Macronutrient ratios in pollen shape bumble bee (*Bombus impatiens*) foraging strategies and floral preferences

Anthony D. Vaudo[a,1], Harland M. Patch[a], David A. Mortensen[b], John F. Tooker[a], and Christina M. Grozinger[a]

[a]Department of Entomology, Center for Pollinator Research, The Pennsylvania State University, University Park, PA 16802; and [b]Department of Plant Science, Center for Pollinator Research, The Pennsylvania State University, University Park, PA 16802

Edited by John G. Hildebrand, University of Arizona, Tucson, AZ, and approved June 1, 2016 (received for review April 19, 2016)

To fuel their activities and rear their offspring, foraging bees must obtain a sufficient quality and quantity of nutritional resources from a diverse plant community. Pollen is the primary source of proteins and lipids for bees, and the concentrations of these nutrients in pollen can vary widely among host-plant species. Therefore we hypothesized that foraging decisions of bumble bees are driven by both the protein and lipid content of pollen. By successively reducing environmental and floral cues, we analyzed pollen-foraging preferences of *Bombus impatiens* in (*i*) host-plant species, (*ii*) pollen isolated from these host-plant species, and (*iii*) nutritionally modified single-source pollen diets encompassing a range of protein and lipid concentrations. In our semifield experiments, *B. impatiens* foragers exponentially increased their foraging rates of pollen from plant species with high protein: lipid (P:L) ratios; the most preferred plant species had the highest ratio (~4.6:1). These preferences were confirmed in cage studies where, in pairwise comparisons in the absence of other floral cues, *B. impatiens* workers still preferred pollen with higher P:L ratios. Finally, when presented with nutritionally modified pollen, workers were most attracted to pollen with P:L ratios of 5:1 and 10:1, but increasing the protein or lipid concentration (while leaving ratios intact) reduced attraction. Thus, macronutritional ratios appear to be a primary factor driving bee pollen-foraging behavior and may explain observed patterns of host-plant visitation across the landscape. The nutritional quality of pollen resources should be taken into consideration when designing conservation habitats supporting bee populations.

foraging behavior | nutritional ecology | pollen | pollinator | preferences

Foraging animals must obtain appropriate nutrients for growth, development, and reproduction from their environments. Bees forage in a very complex and changing environment, where floral nutritional resources (nectar and pollen) vary widely in quality and quantity among plant species (1). These resources are accompanied by myriad floral cues, including floral odors, color, morphology, and display area, and can vary dramatically in spatiotemporal availability; all these factors may influence and reinforce foraging decisions (2, 3). Worldwide declines in populations of bees and other pollinators have been linked to reduced diversity and abundance of host-plant species, likely placing bees under nutritional stress (4, 5). To develop strategic conservation protocols that preserve or restore foraging habitat that supports healthy pollinator populations, we must understand how bees forage in their environments to meet their nutritional needs. It is well established that solitary and social insects can forage selectively and regulate their intake of synthetic diets spanning a range of macronutrient nutritional qualities to reach their optimal, species-specific nutritional intake (6–8). Here we examine whether the generalist bumble bee species *Bombus impatiens* Cresson forages selectively among different plant species and pollen sources for specific macronutrient ratios.

Pollen is the primary source of proteins, lipids, and other micronutrients for bees and is necessary for brood rearing, reproduction, and health (1, 9–17). However, pollen nutritional quality varies widely among plant species, ranging from 2–60% protein and 1–20% lipids by weight (10, 18); thus, it likely is critical that bees selectively collect pollen species with the necessary nutritional quality to support their needs (1). The protein and amino acid concentrations of pollen modulate immunocompetence in honey bees (16, 19) and reproduction (ovary activation and larval development) in bumble bees (12, 14, 20–22). Furthermore, lipids are key to a variety of physiological processes in insects, including molting hormone production (23), and high sterol content in pollen may increase bumble bee larval size and growth (21). Recently, deficiency in linolenic fatty acid (an essential fatty acid) in honey bees has been linked to reduced learning and development of brood-food–producing glands (24).

