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Original Article

Nutritional complexity and the structure of bee foraging bouts

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How foragers cope with complexity in both needs and resources is a major question in behavioral ecology. When faced with nutritionally diverse resources, or when foraging for offspring with divergent nutritional needs, animals must meet the challenge of how to structure their foraging bouts, including *what* resources to forage for and in what order (*how*) to collect them. We investigated how nutritional variation in resources and requirements shapes the structure of bumble bee foraging bouts. Bumble bee workers collect 2 nutritionally distinct resources for consumers with different nutritional needs, floral nectar (largely carbohydrates) for their own needs and that of larvae, and pollen (largely protein) that is used primarily by larvae. We maintained colonies of the Eastern Bumble Bee (*Bombus impatiens*) in the laboratory on either protein-rich or protein-limited diets and assessed bees' foraging bout structure on artificial flowers that offered low, medium, or high ratios of pollen to nectar. We analyzed bout structure using both traditional floral constancy metrics as well as hierarchical Bayesian analyses. Bees from pollen-satiated colonies responded to variation in floral pollen:nectar ratios, tending to collect pollen consecutively when nectar volumes were high. In contrast, foragers from pollen-limited colonies were relatively insensitive to floral reward ratio, tending to collect pollen in long runs regardless of nectar volume. We discuss the implications of these findings for the pollination services that bees provide plants.

Key words: floral constancy, floral rewards, foraging, nectar, nutritional ecology, pollen, pollination, social insect.

INTRODUCTION

Foraging behavior mediates trophic interactions and can dramatically impact the structure and stability of both populations (reviewed in Wolf and Weissing 2012) and communities (Miner et al. 2005). Understanding what drives foraging behavior can provide a mechanistic perspective on community-level processes such as competition, coexistence, and habitat partitioning (Kotler and Brown 2007). Traditionally, foraging models attempting to predict resource or patch selection have assumed that organisms maximize a single currency (MacArthur and Pianka 1966; Charnov 1976; Thomson 1986a), such as net energy gain (van Gils et al. 2003; Houston and McNamara 2014; Kohli et al. 2014), efficiency (Schmid-Hempel et al. 1985), or probability of foraging success (Biernaskie et al. 2009; Lai et al. 2011). Though single-currency models may be sufficient when organisms are foraging for themselves on nutritionally similar resources, nutrients are rarely homogeneously distributed among resources. Furthermore, organisms often forage for secondary consumers such as offspring or members of their social group, which may have different nutritional needs.

Indeed, a growing literature suggests that individuals balance intake of multiple nutrients, foraging more intensively for whichever nutrient is currently limiting (Jensen et al. 2012; Simpson and Raubenheimer 2012; Walker et al. 2014). In such circumstances, animals may be foraging suboptimally from a strictly energetic perspective (Belovsky 1978), and single-currency foraging models may not be appropriate (Mayntz et al. 2005). To further complicate the picture, the optimal balance of nutrients may depend on factors such as the animal's physiological state (Simpson and Raubenheimer 2012) or life-history stage (Paoli et al. 2014). The latter is particularly important when parents and offspring have disparate nutritional needs (e.g., Fewell and Winston 1992), forcing parents to apportion their foraging effort between resources for themselves versus their offspring. Behavioral mechanisms for coping with variation in need include separate foraging trips for self- versus offspring-optimal resources (e.g., Welcker et al. 2009), or foraging proportionately more for offspring as their demand increases (e.g., Eckert et al. 1994; Dussutour and Simpson 2009).

Faced both with the need to collect multiple resources and to feed offspring, 2 major questions arise: 1) *what* resource(s) (and how much of each resource) should organisms collect during a foraging bout? (hereafter bout composition) and 2) *how* should they organize

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collection of each resource over time (hereafter bout order)? Taken together, composition and order shape the types of transitions a forager makes (hereafter bout structure). The resources an organism chooses invariably affect its ecological interactions (Kotler and Brown 2007), but the role of bout order may be less apparent. When switching between resources organisms can be less efficient because of a loss of attention (e.g., Dukas and Kamil 2001), increased search time (e.g., Chittka et al. 1997), or increased handling time (e.g., Woodward and Laverty 1992). Though the relative individual costs of each of these may be minimal, the total cost of switching between resources is likely nonnegligible. Although both composition and order generate the overall structure of a bout and shape its ecological consequences, these 2 components of structure are often studied in isolation.

Bees (Apidae), as long-time models for the study of foraging behavior (e.g., Heinrich 1976; Pyke 1978; Dukas and Real 1991), represent an opportunity to investigate how nutritional diversity in both resources and requirements interact to structure individual foraging bouts. Both social and solitary bees forage for pollen and nectar as sources of protein and carbohydrates, respectively (Roulston and Cane 2000), and nutritional needs vary greatly between adults and the larvae for which adults forage. Larvae are the primary consumers of pollen (diet estimated as ~1:15 protein:carbohydrate in *Bombus terrestris*; Pereboom 2000), whereas adults consume relatively more nectar (diet estimated as 1:149 protein:carbohydrate in *B. terrestris*; Stabler et al. 2015). Likewise, bee-pollinated flowers show great diversity in reward composition (reviewed in Renner 2006): for example, some plants offer primarily carbohydrate rewards (nectar, as in *Asclepias* or certain *Orchidaceae*), others primarily protein rewards (pollen, as in *Solanum* or *Papaver*), or both resources (e.g., many *Asteraceae*).

