June 14- 27	June
2	July 1- 13	July
3	July 23- August 2	July
4	August 8- 25	August
5	August 30- September 13	September

Table 2: The number of days where pollen was collected, and the total number of pollen pellets included in these analyses during each period and at each site for honey bees and bumble bees.

Period	Site	Bumble Bee		Honey Bee	
		Number of Days	Total Pollen Pellets	Number of Days	Total Pollen Pellets
1	1	2	36	2	76
	2	2	41	3	119
	3	3	34	3	118
2	1	3	58	3	119
	2	3	59	3	88
	3	3	55	3	115
3	1	1	9	0	0
	2	1	3	3	100
	3	1	4	2	76
4	1	3	27	2	18
	2	2	26	2	66
	3	2	37	1	38
5	1	2	36	2	47
	2	3	22	2	29
	3	3	39	2	39
Total:		**34**	**486**	**33**	**1048**

Table 3: Results of linear mixed models testing the effects of site, period, species, and their two-way interactions on the proportion of bees that are constant foragers indicated by pollen morphotypes or families. The percent illustrates the threshold used to accept a plant morphotype or family as part of a pollen pellet. Statistically significant factors are indicated in bold.

Morphotype	One Percent			Three Percent			Five percent		
Constancy	DF	F	P	DF	F	P	DF	F	P
Site	2,7	0.25	0.787	2,7	0.56	0.593	2,7	0.28	0.763
Period	**4,7**	**4.22**	**0.047**	**4,7**	**5.06**	**0.031**	**4,7**	**4.51**	**0.040**
Species	**1,7**	**11.56**	**0.011**	**1,7**	**20.41**	**0.002**	**1,7**	**19.05**	**0.003**
Period x Species	4,7	1.08	0.434	**4,7**	**5.37**	**0.026**	**4,7**	**4.63**	**0.038**
Site x Species	2,7	0.33	0.728	2,7	1.83	0.229	2,7	2.21	0.180
Site x Period	8,7	1.20	0.410	8,7	1.39	0.338	8,7	1.29	0.373
Family Constancy									
Site	2,7	0.17	0.844	2,7	0.29	0.756	2,7	0.05	0.953
Period	4,7	3.13	0.089	4,7	2.95	0.101	4,7	1.94	0.208
Species	**1,7**	**13.38**	**0.008**	**1,7**	**24.19**	**0.001**	**1,7**	**20.04**	**0.002**
Period x Species	4,7	1.04	0.449	**4,7**	**5.34**	**0.027**	4,7	3.97	0.054
Site x Species	2,7	0.30	0.752	2,7	1.85	0.227	2,7	1.64	0.261
Site x Period	8,7	1.15	0.432	8,7	1.15	0.433	8,7	0.85	0.590

Table 4: Results of linear mixed models testing the effects of site, period, species, and their two-way interactions on pollen richness, calculated based on morphotypes or families. Significant factors are indicated in bold.

Morphotype Richness	One Percent			Three Percent			Five percent		
	DF	F	P	DF	F	P	DF	F	P
Site	2,7	0.35	0.716	2,7	0.26	0.779	2,7	0.44	0.663
Period	4,7	1.16	0.404	4,7	0.81	0.555	4,7	0.91	0.508
Species	1,7	0.66	0.444	1,7	0.78	0.405	1,7	0.77	0.407
Period x Species	4,7	3.45	0.073	4,7	3.71	0.062	4,7	3.39	0.076
Site x Species	2,7	0.40	0.681	2,7	0.81	0.481	2,7	0.90	0.450
Site x Period	8,7	1.55	0.289	8,7	1.13	0.443	8,7	0.94	0.541
Family Richness									
Site	2,7	0.23	0.799	2,7	0.13	0.876	2,7	0.26	0.779
Period	4,7	0.65	0.646	4,7	0.44	0.777	4,7	0.66	0.640
Species	1,7	0.77	0.409	1,7	1.35	0.282	1,7	1.51	0.258
Period x Species	4,7	3.47	0.072	4,7	4.11	**0.050**	4,7	3.49	0.071
Site x Species	2,7	0.51	0.621	2,7	0.66	0.544	2,7	0.67	0.540
Site x Period	8,7	1.25	0.389	8,7	0.81	0.613	8,7	0.59	0.765

Table 5: Results of linear mixed models testing the effects of site, period, species, and their interactions on pollen diversity, calculated based on morphotypes and families. Significant factors are indicated in bold.

	One Percent			Three Percent			Five percent		
Morphotype Diversity	DF	F	P	DF	F	P	DF	F	P
Site	2,7	0.16	0.851	2,7	0.17	0.849	2,7	0.16	0.853
Period	4,7	2.63	0.124	4,7	2.60	0.127	4,7	2.58	0.129
Species	1,7	3.31	0.111	1,7	3.27	0.113	1,7	3.22	0.115
Period x Species	4,7	0.52	0.726	4,7	0.52	0.726	4,7	0.51	0.731
Site x Species	2,7	0.56	0.596	2,7	0.55	0.597	2,7	0.55	0.598
Site x Period	8,7	0.57	0.779	8,7	0.57	0.779	8,7	0.56	0.780
Family Diversity									
Site	2,7	0.18	0.836	2,7	0.19	0.830	2,7	0.19	0.830
Period	**4,7**	**4.72**	**0.036**	**4,7**	**4.70**	**0.036**	**4,7**	**4.66**	**0.037**
Species	**1,7**	**11.19**	**0.012**	**1,7**	**11.10**	**0.012**	**1,7**	**10.98**	**0.012**
Period x Species	4,7	2.00	0.199	4,7	2.00	0.199	4,7	1.99	0.200
Site x Species	2,7	0.13	0.877	2,7	0.13	0.880	2,7	0.12	0.884
Site x Period	8,7	0.69	0.693	8,7	0.69	0.694	8,7	0.68	0.701

Table 6: Results of linear mixed models testing the effects of site, and survey period on the richness and diversity of flowering plant resources at the species, family, and morphotype level. Significant effects are indicated in bold.

	Species Level			Family Level			Morphotype Level		
Richness	DF	F	P	DF	F	P	DF	F	P
Site	2,5	1.52	0.304	2,5	5.95	**0.047**	2,5	1.93	0.23
Survey Period	3,5	0.78	0.556	3,5	1.18	0.404	3,5	1.17	0.40
Diversity									
Site	2,5	0.46	0.6538	2,5	2.82	0.1511	2,5	0.05	0.9552
Survey Period	3,5	0.91	0.5005	3,5	3.28	0.1170	3,5	1.67	0.2881

Table 7: Bumble bee pollen preference. Results of chi-square statistics performed without the 'other' category which grouped the low frequency morphotypes. Positive Pearson residual values are in bold, indicating preference by bees for that morphotype.

Period	Morphotype	Observed	Expected	Pearson Residual
All	Fabaceae_Other	229	92.18	**14.25**
	Apiaceae_Type	14	57.14	-5.71
	Trifolium pratense	19	41.81	-3.53
	Lotus corniculatus	20	60.75	-5.23
	Asteraceae_Spines	105	89.92	**1.59**
	Asteraceae_Lophate	42	27.22	**2.83**
1	Fabaceae_Other	49	15.53	**8.5**
	Trifolium pratense	9	18.85	-2.27
	Asteraceae_Spines	5	27.23	-4.26
2	Fabaceae_Other	95	53.49	**5.68**
	Trifolium pratense	7	52.66	-6.29
	Lotus corniculatus	9	16.63	-1.87
	Asteraceae_Spines	43	5.267	**16.44**
	Asteraceae_Lophate	22	0.278	**41.26**
3	Fabaceae_Other	6	3.65	**1.23**
	Asteraceae_Spines	6	0.36	**9.41**
4	Apiaceae_Type	7	25.58	-3.67
	Fabaceae_Other	40	17.90	**5.22**
	Lotus corniculatus	8	18.30	-2.41
	Asteraceae_Spines	34	12.40	**6.14**
	Asteraceae_Lophate	8	3.74	**2.20**
5	Fabaceae_Other	39	6.69	**12.49**
	Asteraceae_Spines	17	5.82	**4.63**
	Asteraceae_Lophate	9	15.23	-1.59

Table 8: Honey bee pollen preferences. Results of chi-square statistics performed without the 'other' category which combined the low frequency morphotypes. Positive Pearson residual values are in bold, indicating preference by bees for that morphotype.

Period	Morphotype	Observed	Expected	Pearson Residual
All	Fabaceae_Other	447	214.86	**15.84**
	Apiaceae_Type	144	133.19	**0.94**
	Trifolium pratense	49	97.44	-4.91
	Lotus corniculatus	250	141.61	**9.11**
	Asteraceae_Spines	104	209.60	-7.29
	Asteraceae_Lophate	6	63.44	-7.21
1	Fabaceae_Other	238	77.63	**18.20**
	Lotus corniculatus	32	74.64	-4.94
	Trifolium pratense	39	94.25	-5.69
	Asteraceae_Spines	6	136.15	-11.15
2	Fabaceae_Other	93	106.07	-1.27
	Apiaceae_Type	89	81.34	**0.85**
	Trifolium pratense	6	7.08	-0.41
	Lotus corniculatus	155	32.98	**21.25**
	Asteraceae_Spines	6	10.44	-1.37
3	Fabaceae_Other	66	46.50	**2.86**
	Apiaceae_Type	43	35.66	**1.23**
	Lotus corniculatus	30	14.46	**4.09**
	Asteraceae_Spines	14	4.58	**4.40**
4	Apiaceae_Type	9	25.58	-3.28
	Fabaceae_Other	27	17.90	**2.15**
	Lotus corniculatus	33	18.30	**3.44**
	Asteraceae_Spines	28	12.40	**4.43**
5	Fabaceae_Other	23	8.03	**5.28**
	Asteraceae_Spines	50	6.99	**16.28**
	Asteraceae_Lophate	5	18.28	-3.11

Chapter 3

Percent protein and amino acid composition of pollen collected by honey bees and bumble bees in a shared landscape

