

Bumble bees exhibit daily behavioral patterns in pollen foraging

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Abstract In response to global declines in bee populations, several studies have focused on floral resource provisioning schemes to support bee communities and maintain their pollination services. Optimizing host-plant selection for supplemental floral provisioning requires an understanding of bee foraging behavior and preferences for host-plant species. However, fully characterizing these preferences is challenging due to multiple factors influencing foraging, including the large degree of spatiotemporal variability in floral resources. To understand bee pollen foraging patterns, we developed a highly controlled mechanistic framework to measure pollen foraging preferences of the bumble bee *Bombus impatiens* to nine plant species native to Pennsylvania. We recorded continuous observations of foraging behavior of the experimental bee community and individual bees, while simultaneously standardizing for the number of foragers in the environment and differences in floral display of each plant species, while controlling for flowering phenology such that bees only foraged when all plant species' flowers were open. Our results demonstrate that *B. impatiens* exhibit predictable daily patterns in their pollen foraging choices, and their preferences are dominated by the host-plants they

visit first. We hypothesize that these patterns at the community and individual levels are driven by the interplay between pollen abundance and quality. We recommend that daily cycles of host-plant visitation be considered in future studies to ensure precise and accurate interpretations of host-plant preference. Such precision is critical for comprehensive analyses of the proximate and ultimate mechanisms driving bee foraging behavior and the selection of host-plant species to use in habitat restoration protocols.

Keywords *Bombus impatiens* · Daily phenology · Foraging preferences · Native bee conservation · Pollination ecology

Introduction

Global bee declines have been linked to agricultural intensification, which decreases nesting habitat and the diversity and abundance of flowering plant species on which bees rely (Ricketts et al. 2008; Potts et al. 2010). As bee populations decrease, there is concern that pollination services to ecosystems and agricultural crops will diminish (Kremen et al. 2002; Klein et al. 2007; Aizen et al. 2009; Carvalheiro et al. 2010; Eilers et al. 2011). To mitigate loss of pollination services in agricultural ecosystems and maintain crop productivity, there is an increased interest in developing approaches to conserve natural habitat in proximity to crop fields, including establishing floral resource provisioning systems to support native bee communities (Decourtye et al. 2010; Carvalheiro et al. 2011, 2012; Holzschuh et al. 2012).

The primary challenge of designing floral resource provisioning schemes is including plant species that will

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provide pollen and nectar to a diverse bee community. Practitioners and researchers typically select plants based on apparent preferences of bees to particular plant species in the field, and these “preferences” are often determined by summing the number of bees visiting a particular plant species during a sampling period (Pontin et al. 2006; Tuell et al. 2008; DeBarros 2010). There is, however, a lack of consensus on appropriate sampling methodology to evaluate preferences, because different methods can lead to substantially different results (Gibson et al. 2011). Furthermore, simply summing bee abundance on different plant species overlooks many factors that influence bee foraging behavior, possibly leading researchers to misread preferences for certain plant species. These factors can include flower color (Lunau et al. 1996; Raine et al. 2006), scent (Dobson 1987), morphology (Johnson et al. 1998), size of floral display (Mitchell et al. 2004; Nattero et al. 2011), competition with other floral visitors (Greenleaf and Kremen 2006), learning and habitual behavior of individual bees (Waser 1986; Saleh and Chittka 2007), spatiotemporal availability of resources (Burkle and Alarcón 2011; Devoto et al. 2013), and what resource bees collect (pollen and/or nectar) at a particular time (Peat and Goulson 2005). Because these factors are often intertwined, it is difficult to resolve which are most proximately influencing foraging behavior in the field. Furthermore, foraging preference studies that do not discriminate between nectar and pollen foraging tend to overlook an important factor that should be considered when designating nutritional resources for bees, namely that bees use nectar for fueling their activities but rely on pollen for rearing larvae (Michener 2000). Focusing on pollen foraging preferences should provide more robust data on the plant species that best support population growth of individual bee species, whereas selecting plants for floral provisioning based on simple measurements of abundance may result in a community of plants that do not necessarily optimally support bee communities.

Temporal variation in pollinator–plant interactions can also confound assessments of pollinator preference. Long-term studies have demonstrated that patterns of bee visitation to the same plant species can vary through space and time, both within and among seasons (Petanidou et al. 2008; Basilio et al. 2006; Baldock et al. 2011; Russo et al. 2013). Network studies reveal that interactions between pollinator and plant communities change within flowering seasons (Basilio et al. 2006) and even throughout a day (Baldock et al. 2011), probably due to the differences between pollinator- and plant-community phenologies. Moreover, different plant species vary in the time of day that they present pollen and/or nectar, which may serve as a mechanism to reduce competition between plant species relying on the same pollinator species (Mosquin 1971;

Lack 1982; Stone et al. 1998; Raine et al. 2007). Such asynchronous blooming of host-plant species may greatly skew results of field-based pollinator preference assessments, because the resources of the most preferred plant species may be depleted before observations begin. Thus, studies that only sample bee visitation in one or a few observation periods during the day may misrepresent floral preferences for particular bee species and provide a poor basis for conservation recommendations, highlighting the importance of considering the influence of time of day on foraging preferences of individual pollinator species.