Despite this widely observed variation in the nutritional composition of floral rewards, we know little about how bees respond to variation in the relative distribution of pollen versus nectar in their habitat, and if this distribution has consequences for bout structure (and consequently, plant fitness). Bees respond to changes in floral nectar volume by increasing residence time and pollen transfer (Thomson and Plowright 1980; Thomson 1986), yet it is not clear how the relative abundance of pollen and nectar shape the structure of foraging bouts. This is surprising because bout organization greatly impacts the efficacy of pollination (Thomson 1986b; Wilson and Thomson 1991). Like many other pollinators, bees forage in monospecific runs, visiting flowers of a single species consecutively, a behavior termed floral constancy (reviewed in Chittka et al. 1999). Empirical investigations of floral constancy have focused primarily on nectar rewards with an emphasis on understanding how their quality (including nectar volume) and accessibility affect constancy (reviewed in Gegear and Laverty 2001). Although many bees collect both pollen and nectar on foraging bouts (Hagberg and Nieh 2012), the role that nutritionally complex floral rewards per se play in floral constancy is unknown.

We used the Eastern Bumble bee (*Bombus impatiens* Cresson.) to investigate how feedback from both the colony (i.e., nutritional requirements) and plant (i.e., available resources) shape the structure of foraging bouts. We manipulated colony pollen diet and floral nectar rewards in a factorial design, allowing us to evaluate the interplay of requirements and resource availability. Specifically, we assessed the bout structure of workers foraging on a “single-species” array of dual-reward artificial flowers when nectar volumes per flower were low, medium, or high, and when the colony pollen diet was either restricted or mimicked natural pollen foraging returns.

How might foragers integrate both nutritional needs and available resources when deciding what resources to collect and in what order? Bumble bees are clearly sensitive to variation in colony needs (Plowright et al. 1993) and previous work on nectar foraging suggests they should visit the most rewarding flower or resource (Pyke 1978; Harder 1990; Cnaani et al. 2006; Leonard and Papaj 2011). Therefore, in terms of bout composition (the “what”), broadly, we expected foragers to follow a simple economic model, collecting a more offspring-optimal balance of resources (i.e., more pollen, less nectar) both when the colony demand for pollen was high, and when flowers offered a high pollen to nectar ratio (Hypothesis 1, Figure 1). Additionally, we anticipated that changes in composition would lead to different bout structures (i.e., more pollen–pollen transitions; Hypothesis 2, Figure 1).

A shift in bout composition (i.e., *what* a bee collects) would not inevitably affect bout order (i.e., *how* it collects it) because constancy *sensu stricto* is independent of preference (Waser 1986). For example, 2 bees might collect resources in different proportions (i.e., 50% pollen/50% nectar or 70% pollen/30% nectar), but one may collect a single resource consecutively (being highly constant to floral rewards), whereas the other alternates between collection of nectar versus pollen (being highly inconstant). If collecting resources in a constant manner decreases handling or decision-making time (as is argued for floral constancy more generally: Laverty and Plowright 1988; Gegear and Thomson 2004; Gegear and Laverty 2005), however, we expected colony need for pollen might drive foragers to become more pollen constant (Hypothesis 3, Figure 1) thus altering bout structure (Hypothesis 4, Figure 1).

METHODS

Study species

We used 101 foragers from 7 commercial colonies of *B. impatiens* (Koppert Biological Systems, Howell, MI). Each colony contained between 20 and 60 workers and their natal queen on arrival and quickly grew to ~130 bees while testing. The colonies were housed indoors (20–25°C) and were kept on a 15:9 L:D (6:00–21:00 h light) schedule with fluorescent lighting provided by 9 full spectrum bulbs (True-Lite: F32T8-TL, Interlectric Corp., Warren, PA) and 3 “black light” bulbs (F32T8-BL), elevated 1.5 m above the foraging arena. Colonies were attached to a mesh screened foraging arena (0.7 × 0.6 × 1.0 m) and were tested sequentially. The foraging arena received additional lighting during testing and training from a 5-m strip of 150 human-white LEDs (36 watt, LED Wholesalers, China) 0.6 m above the foraging arena floor. Colonies were provisioned with 50% (w/w) sucrose scented with linalool (1 μL/100 mL sucrose solution), pipetted directly into approximately half of the honey pots each evening. Linalool, a common monoterpene floral scent (Knudsen et al. 2006), was added to facilitate foragers’ detection of the sucrose solution. We used the same 50% sucrose solution concentration throughout training and testing, hereafter referred to as nectar.

Colony nutritional state

We divided colonies between 2 pollen diet treatments. Colonies assigned to the “limited pollen” treatment group ($n = 3$, $n_{\text{foragers-tested}} = 44$, average 14.6 ± 2.6 foragers per colony) were given 500 ± 10 mg of homogenized honeybee-collected pollen (Koppert Biological Systems) placed directly into the colony daily. This pollen diet is sufficient to sustain *B. impatiens* colonies without providing