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Abstract

Bees are important contributors to agricultural productivity and global biodiversity. However, many bee populations are in decline. There exist a variety of life history strategies employed by bees to collect nectar and pollen, the primary sources of energy and nutrition, respectively. Honey bees and bumble bees are eusocial central place foragers that must depart their nesting site, locate resources, and return to the nest with these resources. Furthermore, the identity of resources is highly variable in space and time, and so too is the nutritional component of these resources. Foragers must therefore possess strategies to ensure adequate nutritional intake by the hive. Specifically, honey bees use an elaborate dance communication to recruit cohorts of foragers to resource patches that have a high rate of reward, while individual bumble bees develop trapline foraging routes that more evenly distribute foragers among available resource patches. In the context of these foraging strategies we sought to determine if honey bees and bumble bees differ in the overall quality of pollen collected, and if these patterns change throughout a foraging season and among sites in a shared landscape. We consider the percent protein content, concentration of all pooled essential amino acids, and the concentration of all pooled non-essential amino acids as our quality metrics. Furthermore, we sought to test the

following predictions: i) bumble bees will collect pollen of higher quality relative to honey bees across each of our three quality metrics, and ii) because colony needs change through time, we expect both honey bees and bumble bees will show temporal variability in the quality of resources brought back to the hive. To test these predictions, we quantify the average nutritional value of plant families identified in bee collected pollen samples, and integrate these values with the relative proportion of each plant family represented in pollen samples collected by individual bee colonies each day. Overall, bumble bees collected pollen with higher percent protein content relative to honey bees. Furthermore, bumble bees showed no temporal variability in the pooled concentration of essential amino acids, or pooled non-essential amino acids. However, honey bees collected pollen with lower concentrations of both amino acid metrics in mid-July and early September. These results are discussed in the context of honey bee and bumble bee foraging strategies.

Keywords: pollen, protein, essential amino acid, non-essential amino acid, honey bee, bumble bee, foraging

Introduction

Pollinator populations are in decline around the globe, primarily resulting from exposure to pests and pathogens, agrochemicals, and land use intensification (Vanbergen et al. 2013). Furthermore, the negative effects from these stressors are exacerbated by poor nutrition resulting from decreased resource availability (Goulson et al. 2015). Honey bees are a managed pollinator used in mass-flowering crop systems, as their large numbers result in effective pollination (Rader et al. 2009). However wild pollinators may be more effective at increasing yields (Garibaldi et al. 2013). Bumble bees are a particularly prevalent wild pollinator in the northern temperate zones

and have been estimated to be the most abundant wild pollinator group by numbers (Goulson 2010).

Honey bees and bumble bees are eusocial generalist foragers that collect nectar, primarily for energy as it is a source of sugars, and pollen, which serves as the primary source of proteins and amino acids, together with other macronutrients such as starches, lipids, vitamins, and minerals (Roulston and Cane 2000). The amount and quality of pollen consumed may have important consequences for the health of individuals, and of the colony. For example, in the primitively eusocial generalist sweat bee, *Lassioglossum zephyrum* the amount of protein consumed in the larval stage, regardless of the pollen amount, shows a positive relationship with adult body size (Roulston and Cane 2002). And, body size has been correlated with foraging distance (Greenleaf et al. 2007), and efficiency of resource collection (Minahan and Brunet 2018; Spaethe and Weidenmüller 2002). Individual bumble bees within a colony demonstrate large variations in body size (Goulson et al. 2002), which could in part be driven by variability among developing larvae in the protein content of provisions. For example, individual *Bombus terrestris* larvae reared on pollen that contains a higher mean percent protein (15-24%) are larger than larvae fed a lower percent protein diet (< 15%) (Tasei & Aupinel, 2008). In contrast, when protein is limiting in honey bee hives, the colony may instead decrease the number of workers produced (Brodschneider and Crailsheim 2010), resulting in more homogenous body sizes among workers. These studies highlight the importance of pollen protein in the bee diet, and the potential consequences to bee foraging.

The nutritional needs of the hive are informed by intra-colonial feedback mechanisms, and therefore how the worker caste is structured may have consequences for bee foraging choices. Honey bees are well known for their workforce being structured by age polyethism, in which as workers age, they move from in hive tasks such as cleaning brood cells and nursing developing larvae, to out of hive tasks such as guarding the hive and ultimately foraging for resources (Winston 1987). The progression through these stages is accompanied by pronounced physiological changes that are associated with the consumption of nutrients, notably protein and amino acids. For example, newly emerged adult honey bees will consume pollen for the first couple weeks following eclosion, primarily to support development and maintenance of hypopharyngeal glands (HPGs) that are used to process incoming pollen and nectar into jelly to feed developing larvae (reviewed in Haydak 1970). Proper development of these glands likely requires higher quality pollen, as measured by protein content, to be consumed (Di Pasquale et al. 2013). As the need for new foragers increases, nurse bees will stop consuming pollen, which results in decreased body fat and diminished HPGs in their bodies, which is then followed by an increased intake of carbohydrates to ultimately provide the energetics for foraging (Crailsheim et al. 1992).

Bumble bees on the other hand demonstrate various degrees of alloethism, the division of labor by size (Goulson et al. 2002). For example, the body size of *Bombus terrestris* foragers, measured as thorax width, is larger, on average, than workers performing in hive tasks (Goulson et al. 2002). Likewise, *Bombus mori* foragers in Brazil had greater body size, as measured by wing length, than those performing in hive tasks (Garófalo 1978). Evidence for the stability of this division of labor among worker bumble bees includes one study that experimentally

removed the most active *Bombus huntii* workers involved with in-hive thermoregulation, resulting in the remaining thermoregulators increasing their rate of thermoregulation activity, and not by the recruitment of new workers to this task (Gardner et al. 2007). This alloethism may also have a direct effect on resource collection. For example, among small and large *Bombus terrestris* nectar foragers the frequency and duration of foraging bouts remains constant, however the rate of nectar collection (mg/hr) is greater for larger foragers than smaller foragers (Spaethe and Weidenmüller 2002).

Individual preferences may also dictate what bees collect. There is mixed evidence as to whether individual honey bees cue in on the nutrient quality of pollen, or whether other pollen or floral cues are the primary drivers of plant choices. Honey bees for example, don't increase the rate of dancing in response to artificially increased protein content using soy protein, but instead show greater rates of dancing when pollen, as opposed to soy protein is present in the hive (Beekman et al. 2015). Similarly, honey bees interact more frequently with artificial feeders containing smaller, relative to larger, pollen grains, and less frequently with feeders that require longer handling times, while there was no observed effect of protein content offered in the feeders (Pernal and Currie 2002). On the other hand, honey bees deprived of an essential amino acid in their diet, preferentially collect pollen from petri dishes that contained the missing amino acid (Hendriksma and Shafir 2016). Similarly, *Bombus impatiens* foragers preferentially visited plants in a semi-field array (experimental plantings in a field setting), and feeding dishes in a laboratory, that contained pollen with a 5:1 ratio of protein to lipids, suggesting the relative abundance of nutrients may be important, and is something that bees can cue in on (Vaudo et al. 2016). Finally, in choice assays honey bees preferred *Brassica napus* pollen that is higher in

valine, leucine, and isoleucine, relative to pollen from *Vicia faba* (Cook et al. 2003). However, in this latter study bees needed to be fed *B. napus* pollen first, before they demonstrated a preference, which together with the other findings suggests post-consumption mechanisms influencing preferences.

Overall, it seems that bumble bees primarily rely on their individual learning capabilities while selecting resources for collection. Bumble bees may have a more robust ability to discriminate among low- and high-quality resources while foraging, and therefore individuals can focus their efforts on the most rewarding resources. What is determined to be of low or high quality may depend on current colony needs, and this may therefore be dynamic. Furthermore, bumble bees use a trapline foraging strategy, which is the gradual development of routes that maximize resource intake while minimizing distances traveled (Lihoreau et al. 2012). Among dispersed smaller patches of resources this may be a valuable strategy, as individual bees would potentially be collecting from a variety of resources, thereby having the potential to complement nutritional deficits among resources. We would therefore expect bumble bee colonies to both diversify their diet and locate resources that maximize the intake of important nutritional components such as protein and amino acids, the specific complement of which will vary depending on colony needs.

Honey bees on the other hand, rely heavily on colony level communication among the various members of the hive. For example, a scout bee will locate a profitable resource, and return to the hive to communicate the distance and direction to this resource (Seeley et al. 1991). Ultimately, this process is thought to recruit forager cohorts to patches with the highest rate of reward production (Beekman and Ratnieks 2000; Visscher and Seeley 1982). From this foraging

strategy we would expect honey bees to collect resources of variable overall quality and show lower diversity in the resources collected relative to bumble bees (as observed in Chapter 2).

Both honey bees and bumble bees rely on the interplay between colony level feedback and individual foraging strategies to gather resources. However, in this study we are examining whether patterns of protein and amino acid acquisition by honey bees and bumble bees follow predictions based on what is known about their respective foraging strategies. We start by quantifying the percent protein content, and the concentrations of all pooled essential amino acids, and all pooled non-essential amino acids of plant families represented in pollen samples collected by honey bees and bumble bees. We then extrapolate the mean nutritional values for each metric to the observed pollen foraging behaviors of bees (proportion of each resource collected by bee hives each day), and test for differences between species, among sites, and among periods. Our specific metrics of pollen quality are: i) percent protein content, ii) overall amino acid profile, iii) concentration of all pooled essential amino acids, and iv) concentration of all pooled non-essential amino acids. Furthermore, we make the following predictions: i) because individual bumble bees must learn the location of profitable resources, and choose among these resources, individual bumble bees will collect pollen with higher nutrition values for each quality metric relative to honey bees, and ii) because colony needs vary through time we expect both honey bees and bumble bees will show temporal and spatial variability in the quality of pollen brought back to the hive. Our results are discussed in the context of individual and colony level foraging strategies, with suggestions for future research to elucidate the specific mechanisms connecting foraging strategies to the quality of resources collected.