In this study, we developed a highly controlled, mechanistic framework, evaluating visitation rates and visitation durations, to assess bee pollen–foraging preferences for their host-plant species. We addressed whether the eastern bumble bee *Bombus impatiens* Cresson (Fig. 1; Hymenoptera: Apidae) (1) displays distinct pollen foraging preferences among different plant species and (2) exhibits daily patterns of pollen foraging preferences on these plant species. To address these questions, we tracked pollen foraging by *B. impatiens* to nine perennial plant species that are native to central Pennsylvania, USA, and commonly recommended for floral resource provisioning protocols; both *B. impatiens* and the plant species share habitats and seasonal and daily phenologies in Pennsylvania. We conducted our studies in a hoop house with managed colonies of bumble bees, thereby controlling for competition with other pollinator species and competition with other flowering plant species. This approach also allowed us to simultaneously evaluate relative preferences for plant species that present their floral resources at different times of day in the field: we controlled the timing of initiation of foraging such that bees foraged when resources from all plants were simultaneously available. To measure community-level foraging efforts, we tracked how frequently *B. impatiens* workers collected pollen among the plant species. Additionally, we timed how long individual bees collected pollen from each plant species as an indicator of individual-level foraging efforts. If in agreement, these two data sets would indicate that the most preferred plant species were those that were visited most frequently and for longer durations. Finally, to reduce variation in the data, we standardized our assessments by the size of the floral display of each plant species, reflecting flower patch size and relative pollen quantity, and numbers of foraging bees present. Thus, our approach provides a highly controlled, relativized, and standardized system for evaluating foraging preferences. Because we tested *B. impatiens* foraging behavior among plant species directly, we did not control for flower color, scent, and morphology; however, our methods and results will allow us to further test the influence of other factors, including pollen quality and quantity, on bee foraging behavior and make



Fig. 1 *Bombus impatiens* foragers actively collecting pollen from *Senna hebecarpa*, the most preferred host-plant species in 2013. Each forager is recorded as a single pollen foraging visit, and all visits are summed for “community visitation rate.” The time that each forager spends collecting pollen on the individual plant is recorded as “individual visit duration.” Image by Anthony Vaudo

recommendations for host-plant restoration protocols targeting particular bee species.

Materials and methods

Insect and plant species

Bombus impatiens (Fig. 1) is a generalist foraging bumble bee species native to the eastern USA (Mitchell 1962; Lozier et al. 2011), but is also commercially available to pollinate fruit and vegetable crops (Velthuis and van Doorn 2006). It is common in central Pennsylvania and active from spring through fall (Szabo and Pengelly 1973). *Bombus impatiens* is primitively eusocial and its annual colonies produce as many as 500 workers (Michener 2000; Cnaai et al. 2002). In recent observations in central Pennsylvania that informed our selection of plant species for this study, *B. impatiens* was the most abundant bee species (DeBarros 2010; Russo et al. 2013).

Each year, we purchased two *B. impatiens* research colonies (Koppert Biological Systems, Inc., Howell, MI, USA), comprising one queen and approximately 30 workers. Each colony box includes a large bag of sugar water for bees to obtain an ad libitum carbohydrate source. Weekly throughout the study, we counted number of workers in each colony to estimate the total bee, or potential foraging, population. To correct for the foraging population and growth or decay of each colony and its influence on foraging rates, each week we standardized

foraging rates to 100 bees for our analyses of foraging preferences.

To build upon findings from previous work in central Pennsylvania, we chose nine native plant species that spanned a range of observed visitation rates by *B. impatiens* (DeBarros 2010). *Bombus impatiens* visited three of these plant species frequently (>40 individuals collected), three moderately (13–19 individuals collected), and three infrequently (1–6 individuals collected; DeBarros 2010). Note that due to a lack of flowering, three plant species were only used in one of the 2 years of the current study (see Table 1). In addition to the frequency of visitation by *B. impatiens*, we chose the nine plant species based on their synchrony of flowering between July and August. Information regarding each plant species can be found in the United States Department of Agriculture Plants Database (www.plants.usda.gov). We ordered sixteen individuals (7.6-L pots) of each plant species (Meadowood Nursery, Hummelstown, PA, USA); the plants had been grown outdoors and were at least 2 years old. When plants were not being used for foraging observations, they were stored in field cages to prevent unwanted floral visitation and herbivory.