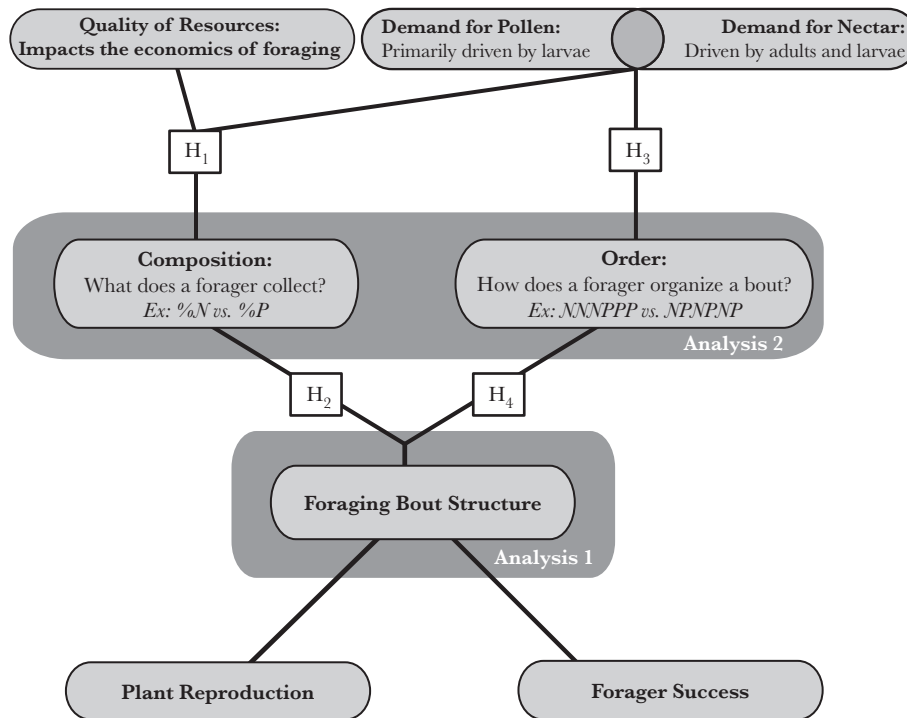


Figure 1

Conceptual model of the causes and consequences of bumble bee foraging bout structure in relation to complex floral rewards. Observed bout structure emerges from decisions bees make regarding which resources to collect and in what order. Although the quality of floral resources depends on the plant, the context in which these rewards are assessed depends on both individual- and colony-level needs. Not all causal linkages are included for clarity. H₁₋₄: hypotheses; N: nectar; P: pollen.

excess pollen for storage (Kitaoka and Nieh 2009). Colonies assigned to the “ample pollen” treatment group ($n = 4$, $n_{\text{foragers-tested}} = 57$, average 14.8 ± 4.7 foragers per colony) were given an amount of pollen proportional to colony size. We estimated colony size by tagging all workers 1–2 days after emergence. We gave each ample-pollen colony 15.3 mg of pollen for every 2 workers per day to mimic natural pollen foraging rates (Plowright et al. 1993). Two colonies in this treatment received a 3:1 mixture of homogenized honeybee-collected pollen and *Prunus avium* pollen (“Bing” variety, Firman Pollen, Yakima, WA, hereafter *Prunus* pollen), and 2 received only honeybee-collected pollen. A pollen mixture was used to encourage discovery of pollen on artificial flowers but was discontinued when it appeared to be unnecessary. These pollen diets did not differ in protein content (determined by Bradford’s assay; methods and results in [Supplementary Material](#)). Further, there was no significant difference in behavioral measures of interest between the bees that received the mixture and the bees that received only honeybee pollen (linear regressions: amount of pollen collected: $t_{1,38} = 1.50$, $P = 0.14$; proportion of visits collecting pollen [arcsine sqrt-transformed data]: $t_{1,55} = 0.82$, $P = 0.414$; constancy: $t_{1,38} = -1.891$, $P = 0.07$). We describe colonies in the scaling treatment as having “ample” pollen because they received more pollen over the course of the experiment (though they received less pollen than pollen-limited colonies until they reached 68 bees). In comparison, bees from pollen-limited colonies clearly acted as though they were pollen-limited (see Results). Further, though honeybee pollen loads contain trace amounts of nectar, all pollen loads were homogenized (using a coffee grinder) to ensure that limited-pollen colonies received less pollen than their ample-pollen counterparts.

Shaping array and floral design

We trained foraging workers to visit an arena (43×9 cm) of 10 artificial shaping flowers. Each flower was composed of a clear acrylic platform ($L \times W \times H$: $4 \times 5 \times 0.5$ cm) placed on a yellow craft foam rectangle (Creatology, Irving, TX) and offered both pollen and nectar. A removable semiopaque plastic “nectary” ($W \times H$: 0.2×0.4 cm) was inset into each artificial flower, and a removable “anther,” constructed from a 2-cm white craft chenille stem (Fibre-Craft Materials Corp., Niles, IL), was inserted into each flower (as in Muth et al. 2015). The nectary and anther were separated by 2 cm, which is greater than a forager’s body length, enforcing collection of one floral reward at a time. Each flower was placed on top of a 7-cm pedestal, painted green with acrylic paint (Shamrock #01219, Craftsmart, Irving, TX).

Each shaping flower offered ad libitum nectar and pure *Prunus* pollen. Colonies had open partially unmonitored access to the shaping array between 15:00 and 9:00 h (6 h of which were illuminated). All foragers observed visiting shaping feeders were marked on the thorax with individually numbered tags (E. H. Thorne Ltd, Wragby, Lincolnshire, UK). Though individual bees were not observed for the entire duration of their access to training arrays, only bees observed collecting pollen and nectar on shaping flowers on the morning of trials were tested, ensuring that all subjects had experience collecting both pollen and nectar on artificial flowers.