Materials and Methods

Study system, bee species, and pollen collection

During the summer of 2016 we placed one honey bee and one bumble bee hive at each of three sites within a shared landscape at the West Madison Agricultural Research Station (WMARS) near Madison, WI, USA. Details on the sites and the hives are presented in the Chapter 2 section *Study Area and Bee Species.* Pollen was collected concurrently from honey bee and bumble bee hives at a site, rotating among sites each day. See Chapter 2 section *Pollen Collection from Individual Bees* for more details.

Selecting pollen pellets for protein and amino acid assays

To perform percent protein and amino acid assays, we selected pollen pellets that were complementary ('b' samples) to those identified as being 100% monofloral (500 of 500 pollen grains all one morphotype) in Chapter 2 ('a' samples). We selected a total of 46 pollen pellets for protein assays, and 27 pollen pellets for amino acid assays. Of these pollen pellets 12 were used in both protein and amino acid assays, while the remainder in each group were used only for the respective assay. For each assay, we used 1mg of dried pollen from each pollen pellet.

One assumption behind using this approach is that both pollen pellets from a single bee contain similar plant morphotype(s). We validated this assumption by selecting an independent set of 42 complementary pollen pellets ("b" samples) representing both bee species, and all time periods and sites. We visually identified the plant morphotypes in these complementary pollen pellets and compared the pollen composition to the one obtained for the other pollen pellet ("a" samples) collected from the same bee and previously identified in Chapter 2. Furthermore, we

selected five additional independent pollen pellets to examine variability in pollen composition among three replicate counts of 500 pollen grains from each pellet We made a separate slide for each of the three replicate counts from a pollen pellet. The methodology used to prepare pollen pellets and identify pollen morphotypes is described in Chapter 2 section *Plant identity in pollen pellets*.

Percent protein content

We determined the percent protein content of 46 monofloral pollen pellets. These included 3 samples each from the plant families Apiaceae, Commelinaceae, Geraniaceae, Iridaceae, Malvaceae, and Scrophulariaceae. We had 4 pollen pellets each from Asteraceae and Brassicaceae, 6 from Poaceae, and 1 each from Onagraceae and Liliaceae. In addition, we had 9 samples from Fabaceae, consisting of 3 each of Fabaceae_Other, *Lotus corniculatus,* and *Trifolium pratense*. Finally, the Poaceae pollen consisted of frozen *Zea mays* pollen used to feed bees in other experiments, as this pollen type was rarely collected by bees in our study (see Chapter 2). We did not have any monofloral pollen pellets for protein assays from Caryophyllaceae, Fagaceae, Lamiaceae, Liliaceae, or Rosaceae.

We determined total protein content using the Bradford Assay (Bradford, 1976) following the Bio-Rad Bradford Assay Microplate Standard Assay protocol (Bio-Rad Laboratories). The Bradford Assay uses Coomasie Brilliant Blue G-250 dye, which changes absorption maxima from red at 465nm to blue at 595nm following the binding of protein (Bradford 1976). The intensity of this absorption is what is measured. In general, the darker the blue color, the more protein is present, and in controls with no added protein the absorption should be minimal.

To determine the protein content of a sample, we first made a standard curve using 2mg/mL Bovine Serum Albumin (BSA) standard (Sigma Aldrich, St. Louis MO). We performed serial dilutions to make concentrations of 500, 250, 125, 62.5, 31.25, and 0 µg/mL of protein. We then placed 5µL of each concentration into individual wells and added 250µL Bradford Dye reagent (Sigma Aldrich, St. Louis MO). Each concentration was run in triplicate. This process was repeated three separate times, each one week apart, and all data were pooled to make our standard curve. A linear regression model was the best model to describe the standard curve using the nine data points obtained at each known concentration (Appendix: Fig. 2). The standard curve relating sample absorbance to protein content was used to estimate the unknown protein content of a sample, in this case pollen pellets, based on its absorbance.

To prepare pollen pellets for the protein assay, we dried individual pellets at 45^{o} C for 24 hours and followed standard protocols (Beekman et al. 2016; Vaudo et al. 2016). We weighed 1mg of each pollen sample and placed it into an individually labeled 1.5mL microcentrifuge tube. We added 2-3 drops of 0.1M NaOH to the sample and rigorously crushed the pollen with a pestle for 1 minute, and then added additional 0.1M NaOH until 1mL of solution was reached. Samples were then vortexed for 30 seconds and refrigerated for 48 hours. After 48 hours each sample was placed in a 100^{o}C water bath for 5 minutes, and then centrifuged at 10,000g for 5 minutes to precipitate pollen pellet debris. Individual samples were then run in triplicate by adding 5µL of each sample to each of three wells, followed by the addition of 250µL Bradford Dye reagent (Sigma Aldrich St. Louis MO) to each well. Samples were mixed with a pipette tip (one for each

sample) and allowed to sit for 5 minutes before being read on a spectrophotometer set to 595nm (BioRad iMark Microplate Absorbance Reader).

Absorbance values corresponding to the 0 µg/mL protein concentration in the standard curve were subtracted from all standard and unknown sample absorbance values to control for any absorbance effects in the reagents. Using this corrected absorbance, we obtained the following equation as the best model to explain the standard curve data:

$$y = 0.001x + 0.0191$$

We then solved for 'x', converted to milligrams, and multiplied by 100 to get the percent protein content of an individual pollen sample.

Amino Acid Composition

We used 27 independent pollen samples for the amino acid assays. Of these, we had 4 samples each from Apiaceae and Asteraceae. Of the Asteraceae, we included one Asteraceae_Lophate, and three Asteraceae_Spines. Furthermore, we included 3 each from Brassicaceae and Scrophulariaceae. Finally, we included 13 from Fabaceae, which included 4 samples each from Fabaceae_Other and *Trifolium pratense,* and 5 samples from *Lotus corniculatus*. We were not able to include samples from Caryophyllaceae, Commelinaceae, Fagaceae, Geraniaceae, Iridaceae, Lamiaceae, Liliaceae, Malvaceae, Onagraceae, Poaceae, or Rosaceae in our amino acid composition analyses.

We used gas chromatography-mass spectrometry (GC-MS) to determine the amino acid content of pollen samples. For sample preparation we dried and weighed 1mg of each pollen pellet

sample and added these to individually labeled 1.5mL microcentrifuge tubes. Following drying and weighing we briefly crushed the pollen samples with a pestle and added 100 µL of 6M HCl to each 1mg sample, then vortexed for 30 seconds to form a slurry (Stabler et al. 2018). We then transferred 50 µL of this slurry into a glass capillary tube and sealed the tube by melting the ends, ensuring that hydrolysis was conducted under hypoxic conditions. These capillary tubes were then placed in a 110^{o}C water bath for 24 hours (Fountoulakis and Lahm 1998). Following hydrolysis, the hydrolysate was collected from each capillary tube and placed into individually labeled 1.5 mL microcentrifuge tubes and then centrifuged at 10,000g for 30 minutes to precipitate pellet debris. The supernatant was then added to a new microcentrifuge tube and diluted with 1mL of ddH_2O to neutralize the sample. Samples were then flash frozen in liquid nitrogen and placed in a lyophilizer for 12 hours to remove all moisture prior to derivatization.

Amino acids are generally non-volatile, resulting in samples failing to vaporize and enter the GC column. To overcome this challenge, we silylated our amino acids prior to analysis. Silylation is a method of derivatization where trimethylsilyl ester bonds are formed at each active hydrogen on a compound, increasing volatility and thermal stability (Halket and Zaikin 2003). This approach to derivatization will not occur in the presence of water, as water molecules will react with polar groups on the amino acids and inhibit the attachment of trimethylsilyl groups during derivatization (Halket and Zaikin 2003). To derivatize our samples, following freeze drying, the residue was resuspended into 50 µL of pyridine containing 8.5 mM norvaline as a standard, and sonicated for 20 minutes. The samples were then centrifuged at 10,000g for 30 minutes to precipitate any remaining pellet debris. Fifty µL of supernatant was then transferred to a 100 µL glass insert placed into a 2mL glass vial, and 50 µL of MTBSTFA silylating reagent containing

1% t-BDMCS was added to each sample (Sigma Aldrich, St. Louis MO). The samples were then placed into an 80^{o}C water bath for 1 hour immediately before being run on the GC-MS (Shimadzu).

Each GC-MS run consisted of 1 µl of sample being injected into a 260^{o}C GC inlet in split mode (Split ratio = 10:1). Volatile compounds were vaporized and carried onto a 30m long (5% phenyl)-methylpolysiloxane column. The column was maintained at 70^{o}C for ten minutes before being gradually heated (5^{o}C /minute) to 300^{o}C, where it was held for 3 minutes. The quadrupole mass spectrometer began detection at 17.5 minutes and scanned between 40 and 600 m/z at a rate of 5 scans per second until the run was complete. Separation based on boiling point and polarity occurred.

Following GC-MS analysis, we used PARAFAC2 based Deconvolution and Identification System (PARADISe) software to determine the identity and concentration of amino acids in each pollen sample (Johnsen et al. 2017). This system requires users to upload raw GC-MS data, select intervals of interest, and then run these data through an automated PARAFAC2 (Parallel Factor Analysis) modelling process to resolve compounds present within each interval (Johnsen et al., 2017). Intervals of interest were defined based on the elution of silylated amino acid standards, and the identities were confirmed via comparison to the NIST ’05 mass spectral library (NIST 2005). We recorded the area of each amino acid peak.

Peak areas were scaled to the area of the internal standard. In our case, the area of each amino acid peak was scaled to the area of the norvaline standard peak in each sample. By scaling to the

internal standard, we can estimate the concentration of each amino acid in our pollen samples, before calculating the absolute abundance of each amino acid in nanograms (ng). After scaling our samples to the internal standard, data was converted to concentrations (ng/mg) of amino acid per pollen sample. We summed the concentration of each essential amino acid to obtain this metric, and likewise for non-essential amino acids.

