Pollen Diet Composition Impacts Early Nesting Success in Queen Bumble Bees Bombus impatiens Cresson (Hymenoptera: Apidae)

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Abstract

Bumble bees are generalist pollinators that typically collect floral rewards from a wide array of flowering plant species. Among the greatest threats to wild bumble bee populations worldwide, many of which are declining, is a loss of floral resource abundance and diversity in the landscapes they inhabit. We examined how composition of pollen diet impacts early nesting success in laboratory-reared queens of the bumble bee Bombus impatiens. Specifically, we provided queens and their young nests with one of three pollen diets, each of which was dominated by a single pollen type, and explored how this diet treatment influenced the length of time until queens initiated nests, total counts of brood in the nest at the end of the experiment (8 wk later), and the size and weight of adult offspring produced. We found that the amount of later-stage brood (pupae and/or adults) produced by recently-initiated nests was strongly impacted by pollen diet. For example, on average 66% fewer later-stage brood were found in nests provided with the Cistus pollen Linnaeas (Cistaceae), relative to the predominantly Asteraceae pollen. This finding suggests that particular pollen diet compositions may delay larval growth, which delays colony development and may ultimately be detrimental for young nests. This study sheds light on how one of the leading stressors for bumble bees (nutritional stress) may negatively impact populations through its influence on brood production during the nest-founding stage of the colony cycle.

Key words: bumble bees, nutrition, nest initiation, larval development

Longer lengthening of bumble bee populations through time (e.g., Westphal et al. 2003, Rundlof et al. 2014, Carvell et al. 2017), some parts of the world are losing forage for bumble bees (e.g., across Britain; Carvell et al. 2006). This trend is being driven by land use changes such as agricultural intensification and urbanization (Grixti et al. 2009, Glaum et al. 2017), and also by global warming-induced phenomena such as phenological mismatches between bees and flowering plants (Thomson 2010, Pyke et al. 2016, Ogilvie and Forrest 2017). Some landscapes, e.g., ones dominated by particular invasive plant species (Liu et al. 1975) or where crops that are frequently visited by bumble bees (e.g., mass flowering canola) are grown monoculturally, may lead to abundant but relatively homogenous pollen diets for larvae in the nests. One proximate explanation for why bumble bee populations are not as well-supported in environments with fewer floral resources is that brood in the nest (specifically, larvae) require not only abundant, but also diverse, pollen in their diet in order to grow and develop optimally. In previous laboratory studies in bumble bees, larvae that were chronically fed pollen derived exclusively or primarily from a single plant species tended to develop more slowly and/or have

Bumble bees (Hymenoptera: Apidae; genus Bombus Latreille) are a geographically widespread group of ~250 species that provide pollination services for a wide variety of flowering plant species. Most bumble bee species are generalists that visit and collect resources from many plant families, with some exceptions (e.g., specialization of Bombus humilis Illiger on Fabaceae; Goulson and Darvill 2004). Whereas nectar is primarily consumed by foragers or stored in pots in the nest for future consumption, pollen (and some nectar) is primarily fed to developing larvae. Pollen provides larvae with the majority of proteins, lipids, and micronutrients they need for early-life growth and developmental processes (Roulston and Cane 2000). In landscapes with diverse floral resources, bumble bee larvae likely feed on a mixture of pollens that reflects the spatial and temporal diversity of floral resources around the nest, and also the unique foraging efforts of pollen-collecting individuals.