There is some evidence that foraging bees can select host-plant species based on pollen protein content. While foraging in the same landscape, bumble bees foraged preferentially on plant species with higher protein content than did honey bees (25), suggesting species-specific differences in protein acquisition. Bumble bee workers can taste and discriminate among diets with different protein or pollen concentrations (26), and their foraging activity has been positively correlated with pollen protein content using modified (diluted with cellulose powder) single-source pollen diets (27, 28) or a single plant species in which pollen protein content varied with soil conditions (20). [Note that in field studies honey bees do not appear to forage preferentially on pollen with higher protein concentrations (29, 30)]. However, diluting pollen with cellulose powder may simply make diets less attractive by reducing all pollen cues, and modifying the soil conditions may alter factors other than pollen protein that may influence bee

Significance

Bees pollinate the majority of flowering plant species, including agricultural crops. The pollen they obtain is their main protein and lipid source that fuels development and reproduction. Bee populations are declining globally, in large part because of landscape-level loss of host-plant species contributing to a nutritional shortage. To mitigate declines, we must understand how the nutritional requirements of bees influence foraging behavior. We demonstrate that bumble bees selectively collect pollen from host-plant species based on the protein:lipid ratios of pollen. Our research indicates that bees evaluate pollen quality and adjust foraging decisions to meet their nutritional needs. To be effective, conservation initiatives must include host-plant species that provide pollen that satisfies the nutritional demands of bees to support their populations.

Author contributions: A.D.V., H.M.P., D.A.M., J.F.T., and C.M.G. designed research; A.D.V. performed research; A.D.V. contributed new reagents/analytic tools; A.D.V., J.F.T., and C.M.G. analyzed data; and A.D.V., J.F.T., and C.M.G. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. Email: adv124@psu.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1606101113/-/DCSupplemental.

Table 1. Host-plant species and associated pollen nutritional values used in host-plant pollen-foraging preference and isolated pollen-feeding preference assays

Species	Family	Common name	Carbohydrate	Protein	Lipid	P:C	P:L
Senna hebecarpa	Fabaceae	American senna	118.47	237.28	51.72	2.00	4.59
Tradescantia ohiensis	Commelinaceae	Spiderwort	70.43	358.25	103.74	5.09	3.45
Veronicastrum virginicum	Scrophulariaceae	Culver's root	55.32	186.72	83.71	3.38	2.23
Echinacea purpurea	Asteraceae	Purple coneflower	101.76	171.43	95.03	1.68	1.80
Symphyotrichum novae-angliae	Asteraceae	New England aster	91.35	91.04	84.03	1.00	1.08
Eutrochium purpureum	Asteraceae	Joe-Pye weed	112.66	146.48	158.76	1.30	0.92
Eupatorium perfoliatum	Asteraceae	Boneset	87.20	78.00	108.01	0.89	0.72

All pollen was collected by hand from freshly dehisced flowers. Note that *S. novae-angliae* did not bloom during data-collection periods for the host-plant foraging-preferences assay, but we used the pollen for the isolated pollen-preference feeding assay. Nutrient concentrations are reported as micrograms of nutrient per milligram of pollen. P:C and P:L ratios are provided. Plant species are listed in order of highest to lowest P:L value (nutritional rank).

choice. Only two studies have demonstrated a correlation between bumble bee foraging preference and pollen protein content in landscapes with multiple plant species (31, 32). Therefore, it is uncertain whether bumble bees truly seek out the host-plant species with higher pollen protein or whether their choice of host-plant species is driven by other factors.

From these previous studies, it is also unclear if bumble bees forage selectively to meet multiple macronutrient needs or to maximize the quantity of a single macronutrient (e.g., protein). Other studies have demonstrated that the pollenkitt, the lipid-dominated, oily outer surface of entomophilous pollen, contains important discriminative stimuli for bees (33–36). Furthermore, given the nutritional importance of lipids, bees may assess the ratios of proteins and lipids when they are foraging for pollen. Indeed, other arthropod species (e.g., beetles and spiders) can regulate their dietary intake and forage selectively to meet specific ratios of lipids and proteins (37–39).