Testing

Foragers were released individually into an arena containing an array ($L \times W$: 30×50 cm) of 30 evenly spaced and equally

rewarding test flowers. We haphazardly assigned foragers to 1 of 3 nectar volume treatments: low (2 μL , $N_{\text{low}} = 34$), medium (4 μL , $N_{\text{medium}} = 36$), and high (8 μL , $N_{\text{high}} = 31$). Each test flower in all treatments additionally offered 2.0 ± 0.2 mg of *Prunus* pollen. These amounts are within the range commonly produced by bee-pollinated plants (reviewed in Willmer 2011).

We filmed the order of reward collection on a Canon HD camcorder (30 fps) and made live behavioral observations, indicating when a forager collected pollen or nectar. We classified nectar collection as any proboscis extension over a nectar well, and pollen collection as anther contact using 3 or more legs (see Muth et al. 2016 for video recording of bees foraging on similar flowers). We defined a collection as complete when the bee left the flower or switched to another floral reward on the same flower. Foragers were allowed to visit flowers freely until they attempted to return to the colony, or until they spent 5 min away from the array, at which time we considered the bout to have ended. If a forager did not land on an artificial flower within 15 min of being released into the arena, it was not tested that day. At the end of a foraging bout, we euthanized the forager and measured both intertegular span (ITS, Cane 1987) and corbicular pollen load mass.

Data analysis

All data analysis was performed in R 3.1.2 (R Core Team 2014). We analyzed bout structure, our primary focus (Analysis 1, Figure 1), and then examined its components, that is, bout composition and bout order, to understand how each shaped overall structure (Analysis 2, Figure 1). For all described analyses, we started with full models but removed interaction terms if they were nonsignificant.

Analysis 1: bout structure

We used the probability of each transition type (i.e., pollen–pollen, nectar–nectar, pollen–nectar, and nectar–pollen) as a measure of overall bout structure. This metric integrates information about both composition and order, providing a succinct numerical descriptor of overall structure. We assessed bout structure differences within a hierarchical Bayesian framework that modeled every individual's preference for a given type of transition and used this to inform a treatment-wide transition bias (10 Markov Chain Monte Carlo [MCMC] simulations, 90 000 iterations each, 5000 burn-in; for a detailed description of this analytic framework, see Forister and Scholl 2012; Gompert and Fordyce 2012). We ensured that the Bayesian posterior distributions were evenly sampled through visual inspection of chains (Forister and Scholl 2012). If there was apparent autocorrelation in MCMC simulations, chains were thinned (Forister M, personal communication).

Analysis 2: components of structure

Bout composition

To determine whether foragers' overall preferences for collecting nectar versus pollen shifted with colony or floral treatment, we calculated the proportion of landings in which a bee attempted to collect pollen. For this analysis, we counted every landing regardless of whether the forager received a reward (i.e., the flower had been previously visited). We modeled differences in proportion of pollen collection attempts using a binomial generalized linear mixed model (GLMM) with colony nested within colony diet treatment as a random effect (R Package lme4: Bates et al. 2014), then tested for the significance of fixed effects using a Type II Wald chi-square test (R Package car: Fox and Weisberg 2011).

As total volume of nectar varied across treatments (60 μL in low; 120 μL in medium; 240 μL in high), it was also important to establish that any differences across treatments were not the result of bees simply switching to pollen collection because they were unable to entirely fill their nectar crops. Although foraging bees do not always fill their nectar crops to capacity (e.g., especially when foraging at proximate patches as in our study; Schmid-Hempel et al. 1985), we used a logistic GLMM (lme4 function glmer, family="binomial"; Bates et al. 2014), to assess whether the pattern of nectar versus pollen collection attempts changed over the course of a bout. In this model, we used resource choice (0 for pollen and 1 for nectar) as a response variable, fixed explanatory variables of colony diet treatment, floral nectar volume, and proportion of bout completed (ranging from 0 to 1), and a random effect of bee. If bees were switching resources over the course of a bout *differentially* in each nectar treatment, we anticipated we would find an interactive effect of floral nectar volume and proportion of bout completed on resource choice.

Finally, to ensure that our pollen-limited colonies were indeed pollen stressed, we standardized the total mass of pollen and nectar collected by each forager by body size (yielding milligrams reward per mm ITS, $N = 85$ bees, 16 samples lost). We used corbicular mass as a proxy for total pollen collected (Plowright et al. 1993; Roulston and Cane 2000). We calculated total nectar collected by multiplying the number of unique nectar wells visited by the volume of nectar in each well. Pilot observations indicated that bees emptied an entire well in a single visit. For both preference and quantity of floral rewards collected, we tested for differences between colony diet and nectar volume treatments (as well as their potential interactions) using permutation analysis of variance (Permutation Anova [permanova] 5000 iterations; Manly 2007).

Bout order and constancy

Bee foraging bout order has traditionally been measured using Bateman's Constancy Index (BI) (Waser 1986) or the "Constancy Index" (CI) (proposed in Gegeer and Thomson 2004). We used "Constancy Index," which was developed to deal with strong innate preference, which foragers may show for certain flower types, colors, or, in this case, rewards. For this analysis, we eliminated all bees that collected exclusively one floral reward type, as all constancy indices are undefined in this case (including CI: Gegeer R, personal communication). Furthermore, it is not possible to separate the effect of bout order and composition on structure when foragers visit only one resource. To detect differences in constancy, we used linear mixed models (Bates et al. 2014) with colony nested within colony diet treatment as a random factor as above and assessed pairwise differences using Tukey's post hoc test.