Declining floral resource availability is among the key threats to bumble bees, many of which are declining worldwide (reviewed in Goulson et al. 2008, Williams and Osborne 2009). Although areas with abundant and diverse floral resources best support bumble bee populations through time (e.g., Westphal et al. 2003, Rundlof et al. 2014, Carvell et al. 2017), some parts of the world are losing forage for bumble bees (e.g., across Britain; Carvell et al. 2006). This trend is being driven by land use changes such as agricultural intensification and urbanization (Grixti et al. 2009, Glaum et al. 2017), and also by global warming-induced phenomena such as phenological mismatches between bees and flowering plants (Thomson 2010, Pyke et al. 2016, Ogilvie and Forrest 2017). Some landscapes, e.g., ones dominated by particular invasive plant species (Liu et al. 1975) or where crops that are frequently visited by bumble bees (e.g., mass flowering canola) are grown monoculturally, may lead to abundant but relatively homogenous pollen diets for larvae in the nests. One proximate explanation for why bumble bee populations are not as well-supported in environments with fewer floral resources is that brood in the nest (specifically, larvae) require not only abundant, but also diverse, pollen in their diet in order to grow and develop optimally. In previous laboratory studies in bumble bees, larvae that were chronically fed pollen derived exclusively or primarily from a single plant species tended to develop more slowly and/or have...
smaller body sizes than those fed more heterogeneous pollen diets (Génissel et al. 2002; Tasei and Aupinel 2008; Baloglu and Gurel 2015; Moerman et al. 2016a,b; Dance et al. 2017; Moerman et al. 2017; Roger et al. 2017a,b; Leza et al. 2018). These effects on larval development might be detrimental for colonies; for example, colonies might have fewer workers if they develop more slowly, and workers that are smaller in size may be less able to collect sufficient resources for the nest (Goulson et al. 2002). Thus, these studies suggest that increasingly homogeneous pollen diets for bumble bee larvae may be one of the mechanisms contributing to bumble bee population declines, which might be addressed by improving foraging habitat restoration schemes (Vaudo et al. 2015). However, some particular monofloral pollen diets appear to be sufficiently nutritious to promote optimal larval growth (Génissel et al. 2002; Tasei and Aupinel 2008; Baloglu and Gurel 2015; Moerman et al. 2016a,b; Dance et al. 2017; Moerman et al. 2017; Leza et al. 2018). These plant pollens may provide adequate nutrients for bumble bee larvae, or may have other beneficial properties, such as protection against pathogens (Giacomini et al. 2018), that promote growth and development. Additional studies are needed to further elucidate relationships between pollen diet, nutrition, and bumble bee population health, as the nutritional content of pollen can vary widely across plant species (reviewed in Roulston and Cane 2000), and the non-nutritive properties of pollen for bees are largely unknown.

Effects of pollen diet composition on bumble bee brood development have been studied primarily using small, queenless micro-colony experiments. An alternative experimental paradigm is to allow queens to become reproductive and produce and rear brood in newly initiated nests in the laboratory (Wu-Smart and Spivak 2017; Leza et al. 2018). This paradigm closely resembles the nest-founding phase of the bumble bee colony cycle, when overwintered queens emerge in spring from their hibernation sites and establish new nests. This stage in the colony cycle is fundamental to nesting success because the failure of young nests precludes the production of foraging workers and reproductive (males and new queens) later in the season. Moreover, the growth and development of the first set of larvae in the nest may be particularly important for nest survival, because these larvae will ultimately become the first workers in the nest. Young nests may be less likely to survive if the production of these workers is delayed, given that they assist the queen with foraging, caring for brood, and other tasks, and there is some evidence that they also directly promote egg laying in queens (Gretenkord and Drescher 1997; Kwon et al. 2006; Gurel and Gosterit 2008; Woodard et al. 2013).

Here, we examined how pollen diet composition impacts brood production and overall nesting success during the nest-founding stage in bumble bees using the laboratory model species Bombus impatiens. We supplied mated, nest-founding queens of this species with one of three heterogeneous, polyfloral mixes of pollens, and then each queen was closely monitored for nest initiation and development across an 8-wk period. At the end of the period, we examined several responses related to the timing and degree of nest development and the quality (weight and size) of workers produced. We hypothesized that queens fed these three pollen diets would exhibit differential nesting success, based on prior studies (primarily using micro-colonies) indicating that pollen diet composition can impact larval development and production (Génissel et al. 2002; Tasei and Aupinel 2008; Baloglu and Gurel 2015; Moerman et al. 2016a,b; Dance et al. 2017; Moerman et al. 2017; Leza et al. 2018). Specifically, we predicted that queens provided with particular pollen diets might initiate nests earlier and ultimately have more brood and workers, and larger-bodied workers, than queens fed other diets.