Hoop house

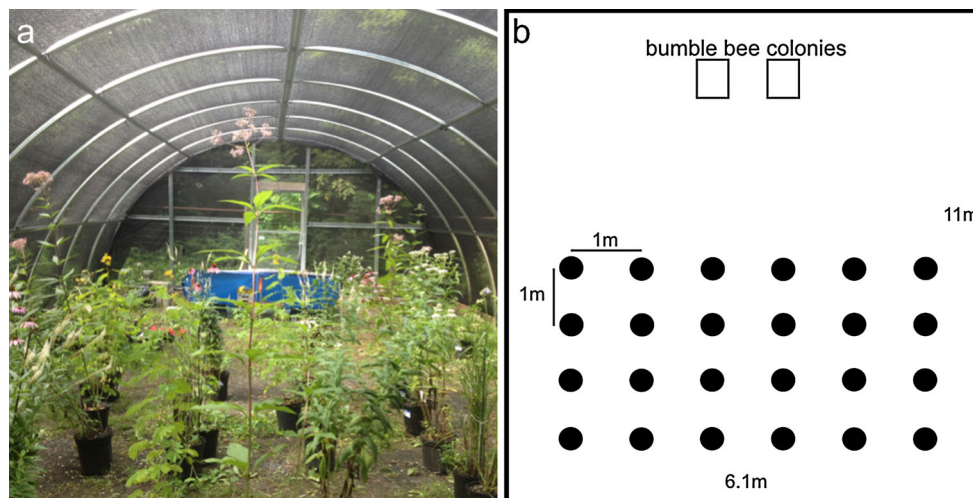
We collected all foraging observation data inside a hoop house, a large flight arena constructed as a semi-cylinder tunnel (11 × 6.1 m, 3.05 m height), covered with a 70 % shade cloth (Fig. 2). With the mesh fabric, the interior of the hoop house was subject to the weather, but sealed so that no foraging bumble bees could escape, nor any other floral visitors enter. Therefore, by introducing purchased colonies, only *B. impatiens* foragers visited the plants we provided. We placed two *B. impatiens* colonies at one end of the hoop house. At the other end, we placed equal numbers of individual plants from each species that were in bloom (either three or four individuals from each species). We randomly arranged the individual plants to positions within a grid (1 m spacing; Fig. 2). Each week, we replaced individual plants with those stored in field cages, but the mixture of the group remained the same (unless a species began or ceased flowering). Each day of observations, we randomized the position of the individual plants in the grid to prevent bee forager learning, traplining, and foot-printing the location of plants (Saleh and Chittka 2007; Saleh et al. 2007; Ohashi and Thomson 2009). Therefore, the bees would always forage from a diversity of plant species, but the locations of the individual plants would change daily and each week bees would forage from new individual plants.

Table 1 Plant species used for *Bombus impatiens* foraging observations

| Visitation frequency (DeBarros 2010) | Species | Family | Common name | Plant code | Reward | Years bloomed |
|--------------------------------------|--|------------------|-------------------|------------|---------------|---------------|
| High | <i>Eupatorium perfoliatum</i> L. | Asteraceae | Boneset | Bon | pollen/nectar | 2012/2013 |
| | <i>Pycnanthemum tenuifolium</i> Scrad. | Lamiaceae | Mountain mint | Pt | pollen/nectar | 2013 |
| | <i>Symphotrichum novae-angliae</i> (L.) G.L. Nesom | Asteraceae | New England aster | Ast | pollen/nectar | 2012 |
| Moderate | <i>Echinacea purpurea</i> (L.) Moench | Asteraceae | Purple coneflower | Ech | pollen/nectar | 2012/2013 |
| | <i>Eutrochium purpureum</i> (L.) E.E. Lamont | Asteraceae | Joe-Pye weed | Jp | pollen/nectar | 2012/2013 |
| | <i>Veronicastrum virginicum</i> (L.) Farw. | Scrophulariaceae | Culver's root | Vv | pollen/nectar | 2012/2013 |
| Infrequent | <i>Monarda fistulosa</i> L. | Lamiaceae | Wild bergamot | Mf | pollen/nectar | 2012/2013 |
| | <i>Senna hebecarpa</i> (Fernald) Irwin & Barneby | Fabaceae | American senna | Sh | pollen | 2013 |
| | <i>Tradescantia ohiensis</i> Raf. | Commelinaceae | Spiderwort | To | pollen | 2012/2013 |

Species were classified for expected visitation frequency based on previous work in our area (DeBarros 2010). “Plant code” used for identification in graphs. “Reward” represents whether each plant species presents both pollen and nectar rewards or pollen only. “Years bloomed” represents years of current study in which plant species bloomed or were available for data collection

Fig. 2 Design of *B. impatiens* foraging preference experiments. **a** An image of the hoop house including the grid of flowering plant species and the tent housing bumble bee colonies in the background. Image by Anthony Vaudo. **b** A schematic of the hoop house (11 m length \times 6.1 m width) and experimental design. Black circles represent individual plants in 7.6 L pots. Individual plants from each plant species were randomized in the grid each day



Foraging data collection

To ensure bees acclimated to the hoop house and learned how to collect pollen from all plant species, all colonies were given 3 days to forage among the plant species prior to data collection. Because this study focused on pollen foraging behavior, we only collected data for bees that were collecting pollen. Even though specific pollen foraging behavior differed between plant species of different flower types, pollen foraging was easily distinguished on all plant species. Generally, pollen foraging included bees actively scraping pollen off anthers with their legs, running in circles collecting pollen on their bodies and legs around open floral displays, or “buzz” pollinating. Nectar collection occurred when bees extended their tongues into floral nectaries. During our observations, pollen collection was

the primary behavior until pollen resources were exhausted. Furthermore, to encourage bees to focus on pollen foraging only, the bees continued to have access to the sugar water source that came with the colonies.