RESULTS

Analysis 1: bout structure

Both colony nutritional state and nectar volume shaped how bees structured foraging bouts; however, bees' response to floral rewards was contingent on colony context. When foragers came from limited-pollen colonies, they showed a bias for pollen-constant transitions regardless of the volume of nectar rewards available on flowers (Figure 2, BayesPref, probability of differences in posteriors = 100% for all floral treatments). In contrast, ample-pollen colonies showed no bias toward pollen- or nectar-constant transitions when flowers offered low and medium levels of nectar but made more pollen-constant transitions when they foraged on flowers with

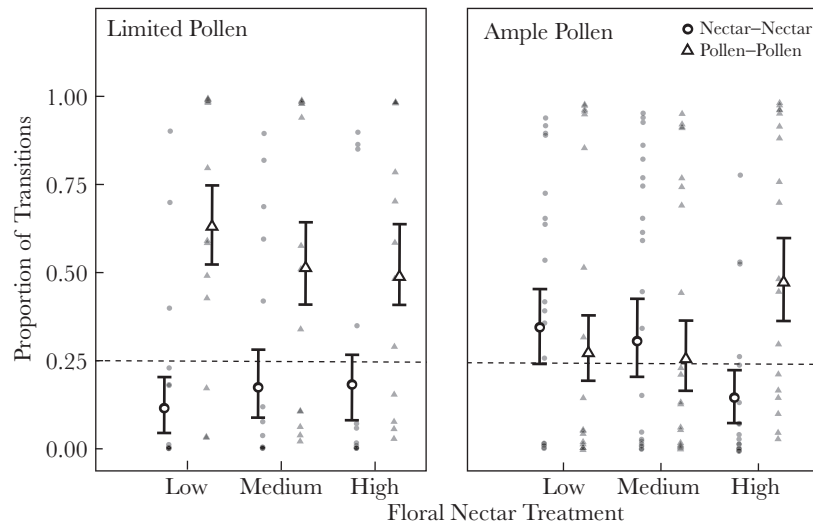


Figure 2

Posterior distributions ($\pm 95\%$ confidence intervals) of transition preferences for nectar–nectar transitions (NN: circles) and pollen–pollen transitions (PP: triangles). Individual posterior estimates indicated by semitransparent points. Foragers from pollen-limited colonies (left) have a high bias toward PP transitions regardless of floral nectar level, whereas foragers from ample-pollen colonies have equal preference for NN and PP transitions at low and medium floral nectar levels, but high bias for PP transitions on high-nectar flowers. Dotted line represents no significant bias for a transition type.

high volumes of nectar (Figure 2, BayesPref, probability of differences in posteriors = low: 30.1%, medium: 34.0%, and high: 100%, respectively). Bees from ample-pollen colonies that foraged on high-nectar flowers were more than twice as likely to make a pollen-constant transition than a nectar-constant transition.

Analysis 2: components of structure

Bout composition

Colony diet and floral rewards had relatively straightforward effects on bout composition. Bees from limited-pollen colonies made proportionately more attempts to collect pollen than foragers from ample-pollen colonies (Figure 3, glmm and Type II Wald chi-square test; $F = 4.65$, $\chi^2 = 4.32$, degrees of freedom [df] = 1, $P = 0.04$, $N_{\text{limited-pollen}} = 44$, $N_{\text{ample-pollen}} = 57$), whereas floral nectar volume did not affect the proportion of pollen foraging attempts (Figure 3, glmm and Type II Wald chi-square test; $F = 1.09$, $\chi^2 = 2.19$, df = 2, $P = 0.33$, $N_{\text{low}} = 34$, $N_{\text{medium}} = 36$, $N_{\text{high}} = 31$).

Across all treatments, bees collected more nectar toward the end of the bout (logistic glmm and Type II Wald chi-square test; $\chi^2 = 39.65$, df = 1, $P < 0.01$), but this effect did not depend on floral reward (Figure 4, logistic glmm and Type II Wald chi-square test; $\chi^2 = 1.13$, df = 2, $P = 0.57$), indicating that bees were not simply emptying low-volume nectar arrays and switching to pollen.

Furthermore, our limited-pollen treatment appeared to be effective; bees from pollen-limited colonies trended toward collecting more pollen per mm of ITS (Figure 5 and Supplementary Figure 2, permanova, $P = 0.06$, $N_{\text{limited-pollen}} = 44$, $N_{\text{ample-pollen}} = 41$), and foragers from ample-pollen collected more nectar (Figure 5 and Supplementary Figure 2, permanova, $P = 0.02$). Unsurprisingly, bees that foraged on medium- and high-nectar flowers collected significantly more nectar than foragers on low-nectar flowers (Figure 5 and Supplementary Figure 2, permanova, $P < 0.01$, $N_{\text{low}} = 25$, $N_{\text{medium}} = 31$, $N_{\text{high}} = 29$). However, the volume of nectar available per flower did not affect how much pollen foragers collected (Figure 5 and Supplementary Figure 2, permanova, $P = 0.38$).