## Methods

### Bee Rearing and Experimental Design

Queen-producing colonies (n = 5) of B. impatiens were supplied by Biobest USA, Inc. (Romulus, MI) and maintained in insectary rooms of the Entomology Building at UC Riverside at room temperature (~21°C) and uncontrolled humidity conditions. Colonies were supplied with mixed-source honey bee-collected pollen (obtained from Brushy Mountain Bee Farm, Moravia Falls, NC) and nectar syrup (provided with colonies by Biobest USA, Inc.) provided ad libitum. A total of 117 queens were removed from their natal colonies on the day of eclosion (detected based on their silvery appearance) and placed in small plastic containers (approximately 15 × 8 × 8 cm). Beginning on the first day after eclosion, queens were provided with one of three pollen diets: primarily (75%) Cistus L. (Cistaceae), primarily (55%) Erica L. (Ericaceae), or primarily (56%) Asteraceae (Table 1). To estimate plant species composition in each of the diets, samples from each diet type (n = 2 each) were stained in fuchsin gel following protocol in (Kearns and Inouye 1993) and the relative amount of pollen from unique plant families was identified based on pollen morphology using the Global Pollen Project (globalpollenproject.org) database as a reference. All pollen was provided as a ball of pollen mixed with 50% sucrose solution and enclosed in honey bee wax. Cistus and Erica pollens were purchased from Pollenenergy (France), a supplier of honey bee-collected, primarily single source pollens, and the primarily Asteraceae pollen was the mixed-source honey bee collected pollen described above. Additional details about this pollen analysis are provided in the Supp. Material. Queens at this stage also began receiving a 50% (w/v) sucrose solution, rather than the commercial nectar described above. Queen natal colonies were fairly evenly represented across pollen diet treatment groups (Supp. Material) and colony of origin was included as a factor in statistical analyses.

Individually caged queens were maintained in an Invictus Drosophila incubator (model number HIS28SD) at 70% RH and 25–27°C. At ages 7–10 d of adulthood, queens were placed in mating cages (approximately 30 × 30 × 15 cm) containing 10 males each, which were supplied from separate male-producing colonies also obtained from Biobest USA, Inc., and their assigned pollen diet treatment and nectar. Queens were maintained in the mating cages for 8 h a day near a window supplying natural light. Queens observed mating on a given day were not placed in mating cages on subsequent days during the 3-d period, and only queens observed mating were included in the experiment. Of 119 queens originally placed on the diet treatments, 57% (n = 69) did not mate and were removed from the experiment. This reduced the total number of queens in the experiment after this stage to 50 (n = 20, 16, and 14 queens on the predominantly Erica, Cistus, and Asteraceae pollen, respectively). Pollen diet did not impact the likelihood that a queen would mate ($\chi^2 = 4.383, df = 2, P = 0.112$) and hereafter, all methods and results only pertain to the 50 mated queens remaining in the experiment.

At ages 12 and 13 d of adulthood, mated queens were subjected to a CO$_2$ narcosis treatment wherein they were kept in a CO$_2$-filled

### Table 1. Pollen diets and their compositions

<table>
<thead>
<tr>
<th>Pollen diet</th>
<th>% Cistus</th>
<th>% Erica</th>
<th>% Asteraceae</th>
<th>% Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primarily Cistus</td>
<td>75</td>
<td>0</td>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>Primarily Erica</td>
<td>43</td>
<td>55</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Primarily Asteraceae</td>
<td>14</td>
<td>3</td>
<td>56</td>
<td>28</td>
</tr>
</tbody>
</table>

*Other*: unidentified but not Cistus, Erica, or Asteraceae pollen.
container for 30 min per day. This treatment has previously been shown to cause bumble bee queens to bypass diapause, develop their ovaries, and become egg laying (Roseler 1985). Although previous studies have observed some negative effects on queens, such as lower egg production than non-treated queens (Gosterit and Gurel 2009) and induced immune response (Amsalem and Grozinger 2017), the method allows for experimental induction of nesting behavior in queens and has been used in previous studies of early nest development (e.g., Wu-Smart and Spivak 2017, Leza et al. 2018).