Quality of pollen collected by bees

To quantify the quality of pollen collected by bee foragers, we linked the individual pollen quality metrics (percent protein, total concentration of essential amino acids, and total concentration of non-essential amino acids) for a plant morphotype or family to the relative proportions of that plant morphotype in the pollen brought back to a colony each day. We first calculated an average value of each quality metric for each plant family. We then multiplied the mean quality value for a plant family by its relative proportion in pollen samples collected by each bumble bee or honey bee hive in a day. We then summed all relative quality values for each plant family collected by a bee hive in a day to obtain an overall estimate of pollen quality. These calculations were completed separately for each quality metric. This resulted in a unique value for each quality metric each day for a bee species, period and site. Data were gathered over three days for each bee species, period and site. As some plant families collected by bees were not found in monofloral pollen pellets, and could therefore not be used in nutritional assays, not all families that bees collected each day were represented in these calculations. This implies that on some days our quality metrics are based on a proportion of the total pollen being returned to the hive. Specifically, we were able to include ten plant families to estimate our quality metric of percent protein, which encompassed 98 ± 1% of bumble bee collected pollen each day, and 93 ±

1% of honey bee collected pollen each day (Appendix: Fig. 1). For our amino acid estimates, we were able to include five plant families encompassing 93 ± 2% of all pollen collected for each day by bumble bees, and 90 ± 3% of all pollen collected by honey bees each day (Appendix: Fig. 1). The discrepancy between the number of plant families used for percent protein and amino acid analyses is due to limited sample numbers, and sample loss during acid hydrolysis.

Statistical Analyses

Variation among plant families and Fabaceae morphotypes

We ran analyses among plant families, and among the three Fabaceae morphotypes (*Trifolium pratense,* Fabaceae_Other, *Lotus corniculatus*) separately. To test for differences in percent protein content, the total concentration of essential amino acids, and the total concentration of non-essential amino acids among plant families, and among Fabaceae morphotypes, we performed one-way analysis of variance (ANOVA) using generalized linear models with the 'proc glm' function in SAS (SAS 9.4). To test for differences in the concentration of each individual amino acid we used Kruskal-Wallis rank sums tests in R (R Core Team 2013), as concentrations of individual amino acids came from non-normal distributions.

To visually examine any differences in the amino acid profiles (relative abundance and quantity of individual amino acids) among plant families, or Fabaceae morphotypes, we performed a Principal Component Analysis (PCA). This also allowed us to extract loading values associated with each principal component dimension and determine the strength of correlation of individual amino acids in their contribution to any observed differences among plant families or Fabaceae morphotypes. To statistically test for differences in amino acid composition among plant families

or Fabaceae morphotypes, we performed permutational multivariate analysis of variance (perMANOVA) using the function 'adonis' in the 'vegan' package in R (R Core Team 2013). We chose perMANOVA over traditional MANOVA, as each of our dependent variables (amino acids) came from a non-normal distribution, and the permutational approach is more robust to non-normality among the dependent variables (Anderson, 2017).

To examine the impact of bee species, period, site, and their interactions on the quality metrics of pollen collected by bee colonies each day, we used linear mixed models with the 'proc mixed' function in SAS (SAS 9.4). The quality metrics we used for the dependent variable in this analysis were i) percent protein, ii) total essential amino acid concentration, and iii) total non-essential amino acid concentration. We included the three-way interaction term species*period*site as a random effect, which served as the model error for which each fixed effect was tested against.

Results

Variation between complementary pollen pellets

From visual observation of 42 complementary pairs of pollen pellets we found that the most abundant pollen type never changed between complementary pollen pellets. In addition, for 36 of the 42 complementary pairs, the identity and relative proportions remained similar (Appendix: Table 1). However, six pellet pairs showed differences in the abundance of the secondary pollen type, and/or in the presence/absence of a tertiary pollen type (Table 1). Specifically, five pollen pellet pairs (pairs 1,2,3,5, and 6 in Table 1) showed the secondary pollen type to be present at contamination levels (< 3%) in one pellet, but then as collected (> 3%) in the other pellet from

the same bee (Table 1). In addition, in one pollen pellet pair (pair 4 in Table 1), the secondary pollen type was different between the two complementary pellets, and whether the third type was considered collected had changed (Table 1). In all pollen pellets that were identified as 100% monofloral (500 out of 500 pollen grains are of one morphotype), the complementary pellet never had a secondary type identified above contamination levels (6 out of 500 pollen grains), which supports our use of complementary pollen pellets for nutritional assays that were identified as being 100% monofloral in Chapter 2.

Variation among samples from the same pollen pellet

For each of the five pellets examined, replicate counts of pollen types from the same pollen pellet provided similar results (Table 2). However, using the 3% threshold level, pellet 4 showed variability among counts in that the secondary pollen type was not identified at the 3% level in sample C. In other words, two out of three counts from the same pollen pellet were classified as bifloral with very low quantity of the second pollen type while the remaining count classified the pellet as monofloral (Table 2). Similarly, for pellet 5, sample A was just below the threshold for inclusion of the secondary pollen type (Table 2). In the other three cases, all triplicates identified the same pollen types in similar proportions (Table 2). If anything, these data suggest that using a 5% rule for inclusion as collected pollen would eliminate variation among subsamples for a single pollen pellet.

Variation among plant families and Fabaceae morphotypes

<u>Percent protein</u>

Plant families differed in percent protein content of pollen ($F_{9,40} = 7.46$; $P < 0.0001$) (Fig. 1). Plants in the Brassicaceae had the greatest percent protein, which was statistically different from all families except Scrophulariaceae (df = 1; t = 1.98; P = 0.616) and Commelinaceae (df = 1; t = 2.26; P = 0.439) (Fig 1). Furthermore, the percent protein in Malvaceae was less than in Scrophulariaceae (df = 1; t = 3.83; P = 0.018) and Commelinaceae (df = 1; t = 3.57; P = 0.034), while Iridaceae pollen had a lower percent protein concentration than Scrophulariaceae (df = 1; t = 3.48; P = 0.042) (Fig. 1). Finally, there were no differences among Fabaceae morphotypes in the percent protein content of pollen ($F_{2,8} = 3.58$; $P = 0.095$).

Amino acids

We identified a total of 15 amino acids in our pollen samples. These included eight amino acids essential to honey bees (methionine, phenylalanine, isoleucine, threonine, leucine, valine, arginine, and lysine), and seven non-essential amino acids (alanine, glycine, proline, serine, aspartic acid, glutamic acid, and tyrosine) (DeGroot 1952). Looking at the profile of all amino acids, the PCA indicates minimal separation among plant families (Fig. 2). The loading values of the different amino acids were similar in their contribution to PC 1, which explained 75% of the variance (Fig. 2; Table 3). Of the separation that we did see, which was mainly in PC 2, lysine had the greatest contribution (Table 3), although PC 2 explained only 8.9% of the variance. Furthermore, we found no statistically significant differences in the overall amino acid profile among the different plant families (perMANOVA: $F_{4,26} = 0.128$; $P = 0.645$). Moreover, none of the individual amino acids differed significantly among plant families (Kruskal-Wallis: df = 4; $P > 0.1$ for all comparisons). Finally, after calculating the total concentration of all pooled essential amino acids, there were no differences among plant families (ANOVA: $F_{4,26} = 0.50$; $P = 0.739$),

nor were there any differences among plant families after calculating the total concentration of all pooled non-essential amino acids (ANOVA: $F_{4,26} = 0.29$; $P = 0.880$).

When we ran PCA on the three Fabaceae morphotypes we saw high variability and detected minimal separation (Fig. 3). In addition, we found no statistically significant differences in the amino acid profile among Fabaceae morphotypes (perMANOVA: $F_{2,12} = 0.62$; $P = 0.633$), or in the concentration of individual amino acids (Kruskal-Wallis: df = 2; $P > 0.2$ for all comparisons). Finally, there were no differences among Fabaceae morphotypes in the total essential amino acid (ANOVA: $F_{2,12} = 0.96$; $P = 0.414$), or total nonessential amino acid concentrations (ANOVA: $F_{2,12} = 01.03$; $P = 0.393$).

Protein and amino acid content of pollen collected by bees

Percent Protein

The two bee species differed in the percent protein they brought back to the hive (Table 4) with bumble bees bringing back pollen with a higher percent protein content, on average, relative to honey bees (Fig. 4). There were no effects of site, period, or any of the two-way interactions on the percent protein content of pollen brought back to the hive. (Table 4).

Amino Acids

There was a species effect, and a period x species interaction effect on the concentration of all pooled essential amino acids (Table 4; Fig. 5a). Specifically, honey bees collected pollen with a similar concentration of essential amino acids relative to bumble bees in all periods, except period five (df = 1,7; t = 5.0; $P = 0.002$) (Fig. 5a). Interestingly, the concentration of pooled

essential amino acids in pollen collected by bumble bees did not change through time (Fig. 5a). However, honey bees collected pollen with a lower essential amino acid concentration during period three than period one (df = 7; t = 3.07; P = 0.018), two (df = 7; t = 2.56; P = 0.034), or four (df = 7; t = 2.74; P = 0.029) (Fig. 5a). Likewise, essential amino acid concentration was lower in period five than periods one (df = 7; t = 4.47; P = 0.003), two (df = 7; t = 3.88; P = 0.006), or four (df = 7; t = 3.86; P = 0.006) (Fig. 5a). There were no effects of site, the associated two-way interactions, or period, on the concentration of essential amino acids in pollen collected by honey bees or bumble bees each day (Table 4).