Therefore it is unknown what are the preferred pollen nutritional qualities for bee species, if the bee's choice of host-plant species is driven by optimal pollen quality on multiple nutritional dimensions (i.e., not specifically maximizing nutrient acquisition), or if the preference for optimal pollen nutrition is maintained in the absence of external floral cues. Our previous work demonstrated that foragers of *B. impatiens* Cresson (Hymenoptera: Apidae), the Common Eastern Bumble Bee, exhibited distinct pollen-foraging preferences among nine host-plant species (40). In the current study, we tested the mechanistic basis for these foraging preferences. First, we determined whether *B. impatiens* pollen-foraging preferences and visitation rates to different host-plant species related to pollen nutritional composition and whether *B. impatiens* foragers evaluate macronutrient (protein, lipid, and carbohydrate) levels individually or in ratios. Second, to determine if *B. impatiens* workers maintained preferences for plant species in the absence of external floral cues, we evaluated bumble bee foraging preferences with isolated pollen. Finally, we modified the protein and lipid concentrations of single-source pollen to determine whether *B. impatiens* worker preferences were related to pollen protein:lipid (P:L) ratios or to increased nutrient concentration.

Results

Host-Plant Pollen-Foraging Preferences. To test if foraging preferences among host-plant species were associated with pollen nutritional quality, we collected extensive simultaneous foraging visitation data for *B. impatiens* colonies to multiple plant species (controlling for phenology and floral area/resource availability) and analyzed the nutritional content (carbohydrate, protein, and lipid concentrations) of each host-plant species' pollen (Table 1). All nutritional values were within the expected range for pollen (18). Foraging data and methodology were previously published in ref. 40 and are described briefly in *Materials and Methods*.

We used multiple regression analysis to quantify the relationship between nutrient concentration and visitation rate because the nutritional components of pollen are not independent of one another. Carbohydrate concentration and the interaction of protein and carbohydrate did not influence foraging rates (carbohydrate: $P = 0.47$; protein × carbohydrate: $P = 0.63$). Interestingly, protein concentration, lipid concentration, and their interaction were significantly associated with foraging rate (protein: $P < 0.01$; lipid: $P < 0.01$; protein × lipid: $P < 0.01$; model: $F_{5,\ 1{,}776} = 57.53$, $P < 0.01$, $R^2 = 0.14$). We explored this interaction further and found that bumble bees exponentially increased their visitation rates to the plant species as the P:L ratio of the pollen increased ($Y = 0.0025e^{1.03 \cdot x}$, $R^2 = 0.96$) (Fig. 1). Importantly, protein:carbohydrate (P:C) ratios, often considered the main nutritional drivers in arthropod herbivore foraging behavior and nutrient regulation (6), did not influence bumble bee host-plant choice ($F_{1,\ 4} = 0.02$, $P = 0.89$, $R^2 = 0.006$). Because this trend may have been driven by the bumble bees' most preferred plant species, we reanalyzed the data excluding *Senna hebecarpa* and found the same trend: As the P:L ratio of pollen increased, so too did visitation rate ($Y = 0.013e^{0.46 \cdot x}$, $R^2 = 0.67$). Furthermore, the peak foraging rate to each plant species throughout the day (see ref. 40 for details) followed the order of the P:L value (highest to lowest) (Fig. 1),

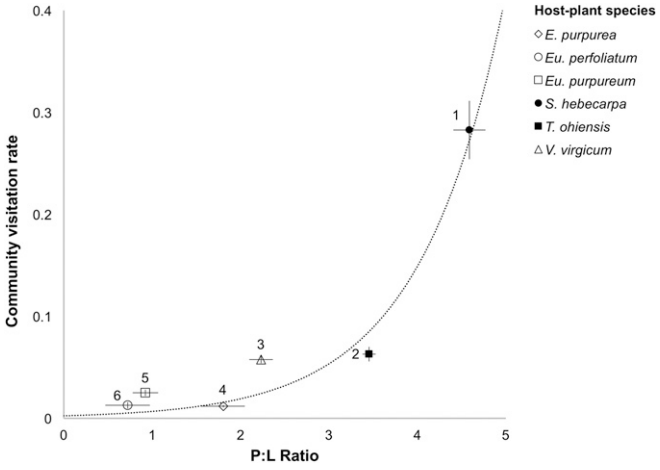


Fig. 1. The relationship between *B. impatiens* pollen-foraging rates and pollen nutritional quality (host-plant pollen-foraging preferences). Community visitation rates are the average number of pollen-foraging visits·min^{-1}·cm^{-2} floral area per 100 bees across the season; see *Materials and Methods* and Vaudo et al. (40) for more information. Data are presented as mean ± SEM. Pollen-foraging rates are exponentially related to the P:L ratio of pollen ($Y = 0.0025e^{1.03 \cdot x}$, $R^2 = 0.96$). Numbers next to each symbol represent the order during the day in which each plant species was most frequently visited, with the exception of *S. hebecarpa*, which experienced consistently higher visitation rates throughout the day.