Nest Monitoring and Dissections
Following CO2 treatment, queens were maintained in the incubator (conditions described above) until they reached the age of 8 wk post-eclosion. During this time, queens were provided with pollen as needed (i.e., when little pollen was remaining) or at a minimum every 3 d. Nectar feeders were replaced every 2–3 d and nests were inspected daily for egg laying and mortality for the first 7 wk of the experiment (with the exception of 6 d during this period) and then inspected every other day for the last week of the experiment. Only two queens died during the entire 8-wk period (one each in predominantly Cistus and Asteraceae groups). For each queen, the dates that eggs and pupae were first observed in the nest were recorded as well as the date that the first male or worker eclosed in the nest (where applicable).

On the date of collection, queens and their entire nests were collected and stored in a −80°C freezer. Colonies were later dissected and the following data were recorded: wet weight of the queen and any workers present; number of eggs, larvae, pupae, males, and workers in the nest; and body size of the queen and any workers in the nest. For the latter, the length of the front wing marginal cell was measured as a proxy for body size. This metric is highly correlated with other metrics used for body size in bumble bees (Knee and Medler 1965, Owen 1988, Yerushalmi et al. 2006). Queen body size was examined as a predictor in statistical analyses (as this is fixed during queen larval development, prior to the onset of the experiment) whereas worker body size was examined as a response to the treatment.

Statistical Analyses
All statistical analyses were performed in R v. 3.3.1 (R Core Team 2018) and the corresponding code is provided in the Supp. Material. Best-fit models were calculated for all analyses using model selection (model.sel in MuMln package) based on Akaike information criterion with a correction for small sample sizes (AICc); models employed for statistical analyses are shown in Table 2. Three variables were evaluated for their effects on nesting success: pollen diet treatment, queen natal colony, and queen body size (using marginal cell of wing as a proxy for body size). To assess how these variables impacted the timing of nest development, we examined the number of days until eggs were first observed in the nest using Cox proportional hazards and Cox mixed effects models with colony as a random factor (survival, coxme packages). For degree of nest development, the total numbers of 1) eggs, 2) larvae, and 3) later-stage brood (pupae and/or adults) in the nest at the time of collection were examined. Egg and larval count data were each sine-transformed to meet assumptions of normality prior to analysis. Data on the number of pupae and adults in the nest were pooled then transformed using Yeo-Johnson power transformation (lambda = 0.5), a Box-Cox family transformation that allows for a high number of zeros, as existed in our data, to meet assumptions of normality. We combined the two oldest stages of development (pupae and eclosed adults) in this analysis because adults only eclosed in a relatively small number of colonies (n = 13), so there were insufficient numbers of nests with adult brood to use these data for a separate analysis. Combining these data is conservative with respect to brood development because it combines a slightly later age class (adults, which typically eclose around age 23–25 d old) with pupae, which are up to ~12–16 d old (developmental timing data from B. impatiens; Cnaani et al. 2002). All brood count data were analyzed using an analysis of variance (ANOVA), with post hoc comparisons using simultaneous tests for general linear hypotheses with Tukey’s post hoc (glht in multcomp package). For our analyses of queen and worker offspring characteristics, the following responses were included in calculation of the best-fit model: worker body size, worker wet weight, and queen wet weight. The best-fit model was then examined using an ANOVA with the relevant factors.