As for non-essential amino acids, we found a statistically significant period x species interaction (Table 4; Fig. 5b). Specifically, honey bees collected pollen with a higher concentration of non-essential amino acids than bumble bees in period one (df = 7; t = 2.64; P = 0.033) and two (df = 7; t = 3.56; P = 0.009) (Fig. 5b), while bumble bees collected pollen with higher non-essential amino acid concentration than honey bees in period five (df = 7; t = 2.41; P = 0.047) (Fig. 5b). There were no differences in the concentration of pooled non-essential amino acids between bee species in periods three and four (Fig. 5b), nor were there differences among periods for bumble bees. However, honey bees collected pollen with a lower concentration of non-essential amino acids during period three relative to periods one (df = 7; t = 2.40; P = 0.047) and two (df = 7; t = 2.47; P = 0.043) (Fig. 5b). Similarly, the concentration of pooled non-essential amino acids in honey bee collected pollen was lower in period five than periods one (df = 7; t = 4.56; P = 0.003), two (df = 7; t = 4.76; P = 0.002), or four (df = 7; t = 3.37; P = 0.012) (Fig. 5b).

Discussion

The quality of pollen collected by bumble bee colonies was not variable through time (periods) or among sites (hives) and this was true for all metrics of pollen quality; percent protein content, overall amino acid profile, and concentration of pooled essential and pooled non-essential amino acids. Honey bees however, while consistent among periods for percent protein content, demonstrated lower pooled essential and pooled non-essential amino acid concentrations in periods three and five compared to periods one, two, and four. Among bee species, bumble bees collected pollen with a higher percent protein content than honey bees. Furthermore, the pooled concentration of essential amino acids was similar between honey bees and bumble bees in each period, except in periods three and five, when the concentrations of both pooled essential and pooled non-essential amino acids declined in honey bee collected pollen samples. Interestingly, the only metric where honey bees had higher quality pollen than bumble bees was for pooled non-essential amino acids in periods one and two. Previous results comparing bumble bees and honey bees observed both *Bombus pascuorum* and *Bombus terrestris* collecting pollen with a higher average concentration of total amino acids and essential amino acids than honey bees (Leonhardt and Blüthgen 2012).

Among plant families, we observed variability in percent protein content. The literature has conflicting results on protein and amino acid differences among plant taxa, which is affected by which plant groups are compared, but also may be partly driven by whether bee collected pollen pellets or hand collected fresh pollen was used to make these inferences. For example, using bumble bee collected pollen samples, Hanley et al. (2008) found notable within plant family variability in percent protein content and essential amino acid concentration, which was

particularly pronounced in the Fabaceae, Asteraceae, and Lamiales. In contrast, Somme et al. (2015) found no differences in the total concentration of amino acids within plant families in bumble bee collected pollen pellets. Furthermore, using hand collected pollen samples from plants identified as being visited by solitary bees, Weiner et al. (2010) found significant variability among plant families in amino acid profiles.

We identified the greatest percent protein content being associated with Brassicaceae (17%), Scrophulariaceae (13%), and Commelinaceae (12%). The pollen with the lowest percent protein was Malvaceae (*Tilia americana*) at only 5.3%. However, it is important to note that sugar additions during foraging can contribute to the dry mass of a pollen pellet (Human & Nicolson 2006), and as a result of this bias, we assume our percent protein values are underestimates of what the true percent protein is for each plant family (Roulston et al. 2000). Furthermore, while we observed differences among plant families in percent protein estimates, the amino acid profile, and concentration of pooled essential and pooled non-essential amino acids did not differ among plant families. Similar results have been observed from honey bee pollen loads, whereby *Brassicaceae* pollen had the largest percent protein (24.08%), but there were no differences in amino acid profiles among families (Szczêsna 2006). We recommend future research perform additional large scale surveys of plant nutritional quality metrics, similar to that done for percent protein by Roulston et al. (2000), and to do this using hand and bee collected pollen samples. This would result in better characterization of how these factors affect quality estimates and clarify and larger scale trends concerning within and between taxon variability across multiple levels of taxonomic ranking.

The nutritional profile of food resources that are ultimately fed to developing bees and in-hive workers is different from what is collected in the environment. For example, the protein content of honey bee collected pollen is generally lower, and the carbohydrate content higher in bee bread compared to pollen collected directly from plants or in bee collected pollen pellets (Wright et al. 2018). There are a variety of factors that can contribute to changes in nutrition between fresh pollen and bee collected pollen, much of which probably arises from the addition of nectar or bee saliva that contains microorganisms such as yeast and bacteria. For example, Standifer et al. (1980) documented decreases in the amounts of individual amino acids of *Prunus dulcis* pollen as it moves from flower, to corbicular pollen load, and into bee bread in the honey bee hive. However, there do not seem to be concomitant changes in percent protein (Herbert and Shimanuki 1978; Standifer et al. 1980). Whether social bees are collecting pollen after perceiving components of its nutritional profile prior to collection, or instead based on correlations with the nutritional profile of food that is consumed inside the hives is unclear, but there is likely an association between the two modes of association and learning.

While the evidence is inconclusive on whether bees can detect pollen quality when foraging, they do detect whether pollen is present in a flower and can detect pollen amounts (Brunet et al. 2015). However, colony feedback mechanisms likely play a critical role in determining what resources foragers should collect. For example, *Bombus impatiens* colonies increase foraging activity when pollen with a high protein content is added to nectar pots in the hive (Kitaoka and Nieh 2009). Furthermore, honey bees increase the allocation of current foragers to the task of pollen foraging in response to low total pollen amount or protein concentration inside the hive

(Pernal and Currie 2001). These studies highlight the importance of communication among nest mates in meeting the nutritional demands of the colony.

To gather resources, bumble bee foragers monitor the resource environment while foraging by learning and following trapline routes, whereby they 'major' on a particular set of resources, while occasionally going off route to sample other areas (Heinrich 1979). In this study, bumble bees collected resources with a higher percent protein content than honey bees. If bumble bees are actively pursuing pollen with a greater protein concentration, then we may expect individuals to more uniformly distribute themselves among the highest quality resource patches available. On the other hand, honey bee distributions may be clustered on resource patches with the highest rate of pollen production, but not necessarily containing the highest percent protein. This hypothesis warrants further empirical testing of the relationship between dynamic colony needs through time, floral resource availability across multiple scales, and evidence that bees visit specific patches.

Our results contribute to the broader understanding of how bee foraging strategies translate into patterns of pollen collection and nutrient acquisition by honey bees and bumble bees. Specifically, we observed that bumble bees tend to collect pollen with a higher percent protein content than honey bees, and show a more stable concentration of essential amino acids through time. Furthermore, this temporally stable concentration of polled essential amino acids in bumble bee collected pollen is in the higher range of estimated values observed in this study. This outcome could result from the systematic search strategy of trapline foraging by bumble bees if all individuals are foraging on the most protein rich pollen available. In a previous study from

these same hives (Chapter 1), we observed that relative to honey bees, bumble bees make more foraging trips and spend a greater total time foraging each day, while also collecting heavier pollen loads (Minahan and Brunet 2018). Taken together, these results suggest an overall greater foraging effort and increased efficiency in gathering higher quality resources by bumble bees compared to honey bees. However, from our experiments we did not quantify the specific requirements of these hives, and how they varied through time. This was particularly true for honey bees, as they were housed in two-frame observation hives, which provides less room to grow than would be found in a full-size hive with at least 9 or 10 frames, or more. Bumble bees on the other hand qualitatively followed a natural progression, whereby the hive began with about 75 workers, increased in size, and produced queens and males towards the end of August. In future studies, relating the amount and identity (male, female, queen) of brood to the quantity, identity, and nutritional quality of resources within bee hives will shed light on what the optimal nutrition intake should be, and how well the bee colonies are meeting this need. Overall, our results provide evidence that the trapline foraging strategy of bumble bees may better facilitate the acquisition of high nutritional quality resources in a patchy environment, compared with the waggle dance of honey bees.

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Figure Captions

Figure 1: Estimated percent protein among pollen families represented in bee collected pollen pellets.

Figure 2: Principal components analysis on the amino acid profile among plant families.

Figure 3: Principal component analysis of the amino acid profile among the three Fabaceae morphotypes.

Figure 4: Percent protein content of pollen brought back to the hive. The center line represents the median of the data points, while the hinges of the boxes represent the first and third quartiles. The lines extend out to 1.5 x IQR (Interquartile Range). Each of the dots represents an outlier.

Figure 5: The interaction between bee species and period on the concentration of essential (A) and non-essential (B) amino acids (mean ± SE) in pollen collected by individual honey bee and bumble bee colonies each day.