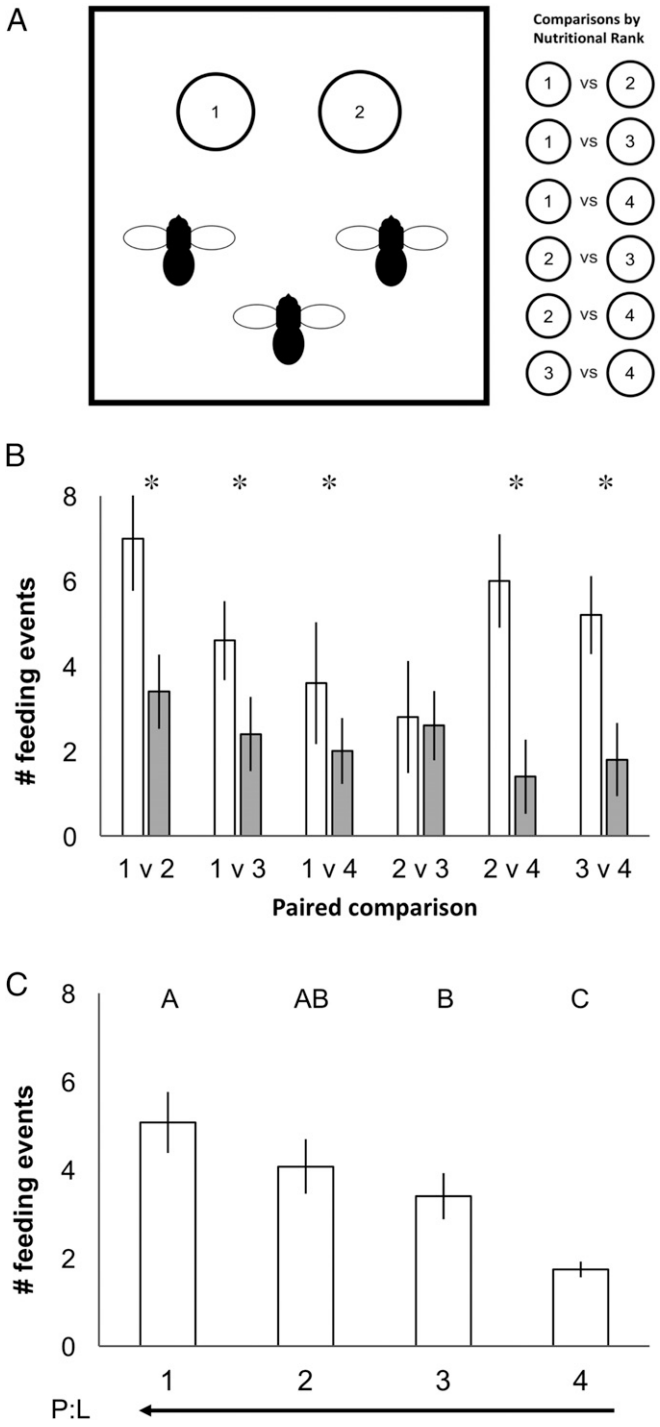


Fig. 2. *B. impatiens* pollen-feeding preferences on isolated pollen are associated with pollen P:L ratios (isolated pollen-feeding preference assay). (*A*) Experimental design for the isolated pollen-feeding preference assay. (*B*) Results of pairwise choice tests according to nutritional rank. Asterisks indicate significant differences within each pair ($P < 0.05$). (*C*) Average feeding events independent of paired comparison across trials. In each trial of the experiment, separate cages containing three bees were presented one of the six possible pairs of four pollen types. Results represent the results of five trials. The pollen nutritional rank represents the pollen species ranked by highest to lowest (1–4) P:L ratio. Data are shown as mean ± SEM. Bars labeled with different letters are statistically different ($P < 0.05$). The arrow under the *x* axis indicates increasing P:L ratio. The nutritional ranks and plant species used were 1, *S. hebecarpa* (trials 1–5); 2, *T. ohiensis* (trials 1–5); 3, *E. purpureum* (trials 1 and 2) and *E. purpurea* (trials 3–5); 4, *E. perfoliatum* (trials 1 and 2), *E. purpureum* (trials 3 and 4), and *S. novae-angliae* (trial 5).