Table 2. Summary of models and statistical tests used to assess impacts of diet, queen natal colony, and queen size on nest initiation and success

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Model type</th>
<th>Factors</th>
<th>z-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to nest initiation</td>
<td>Cox Mixed Effects model</td>
<td>Predominantly Asteraceae vs predominantly Cistus pollen</td>
<td>−1.75</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Predominantly Asteraceae vs predominantly Erica pollen</td>
<td>−1.88</td>
<td>0.061</td>
</tr>
<tr>
<td>Analysis</td>
<td>Model type</td>
<td>Factors</td>
<td>F-value</td>
<td>Pr (&gt;F)</td>
</tr>
<tr>
<td>Number of eggs (sine transformed)</td>
<td>ANOVA</td>
<td>Pollen diet</td>
<td>2.704</td>
<td>0.082</td>
</tr>
<tr>
<td></td>
<td></td>
<td>queen size</td>
<td>0.214</td>
<td>0.647</td>
</tr>
<tr>
<td>Number of larvae (sine transformed)</td>
<td>ANOVA</td>
<td>Pollen diet</td>
<td>1.630</td>
<td>0.211</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Queen size</td>
<td>1.038</td>
<td>0.316</td>
</tr>
<tr>
<td>Number of older brood (Yeo-Johnson power transformation)</td>
<td>ANOVA</td>
<td>Pollen diet</td>
<td>5.083</td>
<td>0.013</td>
</tr>
<tr>
<td>Worker weight</td>
<td>LM</td>
<td>worker body size</td>
<td>25.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Queen weight</td>
<td>ANOVA</td>
<td>Pollen diet</td>
<td>0.58</td>
<td>0.565</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Queen size</td>
<td>47.79</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*For analysis of queen weight, three models were equivalent (ΔAIC scores within five).*
Queen mortality was not examined statistically as a response in the experiment due to the small number of queens that died (n = 2); data from these two nests were excluded from any statistical analyses. A few nests (n = 4) produced both female and male offspring; data from these nests were included in statistical analyses. The presence of male offspring interspersed with female offspring in the first broods produced by a nest can signify diploid males, which can indicate a high degree of relatedness between queens and their mates (Duchateau et al. 1994).

Results

Timing of Nest Development
By the end of the experiment (~6 wk after CO2 treatment), all but two of the queens that survived across the experiment (n = 46 of 48 total surviving queens) had initiated nests. Both queens that failed to initiate nests were in the predominantly Erica pollen diet treatment group. All queens that initiated nests did so by 18–52 d following administration of the CO2 treatment to bypass diapause, with a mean of 28 d to initiation across all treatment groups. Pollen diet did not influence the date of nest initiation (P = 0.052 for the predominantly Asteraceae versus Erica diet, P = 0.662 for predominantly Asteraceae versus Cistus), but queen body size did significantly affect date of nest initiation (P = 0.018), with larger-bodied queens initiating nests earlier in the experiment. Among the nests that produced adult offspring by the end of the experiment (n = 13 nests), all adults emerged within a relatively narrow period of time, irrespective of treatment group; the average queen ages (days post-eclosion) at which adults emerged in the nest fell within the range of 51–52 d for all treatment groups.

Degree of Nest Development
All queens that initiated nests had brood present in the nest at the end of the experiment, although some nests were missing one or more of the following categories of brood at the time they were collected: eggs (n = 17 nests), larvae (n = 7), or pupae (n = 26). Pollen diet did not influence the number of eggs (F = 2.704; df = 2, 33; P = 0.082), mean number of eggs across all treatment groups = 6.19 ± 0.98) or larvae (F = 1.630; df = 2, 33; P = 0.211); mean number of larvae across all treatment groups = 13.06 ± 1.88 SE) present in the nest at the time of collection, but it did influence the number of later-stage offspring (pupae or adult workers and males [F = 5.082; df = 2, 29; P = 0.013]), with the predominantly Asteraceae diet queens producing more of these older stages of brood (mean = 4.23 ± 0.96 SE) than the predominantly Cistus diet queens (P = 0.024, Tukey’s post hoc; mean = 1.39 ± 0.63 SE), but not Erica diet queens (P = 0.790, Tukey’s post hoc; mean = 2.50 ± 0.98 SE) (Fig. 1). Colony of origin and queen body size did not influence the amount of brood in the nest.