Each year, we collected 18 days of bumble bee pollen foraging observations (2012: between 26 June and 30 August; 2013: 5 July–12 August). To control for variation in foraging behavior due to weather (Peat and Goulson 2005), data were only collected on warm and partly cloudy to sunny days. We collected data continuously and divided observations into six discrete collection periods for analysis: 0930–1000, 1000–1030, 1030–1100, 1115–1145, 1200–1230, and 1245–1315 DST. All the plant species had pollen available for collection by 0930, allowing us to directly compare preferences among the plant species (note that *Senna hebecarpa* and

Tradescantia ohiensis bloomed as early as 0630–0700). We did not collect observation data beyond 1,315 because nearly all pollen had been collected from the plants by this time, and the bees started collecting mainly nectar. We determined that pollen was depleted on flowers by visually inspecting the anthers or by brushing anthers with a paintbrush. Colony boxes had entrances with three settings that allowed bees (1) free access to fly in and out of the colony, (2) only to fly in, or (3) no movement in or out of the colony. We opened the entrances for free flight 5 min prior to data collection to prevent bees from collecting pollen before we could make observations. After foraging observations finished for the day, we closed the entrances to only allow bees to return from foraging; thus, all foragers were trapped inside the colony (rarely, a forager or two would not return).

Within each collection period, we observed each individual plant for 1 min and recorded the number of pollen foraging visitors (“community visitation rate”). We collected 2,286 community visitation rate data points in 2012 and 2,104 data points in 2013. Concurrently, we recorded the time individuals spent pollen foraging from each plant species (“individual visit duration”). In 2012, we followed the flight paths of haphazardly selected individual foragers to determine durations of individual visits. We recorded the order of plant species visited and how long foragers spent collecting pollen on each plant. In 2013, rather than following individual bees to determine the visit duration, we observed each individual plant within each collection period and recorded the length of time individual foragers spent collecting pollen from those plants; this approach ensured that we collected data on duration of individual visits for all the plant species. If we observed no foragers on a plant species, that species received a time of zero allowing us to track how visitation to each plant species changes throughout the day. These data, however, were excluded from the analysis of average visit duration, which was independent of time of day. We timed 1,675 individual visits in 2012 and 1,302 data points in 2013. To ensure that we collected continuous foraging observations, if time allowed, we repeated for each individual plant all observations within a collection period.

Floral display

To standardize our foraging observations for the influence of floral display on visitation patterns, each day for each individual plant observed, we measured the area of floral display of the blooming flowers, including only flowers that were presenting pollen. Because the plant species differed in individual flower sizes and types (single or composite), which corresponded to differences in the

amount of pollen available (e.g., single large flowers produced equivalent pollen to many small flowers in a composite display), measuring the area of floral display most accurately accounted for these differences and allowed us to estimate relative pollen quantities between plant species. Using a digital camera (Cannon PowerShot G9; Cannon Inc., Tokyo, Japan), we photographed each flower or cluster of flowers from each individual plant; for reference, we included a ruler in each image. We analyzed images with ImageJ 1.46r software (National Institutes of Health 2012) to calculate the area (cm^2) of the floral display. We analyzed 382 photographs in 2012 and 616 photographs in 2013.

Foraging data metrics

Because we wanted to compare bee pollen foraging preferences among the plant species directly, we created single metrics to analyze *B. impatiens* foraging efforts at both the community and individual bees. These metrics reduce the variation in the data caused by the number of bees in the environment and size of floral display. To analyze bee foraging data, we used two metrics: “community visitation rate” and “individual visit durations.” Community visitation rates indicate how frequently bees visit a particular plant species to collect pollen while individual visit durations indicate how much time individuals spent collecting pollen from each plant species. These two metrics should complement each other to reveal “preferred” (and therefore profitable) plant species if they were visited more frequently and for longer periods of time. We calculated community visitation rate as the number of pollen foraging visits to a plant species per minute per cm^2 of floral display multiplied by a conversion factor (conversion factor = $100/\#$ bees in each colony) to standardize the data for 100 bees in the environment ($\text{visits}/\text{min}/\text{cm}^2/100$ bees). We calculated individual visit duration as the time in seconds spent by a foraging bee collecting pollen at a plant species per cm^2 of floral display (s/cm^2). To determine if there were general differences in foraging rates to each plant species, we first used ANOVA to analyze community visitation rate and individual visit duration data independent of time of day, followed by post hoc analyses to determine differences in foraging rates between each pair of plant species. We then used a two-way ANOVA to analyze community visitation rates and individual visit duration to determine if there was an interaction between plant species and time of day, which would indicate that *B. impatiens* foragers change their foraging efforts to the plant species in different collection periods. Data from 2012 and 2013 were analyzed separately. All statistical analyses were conducted using JMP v.10.0.0 (SAS Institute 2012).