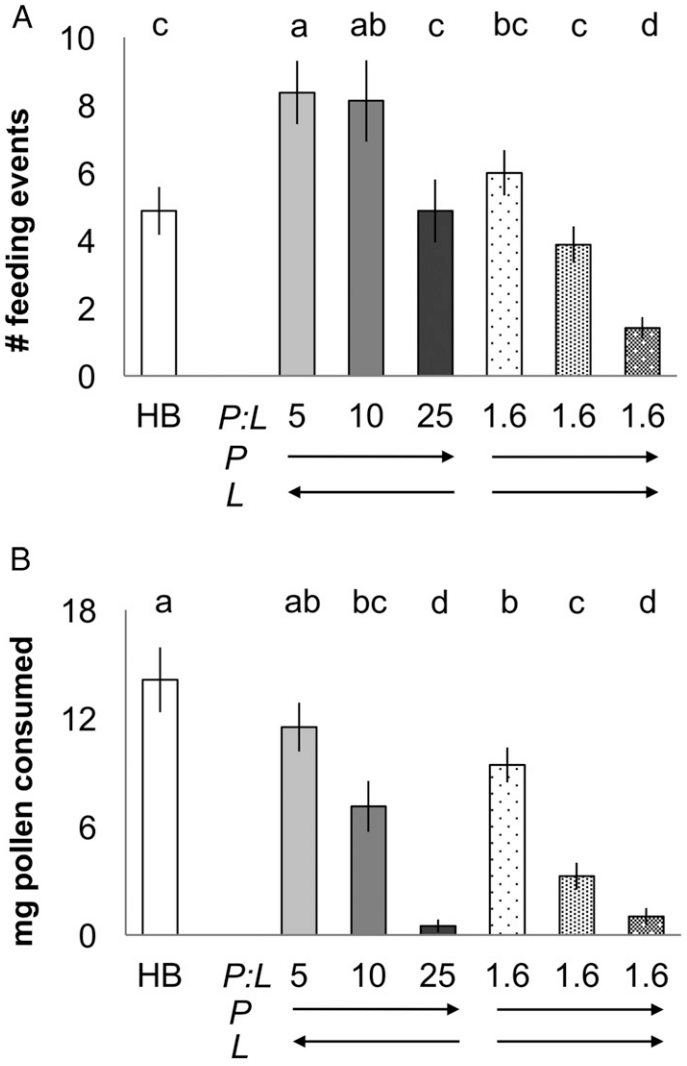
suggesting that bees visit the plant species with the highest P:L ratios first and, once that pollen has been depleted, move on to the plant species with the next highest P:L ratio. Overall, these data indicate that the P:L ratio drives bumble bee preferences for host-plant species. Notably, *S. hebecarpa* (with poricidal anthers) and *Tradescantia ohiensis*, the two most preferred host plant species and those with the highest P:L ratios we tested, were "buzz-pollinated" by the bumble bees (indicating the specialized behavior needed to extract their rewards); these plant species do not produce nectar, suggesting that they may have evolved to produce high-quality pollen as their only reward for pollination.

Isolated Pollen-Feeding Preference Assay. To determine pollen preferences independent of other floral and environmental cues and factors influencing foraging decisions, we hand-collected pollen from six host-plant species (Table 1). Two different pollen species then were presented to caged *B. impatiens* workers in a pairwise choice test (Fig. 2*A*). In each trial, we evaluated caged bees' preferences in all possible combinations of four pollen species. Across the five trials, six possible pollen species were used (it was not possible to compare pairwise combinations across all six pollen species in each trial because of limited amounts of pollen). To integrate the data across all the trials for analysis, we ranked the pollen species within each trial according to P:L ratio (giving the pollen with the highest P:L ratio a rank of 1 and the pollen with the lowest P:L ratio in the trial a rank of 4), allowing us to compare preferences based on relative P:L ratios rather than species of origin.