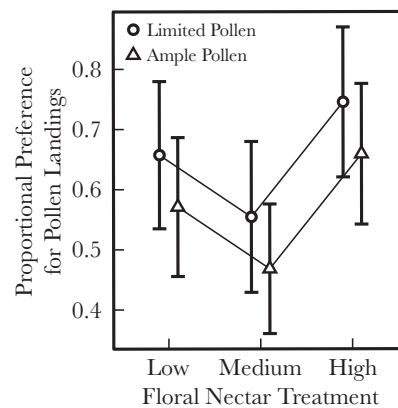


Figure 3

Proportion of landings in which bees collected pollen (least square means $\pm 95\%$ confidence intervals). There is a significant main effect of colony diet treatment ($N_{\text{ample}} = 57$, $N_{\text{limited}} = 44$) but not of floral nectar volume treatment ($N_{\text{low}} = 34$, $N_{\text{medium}} = 36$, $N_{\text{high}} = 31$) or their interaction.

Bout order (constancy)

The way in which foragers ordered the collection of pollen and nectar was driven by nectar availability, but not colony diet. Constancy to each reward type (i.e., bees' tendency to collect a single resource consecutively) decreased with increasing nectar to pollen ratio (lmm and Type II Wald chi-square test; $\chi^2 = 7.05$, df = 2, $P = 0.03$, Figure 6) but was not associated with colony diet (lmm and Type II Wald chi-square test; $\chi^2 = 0.01$, df = 1, $P = 0.91$, Figure 6). Tukey's post hoc comparisons showed that bees were significantly more reward constant on low-nectar flowers than on high-nectar flowers ($P < 0.05$, Figure 6) but constancy on medium-nectar flowers was not different from low- or high-nectar flowers ($P > 0.05$, Figure 6).

DISCUSSION

How does nutritional complexity shape the composition and structure of foraging bouts? We found that high colony demand for a

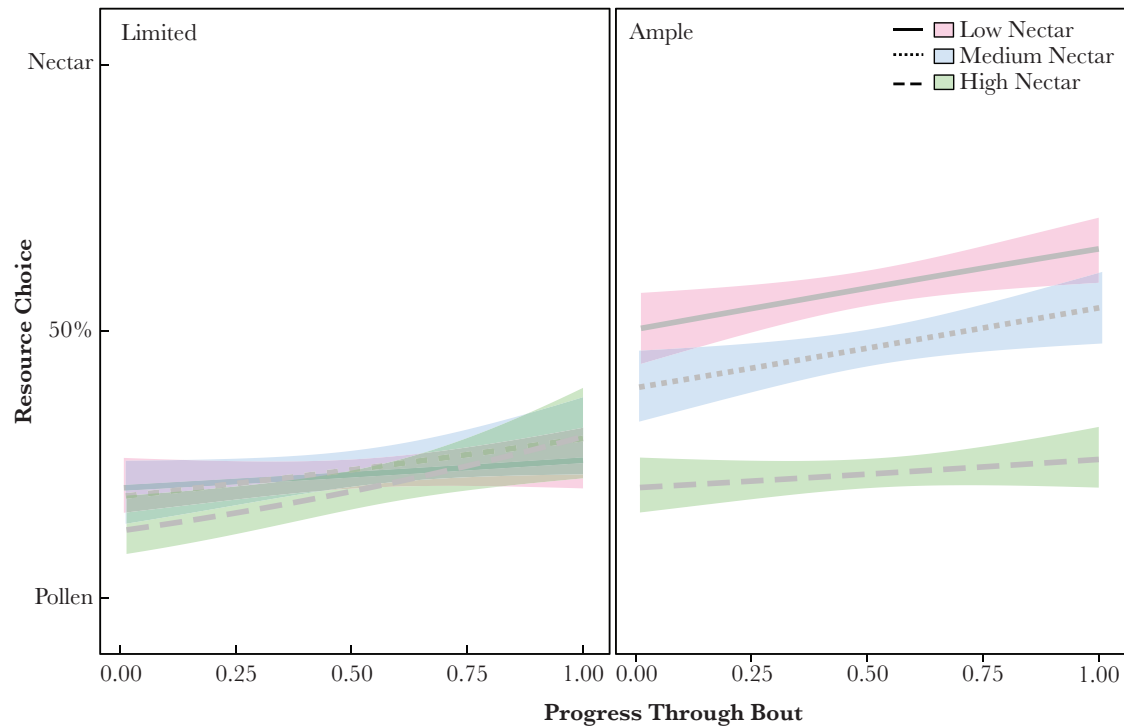


Figure 4

Fit logistic GLMMs (and standard errors) relating resource choice throughout foraging bout by colony diet and floral reward treatment (green/large dash—high nectar, $N_{\text{high}} = 31$; blue/dotted—medium nectar, $N_{\text{medium}} = 36$; pink/solid line—low nectar, $N_{\text{low}} = 34$). Bees collected more nectar at the end of bouts (logistic glmm and Type II Wald chi-square test; $\chi^2 = 39.65$, $df = 1$, $P \ll 0.01$) but did not differentially switch based on nectar treatment (logistic glmm and Type II Wald chi-square test; $\chi^2 = 1.13$, $df = 2$, $P = 0.57$).

single resource shapes *what* an organism (or superorganism) collects, and resource quality shapes *how* it orders a foraging bout. More specifically, whereas nutritional requirements of parents or offspring (in our case colonies) appear to dictate the composition of a bout, relative resource quality dictates its sequence. Past work on foraging behavior has primarily focused on answering where, what, how quickly, and how long animals should eat (Newmann 2007), generally neglecting bout structure. Nevertheless, order (here, constancy) and composition are intimately intertwined, and they are likely to have diverse and varied consequences for plant–pollinator interactions.