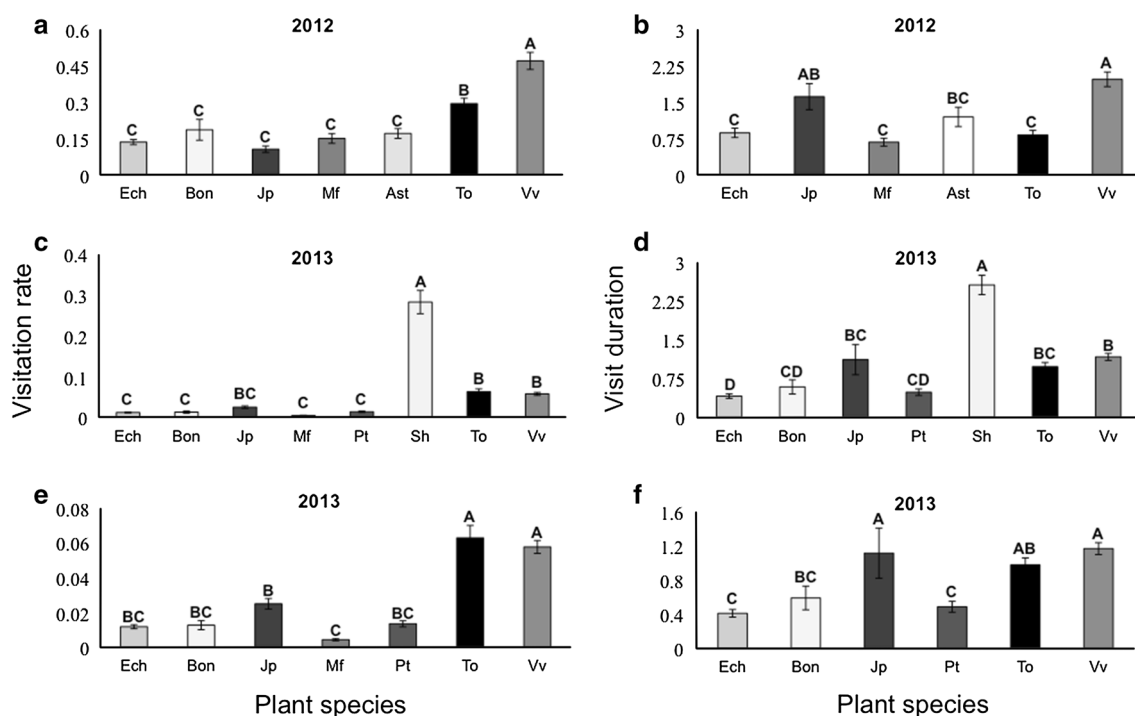


Fig. 3 *Bombus impatiens* general community visitation rates and individual visit durations (mean \pm SE) (independent of time of day) by plant species in 2012 and 2013 (Table 1 defines plant codes). Bars within graphs labeled with different letters are statistically different

($P < 0.05$); see text for details on statistics. **a** community visitation rates in 2012; **b** individual visit durations in 2012; **c** visitation rates in 2013; **d** visit durations in 2013; **e** visitation rates in 2013 excluding *Senna hebecarpa*; **f** visit durations in 2013 excluding *S. hebecarpa*

Results

In both years, the community visitation rates of *B. impatiens* foragers differed significantly among plant species, independent of time of day (Fig. 3a, c; 2012: $F_{6,1219} = 29.7$, $P < 0.0001$; 2013: $F_{7,2095} = 53.0$, $P < 0.0001$). In 2012, *Tradescantia ohiensis* and *Veronicastrum virginicum* were visited most frequently. In 2013, *Senna hebecarpa* dominated the community visitation rates (Fig. 3c); however, exclusion of *S. hebecarpa* from the 2013 analysis (since it was not available in 2012) produced similar results as 2012, with *T. ohiensis* and *V. virginicum* visited more frequently than the remaining plant species (Fig. 3e; $F_{6,1754} = 28.73$, $P < 0.0001$).

When we analyzed community visitation rate data for 2012 by time of day, we detected a significant interaction between plant species and time of day (Fig. 4a; $F_{30,1184} = 3.7$, $P < 0.0001$), indicating that visitation among plant species changed during the course of the day. In 2013, when *S. hebecarpa* bloomed, we found no significant interaction between plant species and time of day (Fig. 4c; $F_{35,2055} = 0.69$, $P = 0.92$), presumably because foragers spent so much time on this particular plant species. When we excluded *S. hebecarpa* from the analysis, a significant

“plant species \times time of day” interaction again emerged (Fig. 4e; $F_{30,1719} = 3.5$, $P < 0.0001$).

When we considered individual visit duration of *B. impatiens* foragers, rather than their community visitation rates (above), we found that for both years, individual visit duration also exhibited significant differences among plant species, independent of time of day (Fig. 3b, d, f; 2012: $F_{5,680} = 16.84$, $P < 0.0001$ [*Eupatorium perfoliatum* excluded due to insufficient observations]; 2013: $F_{6,1178} = 27.08$, $P < 0.0001$ [*S. hebecarpa* included, *Monarda fistulosa* excluded due to lack of sufficient observations]; $F_{5,876} = 7.40$, $P < 0.0001$ [*S. hebecarpa* excluded, and *M. fistulosa* excluded due to lack of sufficient observations]).