When the preferences of bees within each cage were evaluated, the pollen with the higher P:L ratio of each paired choice, independent of plant species, was significantly preferred when assessed by the number of feeding events, i.e., attractiveness [in 73% of the cages, $\chi^2_{(2,\, n\, =\, 30)} = 20.03$, $P < 0.01$]) (Fig. S1) and by milligrams of pollen consumed [in 63% of the cages, $\chi^2_{(2,\, n\, =\, 30)} = 13.6$, $P = 0.011$] (Fig. S1).

Across all pairwise combinations and trials, nutritional rank positively influenced the number of feeding events observed, i.e., the attractiveness of the pollen ($F_{3,\, 52} = 5.52$, $P = 0.002$) (Fig. 2 *B* and *C*): The frequency of feeding events decreased as the P:L ratio of the pollen decreased (Fig. 2 *B* and *C*). Nutritional rank also positively influenced the amount of the pollen consumed; the pollen with the highest P:L ratio was eaten the most, and the pollen with the lowest P:L ratio was eaten the least ($F_{3,\, 52} = 19.18$, $P < 0.0001$) (Fig. S2). Thus, in the absence of floral cues, bumble bees consumed pollen more frequently and in larger quantities as the pollen P:L ratio increased, consistent with their preferences observed in our host-plant foraging study. These results are all the more striking because the bees in this experiment were never exposed to these plant species but still made choices based on the P:L ratio of the pollen.

Modified Pollen-Feeding Preference Assay. Using the same experimental design as in the isolated pollen-feeding preference assay (Fig. 2*A*), we presented *B. impatiens* workers with paired choices of nutritionally modified honey bee-collected (HB) pollen with P:L ratios and protein and lipid concentrations similar to or greater than those in our fresh-collected pollen (Fig. 3). HB pollen alone had a P:L ratio of 1.6:1. We found that the P:L ratio of the diet influenced the number of feeding events observed (see Fig. S3 for details of individual pairwise comparisons of diets and Fig. 3 for compiled results across all pairwise comparisons). Diets with P:L ratios of 5:1 and 10:1 were more attractive (i.e., were visited more frequently) than diets with P:L ratios of 1.6:1 (HB pollen) or 25:1 ($F_{6,\, 157} = 9.43$, $P < 0.0001$) (Fig. 3*A*). However, *B. impatiens* consumed more of the pollen with 1.6:1 (HB pollen) and 5:1 P:L ratios than pollen with 10:1 or 25:1 P:L ratios ($F_{6,\, 157} = 24.20$, $P < 0.0001$) (Fig. 3*B*). These data indicate that diets with P:L ratios of 5:1 and 10:1 are the most attractive and that the higher concentration of protein in these diet



Diet #	Protein mg/g	Lipid mg/g	P:L
HB	66.8	41.7	1.6
2	182.6	36.5	5
3	308.8	30.9	10
4	527.7	21.1	25
5	169.5	105.7	1.6
6	251.3	157.1	1.6
7	409.8	256.1	1.6

Fig. 3. Comparison of *B. impatiens* pollen-feeding events (*A*) and consumption (*B*) of nutritionally modified pollen diets (modified pollen-feeding preference assay). All diets were modified from HB pollen (1:6 P:L ratio). Diets were modified either by adding protein, thereby increasing the P:L ratio (diets 2, 3, and 4) or by adding protein and lipid, thereby increasing nutrient concentrations but maintaining a 1:6 P:L ratio (diets 5, 6, and 7). All diets were modified HB pollen with casein (protein) and canola oil (lipid) used to alter concentrations. The *x* axes represent the P:L ratios of the diets. HB, unmodified honey bee-collected pollen (1.6 P:L ratio). Arrows under *x* axes indicate increasing protein (P) and lipid (L) concentrations of modified pollen diets. Data are shown as mean ± SEM. Bars within graphs labeled with different letters are statistically different ($P < 0.05$). Note that the experimental design is that of the isolated pollen-feeding preference assay (Fig. 2*A*) in which, in each trial of the experiment, cages of three bees were presented one of the 21 pairs of seven pollen diets.

leads to reduced consumption. When we held the P:L ratio constant, concentrations of proteins and lipids above those found in HB pollen reduced feeding events and the amount of pollen consumed (Fig. 3), suggesting that simply increased nutrient concentrations can actually be unattractive to bumble bees. Overall, when considering both attraction and feeding, we infer that *B. impatiens* preferred the diet with a P:L ratio of 5:1, confirming the results of our previous experiments.