Queen and Worker Offspring Characteristics
Worker wing measurements (which are reflective of body size) ranged from 1.9 to 2.8 mm (mean marginal cell width of 2.34 mm ± 0.04 SE), and worker wing size and wet weight (mean of 0.139 g ± 0.011 SE) were strongly positively correlated (adjusted R2 = 0.672; F = 25.54; df = 1, 11; P < 0.001). Pollen diet and queen colony of origin did not impact worker body sizes or weights. Queens were fairly variable in body weight at the time they were collected (0.394–0.756 g). Queen weight was significantly positively correlated with body size (adjusted R2 = 0.57; F = 49.52; df = 1, 36; P < 0.001) and was not impacted by pollen diet or colony of origin.

Discussion
We manipulated the plant species composition of the pollen diet provided to mated, nest-founding humble bee queens and their offspring, and examined how this diet manipulation impacted the timing and degree of brood production and the characteristics (body size and weight) of offspring produced in their young nests. We also explored how queen body size and natal colony impacted these responses in the experiment. Nearly all (~96%) mated queens in our
study produced brood, irrespective of their pollen diet, but queens given one of the diets (predominantly Cistus) had dramatically reduced numbers (on average 66% less) of later-stage offspring (pupae and/or adults) in their nests at the end of the total 8-wk experimental period, relative to the primarily Asteraceae pollen diet. We did not detect an effect of pollen diet on the timing of nest initiation, nor on the numbers of eggs or larvae present in nests at the end of the experiment, or on the characteristics of offspring produced by a nest.

Our finding of more late-stage brood in nests provided with particular mixes of plant pollens strongly suggests that brood develop more rapidly on some pollen diets. We detected this effect in our experiment despite the fact that each of our pollen diets contained pollens from multiple, albeit one dominant (>50%), plant group. There is at least one potential alternative explanation for our finding: queens fed the primarily Cistus pollen diet might have either produced fewer eggs early in the experiment and/or they might have produced similar numbers of eggs, but these eggs did not ultimately reach the pupal stage because they were eaten or the larvae were rejected from the nest. However, we believe that these alternative explanations are less likely given that 1) nests were initiated at a similar time across treatment groups, 2) there were similar numbers of eggs and larvae in the nests of different treatment groups at the end of the experiment, and also 3) there were very few rejected larvae detected during the experiment (data not shown). Further, our findings are consistent with several previous studies in both bumble bees and honey bees that have detected delayed larval growth as a consequence of feeding exclusively or largely on particular monofloral pollen diets (Génissel et al. 2002; Tasei and Aupinel 2008; Baloglu and Gurel 2015; Moerman et al. 2016a,b; Moerman et al. 2017; Leza et al. 2018). Our study extends these findings to worker larvae that are produced and reared by queens during the early nest-founding stage. Future studies that explore the underlying mechanisms of how pollen diet composition impacts larval growth specifically (relative to other life stages) will be important for furthering our understanding of diet effects on early-life developmental processes.

Importantly, our study cannot identify what aspects of the pollen diets were the key drivers of differences in larval growth. With respect to Cistus pollen and its negative effects on larval development, our findings are broadly consistent with previous studies wherein bumble bees were fed this specific plant pollen, including Leza et al. (2018), who found fewer amounts of some brood classes in young B. impatiens nests; Baloglu and Gurel (2015), who observed slower colony development in B. terrestris nests; and Vanderplanck et al. (2014), who found that B. terrestris larvae were significantly smaller than larvae fed exclusively on one of four other monofloral pollen diets. Cistus pollen has a relatively low concentration of essential amino acids (Vanderplanck et al. 2014), which may be driving the effects we observed of this pollen diet on larval development. Whereas Cistus appears to be a relatively poor pollen type for bumble bees, other plant pollens (such as Asteraceae and Erica pollen) may be sufficient to promote optimal larval growth because they are more nutritious, are less protected by secondary compounds, or have some other characteristics that facilitate digestion and nutrient assimilation by larvae. There is also increasing evidence that floral rewards can contain protective secondary compounds that reduce pathogen loads, such as the protection against Cribitida bomby (Trypanosomatidae) by Asteraceae pollen (Giacomini et al. 2018) and by multiple compounds commonly found in floral nectars (Richardson et al. 2015). However, it is difficult to attribute the effects observed in our experiment to a particular pollen type and its constituents, given that our pollen diets were predominantly one pollen type, but contained pollen from multiple plant species. Rather, our data support the overall importance of pollen diet composition for early development, and the hypotheses that pollen mixing (Eckhardt et al. 2014) and preferential foraging on pollens with particular nutrient compositions (Vaudo et al. 2016a,b; Moerman et al. 2017) may be important strategies for generalist bees to avoid relying primarily on pollen types that are insufficient for optimal larval growth and development.