When analyzed by time of day, we found for 2012, a significant effect of the “plant species \times time of day” interaction on individual visit durations (Fig. 4b; $F_{20,631} = 2.80$, $P < 0.0001$ [*E. perfoliatum* and *Symphotrichum novae-angliae* excluded due to insufficient observations]). In 2013, when we included *S. hebecarpa* in the analysis, we did not detect a significant “plant species \times time of day” interaction (Fig. 4d; $F_{30,1236} = 1.11$, $P = 0.31$) likely due again to the dominant visitation to *S. hebecarpa*. Again, however, when we excluded *S. hebecarpa* from the

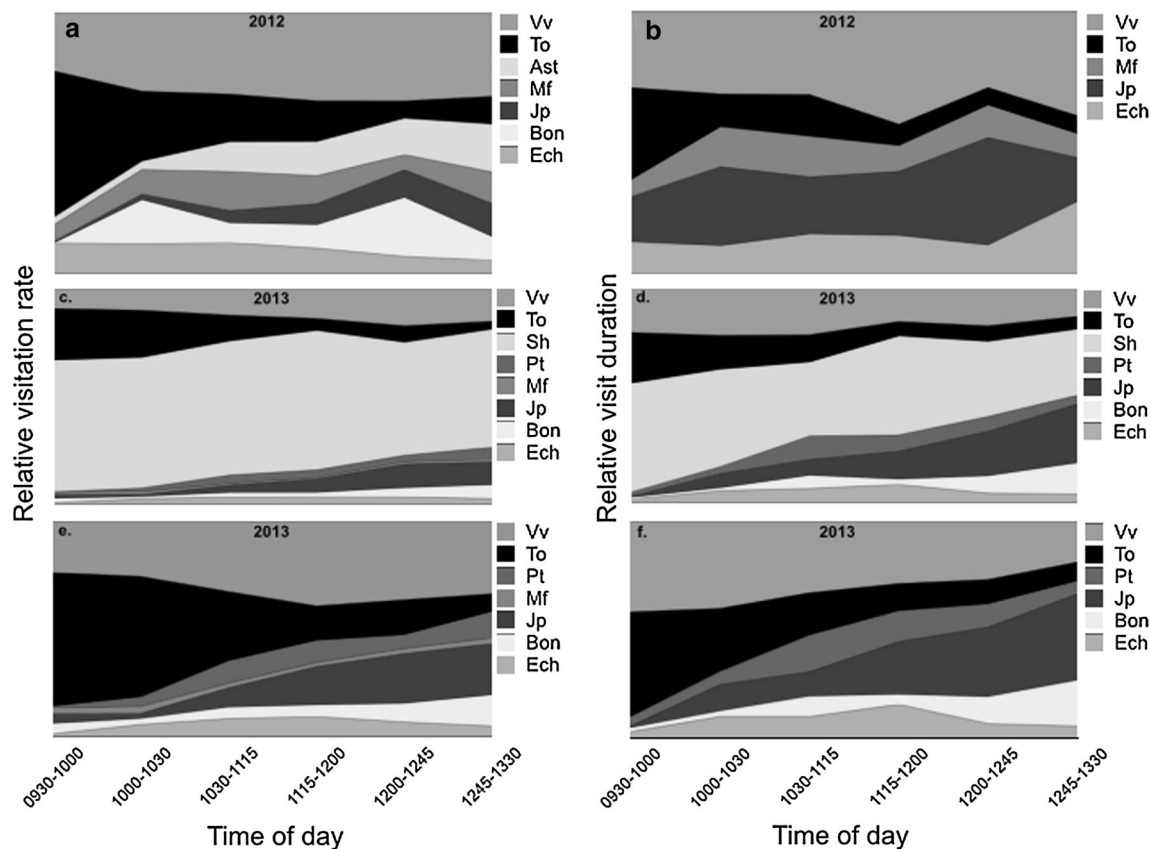


Fig. 4 Interactions of community visitation rates and individual visit durations by plant species and time of day in 2012 and 2013 (Table 1 defines plant codes). Data are represented as relative means totaling 100 % at each time period. **a** community visitation rates in 2012;

b individual visit durations in 2012; **c** visitation rates in 2013; **d** visit durations in 2013; **e** visitation rates in 2013 excluding *Senna hebecarpa*; **f** visit durations in 2013 excluding *S. hebecarpa*

analysis, the interaction was evident (Fig. 4f; $F_{25,939} = 1.72$, $P < 0.02$ [*M. fistulosa* excluded due to insufficient observations]).