Figures

Figure 1

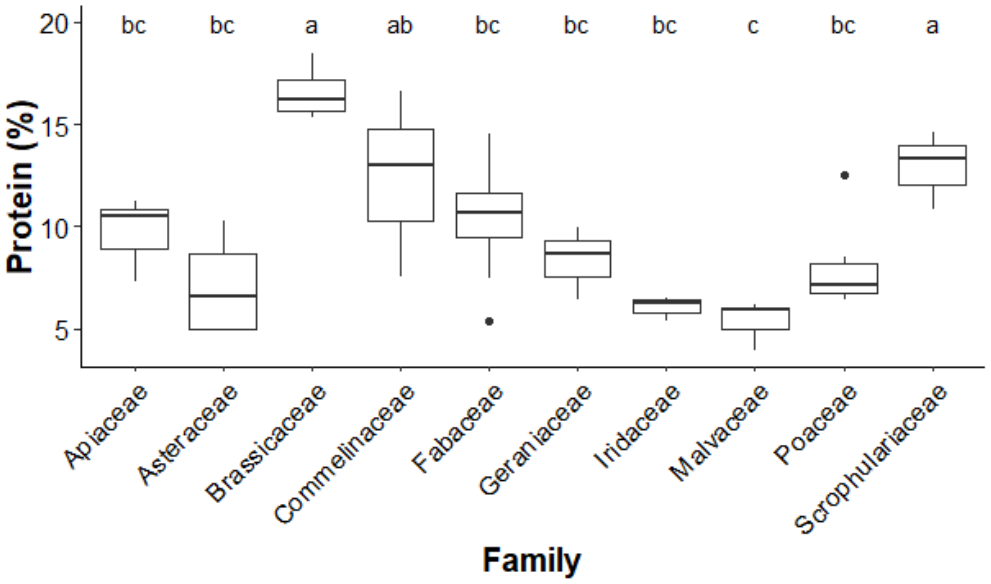


Figure 2

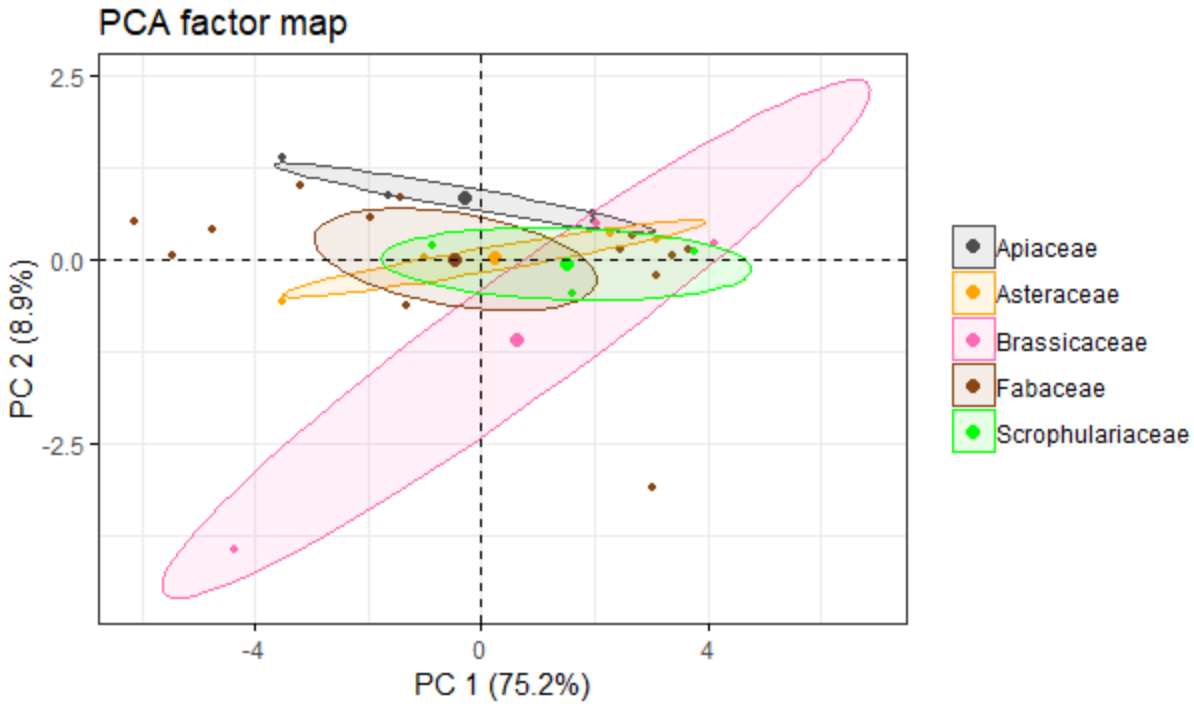

PCA factor map
2.5
0.0
-2.5
PC 2 (8.9%)
-4
0
4
PC 1 (75.2%)
Apiaceae
Asteraceae
Brassicaceae
Fabaceae
Scrophulariaceae

Figure 3:

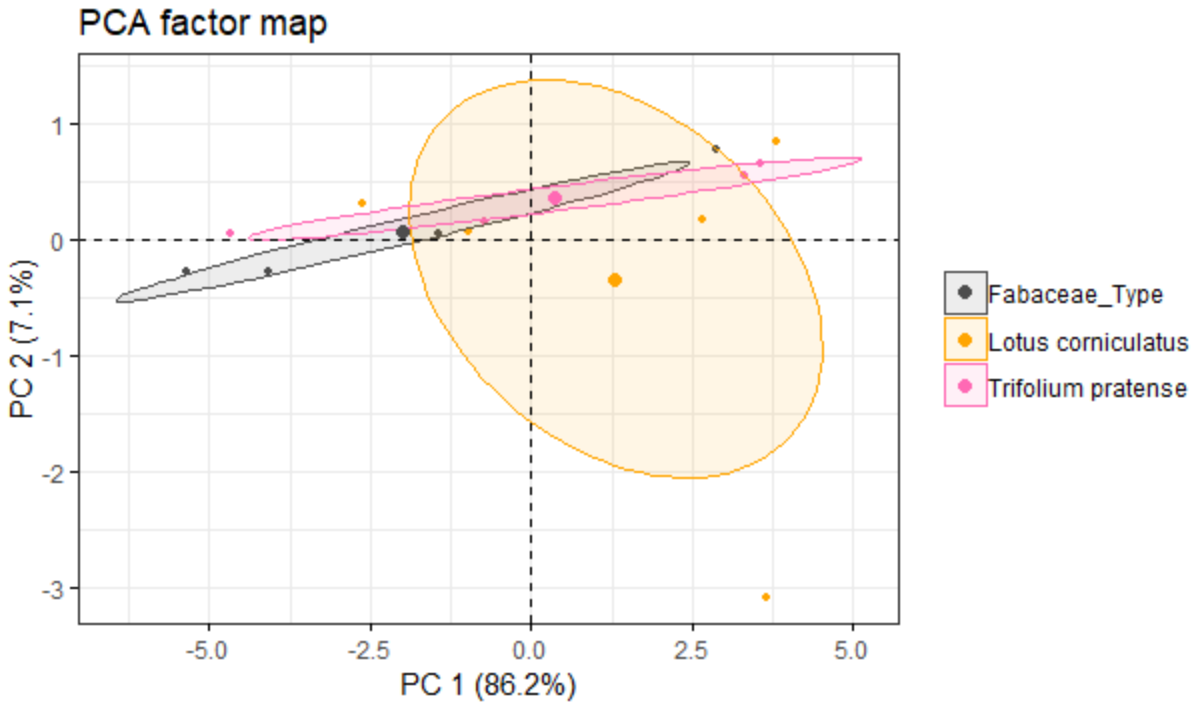

PCA factor map
PC 2 (7.1%)
PC 1 (86.2%)
1
0
-1
-2
-3
-5.0
-2.5
0.0
2.5
5.0
Fabaceae_Type
Lotus corniculatus
Trifolium pratense

Figure 4:

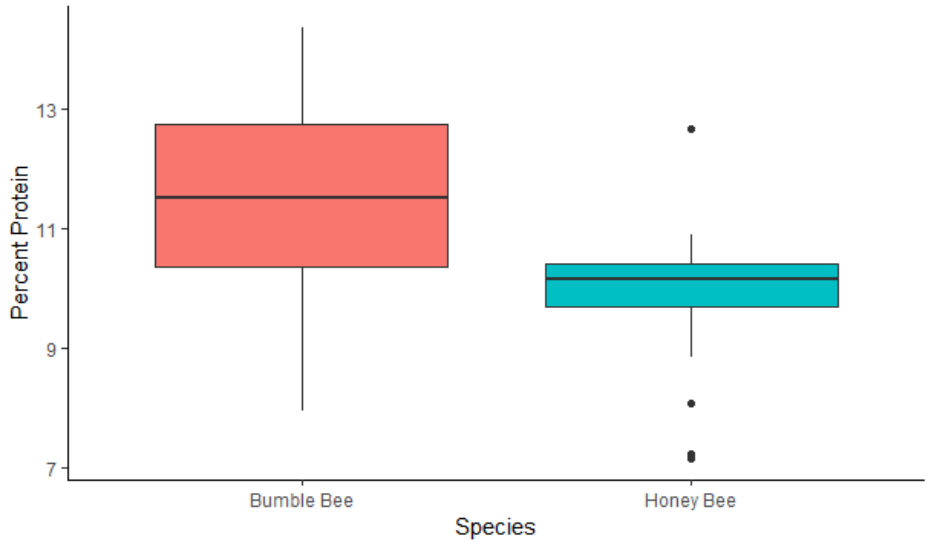
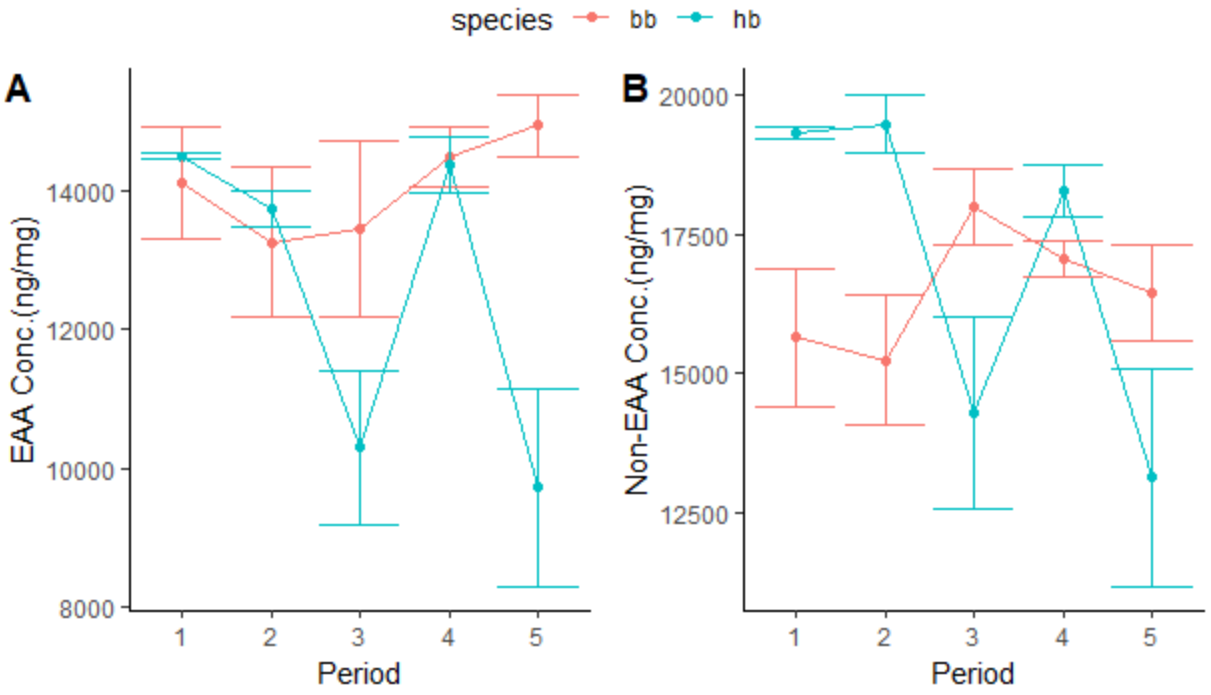


Figure 5:


species
bb
hb
A
B
EAA Conc.(ng/mg)
Non-EAA Conc.(ng/mg)
Period
Period
8000
10000
12000
14000
12500
15000
17500
20000
1
2
3
4
5

Tables:

Table 1: Eight of the 42 pollen pellets examined to compare the identity and relative proportion of pollen types found in complimentary pollen pellets from individual bees. Samples (A) and (B) represent complementary pollen pellets from the same bee.