Because pollen consumption of *B. impatiens* appeared to be influenced significantly by nutrient concentration (Fig. 3*B*), we analyzed the effect of protein and lipid concentration on the amount of each diet consumed. As the absolute protein concentration increased across all diets, the consumption of the diet decreased linearly ($F_{1,\ 166} = 108.17$, $P < 0.0001$, $R^2 = 0.44$) (Fig. 4*A*), suggesting that *B. impatiens* could obtain similar levels of proteins by eating larger amounts of low-concentration diets or small amounts of high-concentration diets. However, lipid concentration had a biexponential effect on the amount of pollen consumed: Bees increased their pollen consumption as lipid increased in diets with low lipid concentrations but ate less in diets with high lipid concentrations ($Y = 1558 \times e^{-26.8x} - 1600 \times e^{-28.0x}$, $R^2 = 0.44$) (Fig. 4*B*). These data indicate that the lipid concentration is responsible for attractiveness and phagostimulation at low concentrations but that increased concentrations could lead to satiation, similar to findings in the protein-concentration data.

Discussion

Our results demonstrate that the macronutrient ratios in pollen are a key factor determining bee foraging behavior. *B. impatiens* discriminated among plant species based on the pollen nutritional quality and in field and laboratory assays exhibited preferences for the species or for the isolated pollen with the highest P:L ratios (~4.6:1) (Figs. 1 and 2). When presented pollen with altered P:L ratios or protein and lipid concentrations (including a 25:1 ratio, which was higher than produced by the plant species in our study) or pollen with the same ratio but increasing protein and lipid concentrations, *B. impatiens* still preferred the diets with the 5:1 and 10:1 P:L ratios, suggesting that *B. impatiens* workers seek to optimize their nutritional intake and do not simply try to maximize the