Discussion

The implications of daily foraging patterns for studies of plant–pollinator interactions

By restricting bee foraging inside a hoop house and limiting the variability typically associated with field-based studies, our experiments provided a controlled setting in which to accurately determine relative pollen foraging preferences among the plant species tested. Though we did not control for every potential factor influencing bee foraging behavior, we measured foraging preferences by controlling and standardizing for important factors such as flower patch size, specific resource foraging, interspecies competition, and spatial memory and marking of the location of resources. Importantly, by only giving *B. impatiens* foragers access to flowers when

all species were presenting their floral resources, we were able to directly evaluate foraging preferences for plant species that otherwise bloom at different times of the day. Our results indicate that *B. impatiens* foragers exhibited observable and habitual patterns of pollen foraging on the plant species we offered. The foragers showed distinct pollen foraging rates at the community and individual levels, regardless of the time of day (Fig. 3), but notably, showed daily patterns in pollen foraging (Fig. 4), exhibiting different foraging rates to the flowering plant species at different times of day.

With our controlled and continuous foraging observations, we revealed often-overlooked patterns in bee foraging behavior. Our data suggest that future assessments of pollinator preference need to be mindful of daily cycles in bee foraging behavior and host-plant resource presentation (Mosquin 1971; Lack 1982; Stone et al. 1998, 1999; Raine et al. 2007). For instance, from the current study if we only collected foraging preference data at 1300, we would interpret bee foraging preference data much differently than if we only collected the data at 0930. Indeed, previous data from our area indicated that *B. impatiens* infrequently

visited *Senna hebecarpa* and *Tradescantia ohiensis* while frequently visiting *Eupatorium perfoliatum* (DeBarros 2010; Russo et al. 2013); however, our data show the opposite. This disparity likely resulted from three factors: (1) aggregating daily observations, (2) the associated assumption that patterns of foraging are consistent throughout the day (and year) (Vázquez and Aizen 2004; Jordán and Osváth 2009), and (3) by having missed foraging that occurred prior to mid-day observation periods. Moreover, the disparity must also have been influenced by our methodology of controlling for flowering phenology by limiting access to flowers until the same time each day and tracking a single pollinator species, approaches that differ from typical community network studies. Field studies, of course, provide a realistic view of community-level distribution of bee foraging for host-plant species in a particular area. But ecological differences between field sites could perhaps obscure what are the underlying mechanisms that shape pollinator preferences for certain plant species. These factors can include overall plant-pollinator community composition, daily and seasonal plant species blooming phenologies, daily and seasonal bee foraging activity, and species interactions, all of which combine to shape pollinator communities throughout the day (Baldock et al. 2011). Our methodology therefore provides a framework to test the primary mechanisms (such as resource quantity and quality) that drive pollinator host-plant choice, and our results reveal patterns of host-plant visitation that beg a mechanistic explanation.

Nectar is the primary carbohydrate source for bees and is often used to fuel foraging, whereas pollen is their primary source of proteins, lipids, and micronutrients and is essential for rearing offspring and is often presented by flowers in limited quantities (reviewed in Nicolson 2011). Studying pollen foraging preferences therefore will provide insight on host-plant species that may best support future generations of specific bee species. In this study, the behavioral differences between pollen and nectar foraging were easily observed, and in the future, it will be valuable to distinguish between the two types of foraging when collecting data to accurately report bee foraging efforts and determine host-plant preferences. By differentiating between pollen and nectar foraging, we resolved differences in pollen foraging preferences between host-plant species. For example, *B. impatiens* visits to *Echinacea purpurea* and *Monarda fistulosa* especially were dominated by nectar collection (data not shown); therefore, if we merely summed all bee visits (for both pollen and nectar) to those plant species, then we may not have been able to quantify differences in community and individual pollen visitation rates among the plant species. This level of resolution will allow us to subsequently test the mechanisms driving the pollen visitation rates we observed.

Foraging patterns reveal interplay between pollen quality and abundance

Observing bumble bees at both community and individual levels revealed interesting patterns in pollen–foraging behavior. We observed that, generally, the community visitation rates and individual visit durations to each plant species were similar independent of time of day. But importantly, *B. impatiens* foragers appear to visit their preferred pollen host-plant species early in the day and then, after exhausting those host-plant resources, move on to less preferred species. We hypothesize that these interactions may be driven by tradeoff between resource (pollen) quality and abundance.

When *B. impatiens* foragers visited *Veronicastrum virginicum* in 2012 and *S. hebecarpa* in 2013 (their most “preferred” host-plants in each year independent of time of day; Fig. 3a–d), their community and individual-level foraging behavior did not change significantly through the day (unlike the remaining plant species, Fig. 4a–d), indicating their preferred status. These results suggest that these host-plant species were the most rewarding for *B. impatiens*, which consistently tried to collect their resources even when we observed that their pollen stores had been depleted. We hypothesize that these plant species produced high-quality pollen that evoked consistent foraging behavior by *B. impatiens*.