Pellet	Sample	Type One	Proportion	Type Two	Proportion	Type Three	Proportion
1	A	Asteraceae_Spines	0.990	Fabaceae_Other	0.010	-	-
	B	Asteraceae_Spines	0.926	Fabaceae_Other	0.074	-	-
2	A	Asteraceae_Lophate	0.962	Fabaceae_Other	0.038	-	-
	B	Asteraceae_Lophate	0.988	Fabaceae_Other	0.012	-	-
3	A	Lotus corniculatus	0.974	Asteraceae_Spines	0.024	Unknown	0.002
	B	Lotus corniculatus	0.844	Asteraceae_Spines	0.156	-	-
4	A	Fabaceae_Other	0.770	Trifolium pratense	0.220	Asteraceae_Lophate	0.010
	B	Fabaceae_Other	0.900	Brassicaceae_Type	0.070	Asteraceae_Lophate	0.030
5	A	Fabaceae_Other	0.988	Asteraceae_Spines	0.012	-	-
	B	Fabaceae_Other	0.942	Asteraceae_Spines	0.058	-	-
6	A	Geraniaceae spp.	0.974	Lotus corniculatus	0.026	-	-
	B	Geraniaceae spp.	0.776	Lotus corniculatus	0.222	Asteraceae_Spines	0.002

Table 2: Variation between multiple independent counts of pollen morphotypes from individual pellets. The letters (A), (B), and (C) indicate independent samples taken from the same pollen pellet. 500 pollen grains were counted in each pellet.

Pellet	Sample	Type One	Proportion	Type Two	Proportion	Type Three	Proportion
				Variability Within Pollen Pellets			
1	A	Brassicaceae_Type	0.980	Fabaceae_Other	0.020	-	-
	B	Brassicaceae_Type	0.974	Fabaceae_Other	0.026	-	-
	C	Brassicaceae_Type	0.992	Fabaceae_Other	0.008	-	-
2	A	Tilia americana	1.000	-	-	-	-
	B	Tilia americana	0.984	Brassicaceae_Type	0.012	Asteraceae	0.004
	C	Tilia americana	0.994	Asteraceae_Spines	0.006	-	-
3	A	Lotus corniculatus	0.994	Asteraceae_Spines	0.006	-	-
	B	Lotus corniculatus	0.994	Geraniaceae spp.	0.004	Fabaceae_Other	0.002
	C	Lotus corniculatus	1.000	-	-	-	-
4	A	Lotus corniculatus	0.956	Fabaceae_Other	0.044	-	-
	B	Lotus corniculatus	0.970	Fabaceae_Other	0.030	-	-
	C	Lotus corniculatus	0.990	Fabaceae_Other	0.010	-	-
5	A	Geraniaceae spp.	0.974	Lotus corniculatus	0.026	-	-
	B	Geraniaceae spp.	0.950	Lotus corniculatus	0.050	-	-
	C	Geraniaceae spp.	0.962	Lotus corniculatus	0.038	-	-

Table 3: The amino acid loading values for PC1 and PC2 associated with the plant family Principal component analysis (PCA). Lysine contributed the most to PC2.

	PC1	PC2
Amino Acids	All Families	
alanine	-0.303	0.079
glycine	-0.284	-0.250
valine	-0.303	0.102
leucine	-0.106	0.148
isoleucine	-0.297	0.147
proline	-0.214	0.349
methionine	-0.294	0.053
serine	-0.302	-0.037
threonine	-0.271	-0.233
phenylalanine	-0.293	0.134
asparticacid	-0.263	-0.268
glutamicacid	-0.303	-0.009
lysine	-0.061	**-0.776**
tyrosine	-0.298	0.074

Table 4: The quality of pollen collected by bee hives each day as inferred from average percent protein content, essential amino acid content and non-essential amino acid content

Factor	Percent Protein			Essential Amino Acids			Non-essential amino acids		
	DF	F	P	DF	F	P	DF	F	P
Site	2,7	0.15	0.8608	2,7	0.53	0.6114	2,7	0.62	0.5634
Period	4,7	1.75	0.2436	4,7	3.45	0.0735	4,7	3.13	0.0896
Species	1,7	21.14	**0.0025**	1,7	8.87	**0.0206**	1,7	0.64	0.4516
Period x Species	4,7	3.60	0.0673	4,7	5.70	**0.0232**	4,7	6.00	**0.0203**
Site x Species	2,7	0.93	0.4386	2,7	0.75	0.5069	2,7	3.78	0.0772
Site x Period	8,7	1.20	0.4117	8,7	1.58	0.2801	8,7	1.27	0.3825

Appendix

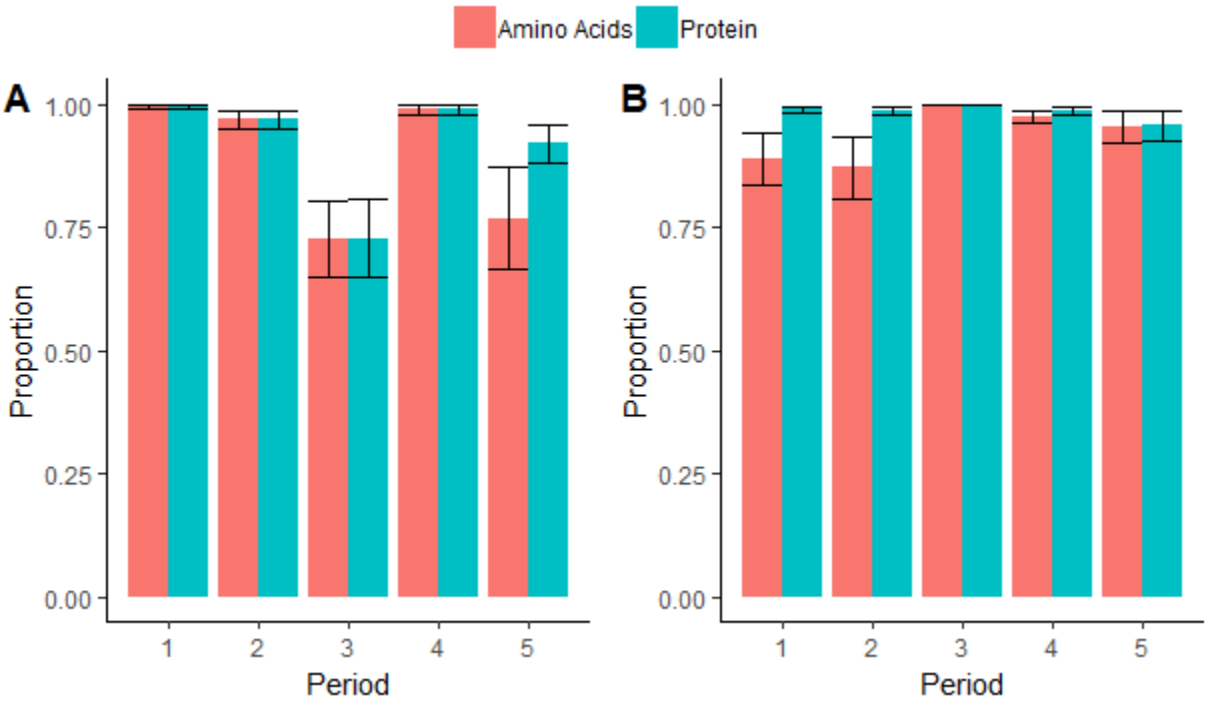


Figure 1: The proportion of all pollen collected by honey bees (A) and bumble bees (B) in each period (mean ± SE) that were used to calculate the quality of resources. Ten plant families were included in percent protein estimates (blue), while five plant families were included in essential/non-essential amino acid concentration estimates (red).

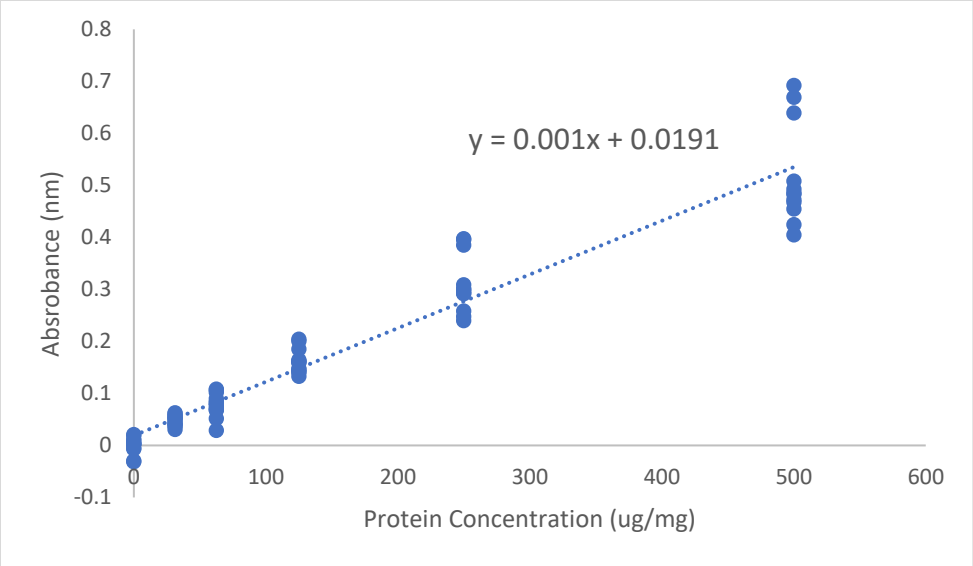


Figure 2: Linear standard curve to estimate the concentration of protein found in bee collected pollen pellets. Concentration values were converted to percent protein prior to analysis.