In terms of bout order, our results show firstly that bumble bees are not inherently constant to floral rewards, that is, CI was not different from zero across all treatments, and secondly, that bees tend to switch between collecting pollen and nectar more often as nectar volume increases. The first finding is notable because when choosing among flower species, floral colors, or artificial flowers requiring different handling techniques, bees tend to be constant (Waser 1986; Gegeer and Laverty 2001; Grüter and Ratnieks 2011). To our knowledge, this study is the first to ask whether bees show similar constancy to reward types; overall, their collection sequences could neither be characterized as constant nor true task switching (inconstancy). Nevertheless, bees switched between pollen and nectar collection significantly more often as nectar volume increased, a finding in line with previous work on floral color constancy more broadly where bees that receive high-nectar rewards show lower floral constancy (Gegeer and Thomson 2004; Grüter et al. 2011). In both cases, constancy (to colors or rewards) may reflect an economic calculus, as the absolute cost of bypassing a nectary or anther is

higher when their rewards are greater. An alternate interpretation of this pattern may be a version of the restricted search area hypothesis. On encountering high-quality flowers, bees tend to restrict their search area (Keasar et al. 1996). The decreases in reward constancy we observed may be a micro-scaled version of this phenomenon in which bees encounter a highly rewarding floral nectary (rather than flower), restrict their search area, and are thus more likely to visit the anther.

It is surprising that reward constancy did not respond to colony demand. Floral constancy is at least partially the result of selective pressure to maximize foraging efficiency (Gegeer and Thomson 2004), and if it increases foraging rate, might be expected to increase in response to colony need. The costs incurred through floral inconstancy may include increased search time; for example, bees make longer interfloral flights when making inconstant transfers (Goulson 2000; Raine and Chittka 2007). Thus, we may have found reward inconstancy because when bees are faced with a decision of switching between intrafloral rewards rather than between floral species, the cost of inconstancy is lower. In field-based experiments, foraging bumble bees show stronger constancy to color than handling strategy (Wilson and Stine 1996), supporting the assertion that switching between pollen and nectar handling may have a relatively low cost. Nevertheless, though pollen and nectar are often offered in close proximity on flowers, many angiosperm species offer only a single reward to pollinators, and in these cases the cost of searching for a different reward may be higher. Further, there is evidence that the presence of nectar on a flower may inhibit the learning of pollen visual stimuli but not vice versa (Muth et al. in preparation) and thus may affect apparent reward constancy when foragers are learning about nutritionally complex floral rewards in multispecies contexts.

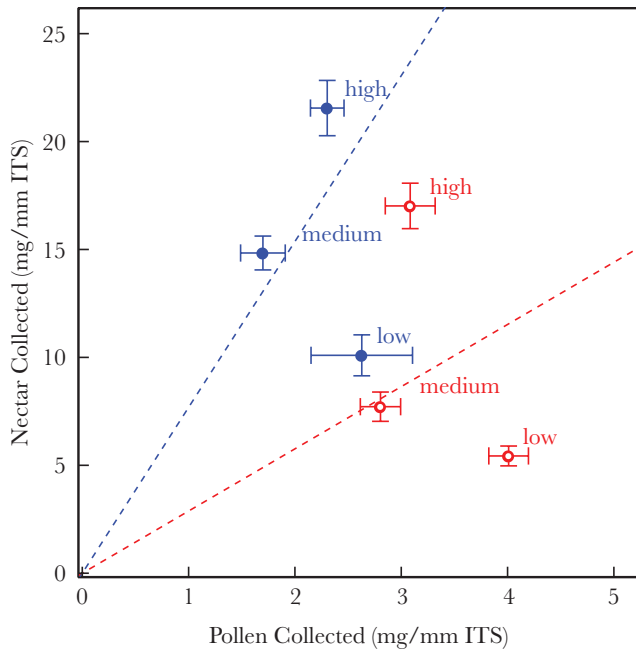


Figure 5

Amount of nectar (y axis) and pollen (x axis) collected, standardized by forager body size (means ± 1 standard error) plotted into nutritional resource space. Limited-pollen colonies are plotted in open red points, and ample-pollen colonies are plotted in closed blue points. There is a significant difference in nectar collection between limited-pollen and ample-pollen colonies as well as between floral nectar levels. There is no significant difference in pollen collection between floral nectar treatments, and a nonsignificant trend toward limited-pollen colonies collecting more nectar than ample-pollen colonies (see Supplementary Figure 2 for additional single-nutrient plots).

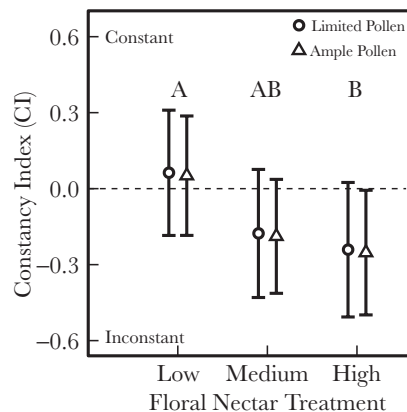


Figure 6

Constancy Index (least square means $\pm 95\%$ confidence intervals) in response to colony pollen diet (ample: triangles, $N_{\text{ample}} = 57$; limited: circles, $N_{\text{limited}} = 44$) and floral reward. There is no significant effect of colony diet treatment on reward constancy. In contrast, reward constancy significantly decreases with increasing floral nectar. Letters indicate results of Tukey's post hoc comparisons ($P < 0.05$, $N_{\text{low}} = 34$, $N_{\text{medium}} = 36$, $N_{\text{high}} = 31$).