For the remaining plant species, *B. impatiens* still visited their next most preferred plant species early, moving on to less preferred species as the day progressed. For example, in both 2012 and 2013, independent of time of day, *T. ohiensis* received the second highest community visitation rates, but relatively lower individual visit durations (Fig. 3a vs. b, c vs. d). But with respect to time of day, at both the community and individual levels, *B. impatiens* collected pollen from *T. ohiensis* most frequently early in the day and then visits decreased as the day progressed (Fig. 4). We hypothesize that *T. ohiensis* provided high-quality pollen that was not very abundant (indeed, its floral display was relatively small); therefore, the many foragers that collected pollen from *T. ohiensis* early in the day exhausted its pollen supply and then moved to a different pollen source. Nevertheless, a few *B. impatiens* still attempted to collect pollen from *T. ohiensis* later in the day. Surprisingly, these continued visits to *T. ohiensis* and also *S. hebecarpa*, even when their pollen was depleted and pollen was available from the other plant species, included pollen robbing. Foragers would use their mandibles to cut into unopened flowers to collect pollen (Hargreaves et al. 2009; Irwin et al. 2010). *Senna hebecarpa* and *T. ohiensis* do not provide floral nectar (Faden 1992; Marazzi and Sanderson 2010), and therefore, we hypothesize that these plant species produce high-quality pollen as their only

reward. And in a setting with limited resources such as a hoop house, this can lead to pollen robbing behavior if the remaining pollen available from other plant species is of lesser quality.

It is important to consider the relationship between host-plant flowering phenology and time of bee activity. In some settings, few plant species bloom and few pollinator species are active early in the morning (Baldock et al. 2011), but bumble bees forage earlier in the day and under cooler conditions than most other bee species in temperate environments (Goulson 2009). It is interesting therefore that the most preferred host-plant species of *B. impatiens* in our work (*S. hebecarpa*, *T. ohioensis*, and *V. virginicum*) were the species that bloomed earliest in the day (personal observation). These apparently corresponding phenologies may reflect a coevolutionary relationship between *B. impatiens* and these host-plant species, and their pollen quality may be particularly suitable for *B. impatiens*.

As the day progressed, *B. impatiens* increased their community and individual visitation to *Eu. perfoliatum*, *Eutriochium purpureum*, and *Symphyotrichum novae-angliae* (Fig. 4a, b, e, f), suggesting that these lesser preferred species were only visited after the pollen of more preferred species had been collected. We again hypothesize that pollen quality and abundance drove this pattern. Community visitation rates to these host-plant species, which may have lower quality pollen, appeared to increase only after the most preferred plant species were depleted; individual-level visit durations to these plant species also increased as the day progressed, suggesting that these species produced high quantities of pollen that sustained longer individual visits. *B. impatiens* foraging behavior to *Eu. purpureum* may have been an exception to the overall consistency in the community and individual visit durations; but this discrepancy only appeared when considering the behavior independent of time of day (its community visitation rates were low compared with the large amount of time individuals collected its pollen; Fig. 3a, e vs. b, f). This pattern could arise if *Eu. purpureum* produces low-quality pollen, therefore eliciting low community visitation rates, but high quantities of pollen, therefore ensuring long individual visit durations. Notably, the floral displays of *Eu. purpureum* were of the largest of the plant species tested (data not shown) and also produced a vast amount of pollen (determined by our efforts to collect fresh pollen for a separate study); therefore, it is conceivable that any bee collecting pollen would need to spend more time to adequately cover an individual plant (Mitchell et al. 2004).

Flower handling time is one potential constraint to our interpretation of pollen foraging preferences at the individual level. Indeed, for some plant species, bumble bees must invest considerable time to learn how to effectively obtain floral resources (Raine and Chittka 2007). Among

our plant species, *S. hebecarpa* had the most complex flower, which required “buzz-pollination” (Marazzi et al. 2007), while flowers of the other plant species were “open” with easily accessed resources. Despite the fact that *S. hebecarpa* resources may be more challenging to obtain, our results indicate *S. hebecarpa* was strongly preferred by *B. impatiens* for three reasons: (1) *S. hebecarpa* received both the vast highest community visitation rates and individual visit durations; (2) individual visit durations did not change to *S. hebecarpa* throughout the day, suggesting that even as bees learned how to handle the flowers, they still spent considerable time collecting resources from these plants; and (3) prior to beginning observations, we allowed *B. impatiens* to forage from the plant species so that they had the opportunity to learn handling techniques for all flowers.

Conclusion

Our study explored relative bee foraging preferences for host-plant species through a fine-scale and controlled approach that is unlike many previous studies, including field studies typically used for characterizing plant-pollinator networks. But importantly, our focus differed from that of typical community-level plant-pollinator networks because our methodology provides a fundamental framework to address the mechanisms that drive foraging behavior of a single bee species over time, whereas community-level studies provide information on the outcome of interactions of these mechanisms. By examining bees at both community and individual levels in a controlled setting that considers and standardizes for differences in flowering phenology between host-plant species, our data support the hypothesis that daily patterns in foraging behavior may be driven by the interplay of resource quality and quantity. In turn, these factors may shape overall plant-pollinator community network interactions over time. Our results demonstrate the importance of considering daily foraging patterns and the resource that pollinators are collecting (pollen vs. nectar) when evaluating floral preferences. Differentiating between pollen and nectar sources, considering foraging timing, and scrutinizing plant species based on the quality and abundance of their floral resources will allow us to recommend host-plant species for floral resource provisioning schemes that better support larval development and future generations of bees.

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