Table 1: Identity and relative proportion of pollen morphotypes among complementary pollen pellets from the same bee.

Pellet	Sample	Type One	Proportion	Type 2	Proportion	Type Three	Proportion
1	A	Asteraceae_Spines	0.990	Fabaceae_Other	0.010	-	-
	B	Asteraceae_Spines	0.926	Fabaceae_Other	0.074	-	-
2	A	Asteraceae_Lophate	0.962	Fabaceae_Other	0.038	-	-
	B	Asteraceae_Lophate	0.988	Fabaceae_Other	0.012	-	-
3	A	Lotus corniculatus	0.974	Asteraceae_Spines	0.024	Unknown	0.002
	B	Lotus corniculatus	0.844	Asteraceae_Spines	0.156	-	-
4	A	Fabaceae_Other	0.770	Trifolium pratense	0.220	Asteraceae_Lophate	0.01
	B	Fabaceae_Other	0.900	Brassicaceae_Type	0.070	Asteraceae_Lophate	0.03
5	A	Fabaceae_Other	0.988	Asteraceae_Spines	0.012	-	-
	B	Fabaceae_Other	0.942	Asteraceae_Spines	0.058	-	-
6	A	Geraniaceae spp.	0.974	Lotus corniculatus	0.026	-	-
	B	Geraniaceae spp.	0.776	Lotus corniculatus	0.222	Asteraceae_Spines	0.002
7	A	Brassicaceae_Type	0.856	Fabaceae_Other	0.144	-	-
	B	Brassicaceae_Type	0.760	Fabaceae_Other	0.240	-	-
8	A	Lotus corniculatus	0.956	Fabaceae_Other	0.044	-	-
	B	Lotus corniculatus	0.893360161	Fabaceae_Other	0.094567404	-	-
9	A	Fabaceae_Other	0.862	Brassicaceae_Type	0.138	-	-
	B	Fabaceae_Other	0.836	Brassicaceae_Type	0.164	-	-
10	A	Brassicaceae_Type	0.980	Fabaceae_Other	0.020	-	-
	B	Brassicaceae_Type	1	-	-	-	-
11	A	Brassicaceae_Type	1	-	-	-	-
	B	Brassicaceae_Type	1	-	-	-	-
12	A	Tilia americana	1	-	-	-	-
	B	Tilia americana	1	-	-	-	-
13	A	Tilia americana	1	-	-	-	-
	B	Tilia americana	1	-	-	-	-
14	A	Verbascum spp.	1	-	-	-	-
	B	Verbascum spp.	1	-	-	-	-

Table 1: Identity and relative proportion of pollen morphotypes among complementary pollen pellets from the same bee.

Pellet	Sample	Type One	Proportion	Type 2	Proportion	Type Three	Proportion
15	A	Apiaceae_Type	0.982	Brassicaceae_Type	0.014	Asteraceae_Spines	0.004
	B	Apiaceae_Type	1	-	-	-	-
16	A	Lotus corniculatus	0.994	Asteraceae_Spines	0.006	-	-
	B	Lotus corniculatus	0.98	Asteraceae_Spines	0.02	-	-
17	A	Fabaceae_Other	1	-	-	-	-
	B	Fabaceae_Other	1	-	-	-	-
18	A	Fabaceae_Other	0.996	Asteraceae_Lophate	0.002	Asteraceae_Spines	0.002
	B	Fabaceae_Other	1	-	-	-	-
19	A	Asteraceae_Spines	1	-	-	-	-
	B	Asteraceae_Spines	1	-	-	-	-
20	A	Asteraceae_Spines	0.998	Tradescantia spp.	0.002	-	-
	B	Asteraceae_Spines	1	-	-	-	-
21	A	Hemerocallis spp.	1	-	-	-	-
	B	Hemerocallis spp.	1	-	-	-	-
22	A	Fabaceae_Other	1	-	-	-	-
	B	Fabaceae_Other	1	-	-	-	-
23	A	Fabaceae_Other	1	-	-	-	-
	B	Fabaceae_Other	1	-	-	-	-
24	A	Fabaceae_Other	1	-	-	-	-
	B	Fabaceae_Other	1	-	-	-	-
25	A	Fabaceae_Other	1	-	-	-	-
	B	Fabaceae_Other	1	-	-	-	-
26	A	Trifolium pratense	1	-	-	-	-
	B	Trifolium pratense	1	-	-	-	-
27	A	Fabaceae_Other	0.996	Asteraceae_Spines	0.004	-	-
	B	Fabaceae_Other	0.996	Asteraceae_Lophate	0.002	Asteraceae_Spines	0.002
28	A	Lotus corniculatus	1	-	-	-	-
	B	Lotus corniculatus	0.998	Apiaceae_Type	0.002	-	-

Table 1: Identity and relative proportion of pollen morphotypes among complementary pollen pellets from the same bee.

Pellet	Sample	Type One	Proportion	Type 2	Proportion	Type Three	Proportion
29	A	Apiaceae_Type	0.974	Fabaceae_Other	0.026	-	-
	B	Apiaceae_Type	0.976	Fabaceae_Other	0.014	Asteraceae_Spines	0.010
30	A	Fabaceae_Other	1	-	-	-	-
	B	Fabaceae_Other	1	-	-	-	-
31	A	Fabaceae_Other	0.998	Apiaceae_Type	0.002	-	-
	B	Fabaceae_Other	1	-	-	-	-
32	A	Fabaceae_Other	1	-	-	-	-
	B	Fabaceae_Other	1	-	-	-	-
33	A	Fabaceae_Other	1	-	-	-	-
	B	Fabaceae_Other	1	-	-	-	-
34	A	Geraniaceae spp.	0.948	Fabaceae_Other	0.050	Asteraceae_Spines	0.002
	B	Geraniaceae spp.	0.956	Fabaceae_Other	0.042	Apiaceae_Type	0.002
35	A	Lotus corniculatus	1	-	-	-	-
	B	Lotus corniculatus	1	-	-	-	-
36	A	Geraniaceae spp.	0.946	Brassicaceae_Type	0.054	-	-
	B	Geraniaceae spp.	0.962	Brassicaceae_Type	0.038	-	-
37	A	Geraniaceae spp.	0.994	Fabaceae_Other	0.006	-	-
	B	Geraniaceae spp.	0.99	Unknown	0.01	-	-
38	A	Fabaceae_Other	1	-	-	-	-
	B	Fabaceae_Other	1	-	-	-	-
39	A	Fabaceae_Other	1	-	-	-	-
	B	Fabaceae_Other	1	-	-	-	-
40	A	Brassicaceae_Type	1	-	-	-	-
	B	Brassicaceae_Type	1	-	-	-	-
41	A	Fabaceae_Other	1	-	-	-	-
	B	Fabaceae_Other	1	-	-	-	-
42	A	Irdiaceae_Type	1	-	-	-	-
	B	Irdiaceae_Type	1	-	-	-	-

Thesis Conclusion

Individual bumble bees put forth more effort to foraging than honey bees. We observed that individual honey bees and bumble bees have similar foraging bout duration. However, bumble bees embark on more foraging trips and spend more time foraging each day than honey bees. This coincides with bees collecting larger pollen pellets as well. This implies that individual bees may have a disproportionally large responsibility to assuring colony success relative to honey bees.

Bumble bees collect a greater diversity of pollen than honey bees, and are less flower constant. We observed that bumble bee colonies collect pollen from a greater diversity of plant families than honey bees. This result is primarily driven by more uniform collection of multiple pollen types, as there were no persistent differences between species in the number of pollen types collected. This finding suggests that individual bumble bees may be more evenly dispersed in the landscape, and perhaps visiting a variety of smaller size patches. This foraging pattern may also help to explain the lower rates of flower constancy exhibited by bumble bees relative to honey bees. These observations contrast with honey bees colonies that often collected a dominant pollen type each day and show high rates of flower constancy. These results align with our predictions from bee foraging strategies. Specifically, bumble bees individually develop routes among profitable patches, with foragers likely visiting different patches in the landscape. In contrast, honey bees are recruited to resource patches in cohorts by scout bees. It is probable that honey bees are foraging on larger resources, possibly at larger distances than bumble bees. These results indicate that honey bees might do best where large patches of resources, perhaps resource pulses by one, or a few closely related plant species are prevalent in the landscape. In contrast, bumble bees may do best when more abundant smaller patches are present representing

diverse resources.

The quality of pollen collected by bumble bees is greater than that observed for honey bees. We observed that on average, the percent protein of pollen collected by bumble bees is greater than that of honey bees. Furthermore, bumble bees show remarkable consistency in the total concentration of essential amino acids in their diet. This contrasts with honey bees that show quite drastic temporal variability. Protein is an essential nutrient for bees. It has important consequences on physiology, notably body size of bees, which may affect the capacity for bees to collect enough forage, and even to access resources at farther distances. Furthermore, protein is essential for the development of hypopharyngeal glands in nurse honey bees. This physiological transition is critical for the success of honey bee hives, as it directly effects the quality of food that is fed to the next generation.

Honey bees and bumble bees use their environments differently and therefore conservation efforts should consider foraging strategies when designing easements. When it comes to bee conservation it is important to think about bees as a group, and beyond just the honey bee or the bumble bee. All bees need pollen and nectar from flowers, they need water, and in some cases resins for hive maintenance. Establishing patches of flowers, say in gardens or hedgerows around crops, that consist of native plants that bloom throughout the foraging season is one way to help bees more generally. In addition, maintaining semi-natural areas with frequent and large pulses of native flowers may support honey bees and native populations.