More broadly, constancy is thought to arise from interactions between intrinsic factors (e.g., the cost of organisms acquiring information or limitations driven by search image) and extrinsic factors (i.e., relative resource quality and nutritional composition) (Gegear and Thomson 2004; Raine and Chittka 2007; Grüter

and Ratnieks 2011). In this experiment, we may have minimized the importance of intrinsic cognitive mechanisms by using a single color stimulus and flowers with simple handling strategies. In this case, reward quality played a major role in shaping bout order; however, assessing its relative importance in informationally complex systems (i.e., when bees face many species of flowers varying in both displays and handling strategies) is a clear next step.

When we used a hierarchical Bayesian analysis to consider the combined effects of constancy and composition on bout structure, we discovered an intriguing interplay between colony demand and floral rewards. Colony demand shaped bout structure when it was extreme, through changes in composition, thus resulting in frequent transitions between the most limiting resource (pollen). Alternatively, floral reward shaped bout structure when colony demand was low, likely through its impact on reward constancy. Foragers from pollen-satiated colonies on high-nectar flowers may have shown decreased nectar-nectar transitions due to a resource switching rule, in which bees aim to meet a nutritional target (e.g., a set ratio of pollen: nectar rewards sensu Simpson and Raubenheimer 2012) before switching to pollen foraging. For example, Houston et al. (2011) modeled foraging on 2 nutritionally distinct resources and predicted that organisms should forage exclusively for one resource until they are nutritionally balanced, after which they should rapidly switch between resources. Along these lines, perhaps bees in high nectar volume conditions were reaching a nutrient balance more quickly and thus subsequently spent more time switching between resources.

Our Bayesian analysis of bout structure revealed a large degree of individual variation in response to both colony and floral resources. In limited-pollen colonies, there were consistently individuals with a high preference for pollen-pollen transitions (Figure 2, semitransparent shapes) suggesting that pollen demand does not result in a wholesale shift in foraging effort by the colony, but rather deployment of a limited number of pollen specialists. Social foraging allows for massive individual variation in foraging strategies, and some persistent specialists should be expected (O'Donnell et al. 2000; Jandt et al. 2009; Hagbery and Nieh 2012; Jandt et al. 2014). Additionally, social insects, at least ants, adhere more strictly to nutritional requirements when foraging for offspring needs than when for adult colony mates (Dussutour and Simpson 2008; Dussutour and Simpson 2009), as was likely the case in our limited pollen colonies. Certainly, solitary consumers of floral rewards, including the majority of bees (Michener 2000), mix floral resources, and probably do so to cope with nutritional complexity (Williams and Tepedino 1999). Given this, how nonsocial species might cope with these aspects of floral nutritional complexity is an open question.

What consequences might these shifts in bees' foraging bout structure have for plant fitness? Our results support the idea that plants that produce pollen and nectar as a reward to pollinators face a trade-off between rewarding pollinators and limiting excessive pollen consumption. Further, this trade-off is dependent on nectar quantity and is mediated by bee behavior. Male reproductive success in plants depends on transportation and deposition of high-quality pollen and can be limited by pollinators (Aizen and Harder 2007), yet regulating the amount of pollen that pollinators can remove is thought to be a common selective pressure on plants (Harder and Wilson 1994). In cases where pollinators actively seek pollen and plants produce high levels of nectar, plants may be at risk of consumptive emasculation (i.e.,

the loss of male gametes due to pollen collection; Harder and Thomson 1989; Hargreaves et al. 2009). This nectar–pollen trade-off has been long recognized (e.g., Harder and Thomson 1989), but investigations of the phenomenon have been based largely on situations with passive pollen deposition, whereby high nectar volumes increase pollen removal by encouraging long floral residence times. Alternatively, we have characterized a novel behavioral basis for this trade-off in a case where flowers require active pollen collection; for example, plants that require anther “milking,” “drumming,” “probing,” and most notably buzz pollination, which is observed in more than 8% of angiosperm species across more than 72 families (Buchmann 1983; Thorp 2000).

More broadly, the interdependent response of bees to both colony need and floral rewards highlights that the reproductive potential of a given flower is not solely dependent on the rewards it offers to pollinators but also on the nutritional and community context in which it offers those rewards (e.g., Hersch and Roy 2007). Though resource quality is inherently an individual plant-level (or perhaps patch-level) characteristic, colony nutritional state largely depends on resources available at the landscape scale (Williams et al. 2012). Further, the nutritional landscape available to colonies is increasingly subject to disturbance by human activities (Jha and Kremen 2013). A thorough understanding of bees’ ability to detect and respond to these landscape-level changes will help inform predictions about the broader impacts of anthropogenic change. For example, one implication of our findings is that decreases in the pollen available to bumble bees may not only decrease their reproductive success but may also alter their efficacy as pollinators through changes in fine-scale bout structure. Understanding how the observed effects of colony demand and available resources on bout structure mediate pollen transfer between and among plant species is an avenue of research that may be particularly productive in the future.

DATA ACCESSIBILITY

Data archived at Dryad Repository, doi: 10.5061/dryad.467km.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>

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