There are several factors that may lead to pollen diet homogeneity for bumble bees. There is some evidence that bumble bee queens preferentially search for nesting locations (Svensson et al. 2000, Kells and Goulson 2003), and they may seek out areas with greater floral resources; however, this may not be possible in landscapes where fewer nesting choices are available. Additionally, queens of some species initiate nests very early in the season when fewer plant species may be flowering, and so their foraging options may be more limited at this time relative to later in the season. Given that when nests are first initiated, queens are the sole foragers for the nest, the pollen diet fed to larvae by the queen may also be more homogeneous simply because it is a function of the foraging efforts of a single individual. Bumble bees can exhibit great fidelity to a single foraging patch or flower type (Bowers 1985, Waser 1986, Heinrich 2004), which may result in more homogeneous pollen diets fed to larvae in the nest. This might be especially true in queens, which in some species have been found to exhibit greater monolecitry relative to workers while foraging (Heinrich 1976, Macior 1994). However, there is also evidence that individual bumble bees (including queens) forage from multiple flowering plant species, including within a single foraging bout (Brian 1952, Free 1970, Ranta and Lundberg 1981, Macior 1994). Bumble bees may be seeking out particular plant pollen types to optimize the nutritional content of collected pollen (Leonhardt and Blüthgen 2012; Vaudo et al. 2016a,b), and this might drive their foraging activity on individual or multiple plant species, in ways that are dependent on foraging landscape composition.

We examined nest initiation and development during the early nest-founding stage, which has been relatively under-studied in bumble bees (but see Baloglu and Gurel 2015; Baron et al. 2017a,b; Wu-Smart et al. 2017; Leza et al. 2018). Nests are sensitive to a variety of stressors at this stage, including exposure to neonicotinoid-type insecticides, which can lead to nest failure and potentially drive population extinction (Baron et al. 2017b; Wu-Smart and Spivak 2017; Leza et al. 2018). The more rapidly brood develop and reach adulthood, the sooner a nest transitions from one where the queen is the sole provider of brood care, to a eusocial nest wherein a queen has adult helpers (her daughter-workers) who ultimately perform the majority of work-related tasks in the nest. Workers may also improve nesting success by directly increasing the number of eggs that queens are able to produce (Greterkord and Drescher 1997, Kwon et al. 2006, Gurel and Gosterit 2008, Woodward et al. 2013). Thus, pollen diet diversity and quality may ultimately promote early nesting success specifically via its effects on the rapidity of worker production, and the benefits that this confers.

More broadly, our study supports the notion that a fundamental component of bumble bee conservation efforts is the promotion and management of diverse foraging resources. Nutritional stress stemming from foraging habitat loss appears to be a key driver of bumble bee declines (reviewed in Goulson et al. 2008, Williams and Osborne 2009, Woodward 2017). Higher levels of floral resource diversity increase the opportunities for bumble bees to both forage on multiple, complementary pollen sources, or seek out particular plant pollens that are sufficient to meet their nutritional needs.
Supplementary Data
Supplementary data are available at Environmental Entomology online.

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References Cited