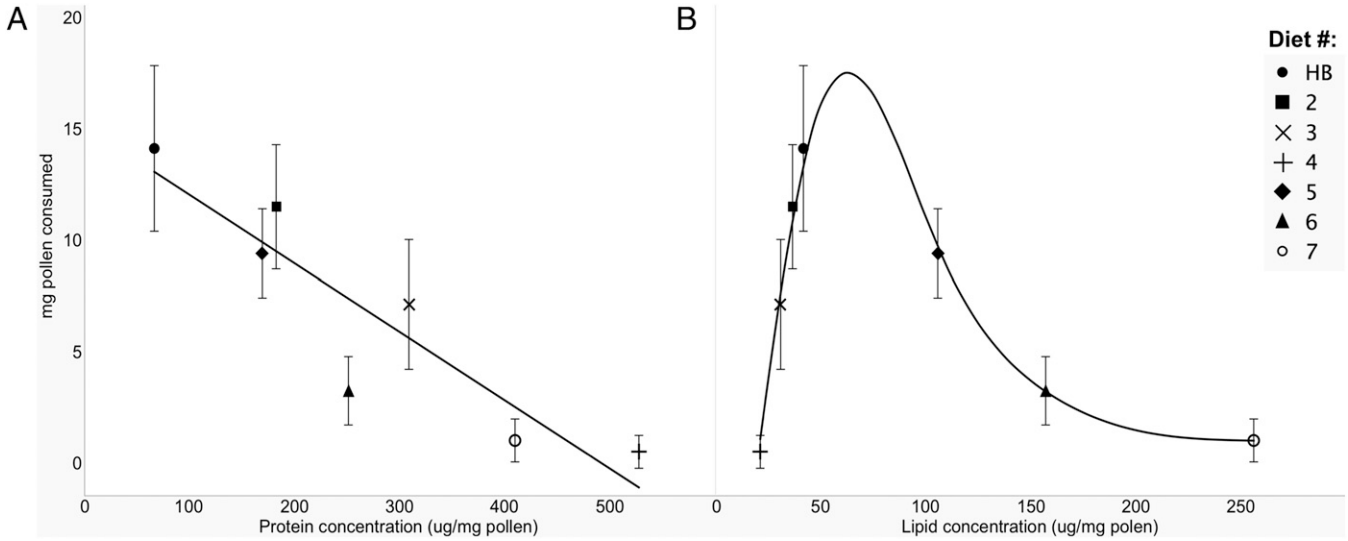


Fig. 4. The relationship between protein (*A*) and lipid (*B*) concentration and the amount of pollen consumed by *B. impatiens* (modified pollen-feeding preference assay). Data are shown as mean and 95% confidence interval. Diets were modified from HB pollen. See Fig. 3 for specific nutrient concentrations. There was a negative linear relationship between protein concentration and pollen consumption ($Y = 15.129607–30.761651 \cdot x$, $R^2 = 0.44$). However, the relationship of lipid concentration to consumption was biexponential, showing increased consumption at low concentrations but decreasing consumption at higher concentrations ($Y = 1558{*}e^{-26.8 \cdot x} - 1600{*}e^{-28.0 \cdot x}$, $R^2 = 0.44$).

amount of protein in their diets. Indeed, increased nutrient concentrations of both proteins and lipids actually led to decreased attraction and consumption of diets overall (Figs. 3 and 4), although lipids appeared to have had a phagostimulatory effect at lower concentrations. Thus, *B. impatiens*, and likely other bumble bee and generalist bee species, appears to have a sophisticated ability to assess pollen nutritional quality and forage selectively to reach nutritional intake targets (6, 41–43).

Making comparisons across plant species, using isolated pollen, and manipulating P:L ratios in pollen allowed us to demonstrate that bumble bee foraging preferences were driven by simultaneous assessments of multiple nutritional components of pollen, specifically the P:L ratio. Among the host-plant species we tested, carbohydrates, proteins, and lipids independently were insufficient to explain bumble bee foraging behavior. Protein and lipid concentrations were significantly associated with foraging rates but also showed a significant interaction. Exploring this interaction further revealed that the ratio of these two components was the major driver of foraging rates. Furthermore, although P:C ratios are commonly associated with nutrient regulation in herbivores (6), these ratios did not correlate with preferences for the pollen of host-plant species. Interestingly, higher protein concentrations in modified diets led to fewer feeding events and reduced pollen consumption. It is important to note that in the previous experiments that found increasing protein concentration increased attraction (27, 28), the protein composition of the pollen was modified by diluting it with cellulose powder, thereby reducing all nutrient concentrations and sensory cues. Furthermore, our studies demonstrate that *B. impatiens* does not simply avoid pollen (or diets) with high lipid content: One of their most preferred plant species, *T. ohiensis*, had relatively high lipid concentrations (Table 1), and they preferentially consumed modified diets with moderate lipid concentrations. Thus, similar to other insects [caterpillars and predators (37, 38, 44)], *B. impatiens* appears to regulate the intake of dietary P:L ratios, which may, at least partially, drive feeding behavior in bees.

Despite the importance of P:L ratios, other components in pollen, such as micronutrients or individual amino or fatty acids, may still influence bee foraging decisions (45–47). Indeed, pollenkitt, which includes free fatty and amino acids that vary among pollen species, is critical for pollen recognition and phagostimulation (34–36, 48, 49). Furthermore, as generalist foragers, bumble bees may avoid or dilute the negative effects of toxic phytochemicals by collecting pollen from multiple host-plant species (50, 51). It is unclear, however, whether micronutrient variation in pollen or secondary plant metabolites can alter bee foraging for macronutrients or whether the concentrations of these compounds are somehow associated with the macronutrient levels we measured. Because we manipulated P:L ratios in our study using the same HB pollen samples, the amounts of micronutrients and other chemicals remained constant (or were reduced in concentration) and thus were likely not a factor in this analysis.

Our results suggest that foraging bumble bees may assess pollen nutritional quality via both pre- and postingestive processes, but additional studies are needed to evaluate fully the proximate mechanisms underlying the preferences we observed. In the field, we observed bees antennating pollen, potentially assessing its quality. Bumble bees appear to determine pollen quality by its protein content through tactile chemoreceptors and show preferences for high-protein pollen (26, 52), whereas honey bees do not appear to share the same preference (25, 29, 30). Although both species may be sensitive to protein quality, the preferences observed in previous studies may reflect species-specific differences in nutritional requirements for protein and lipids. Bees also can discriminate between pollen types and may be able to assess pollen quality via pollenkitt or volatile chemical profiles (34–36, 48, 49). Pollenkitt contains lipids, proteins, and carbohydrates (35), which bees could detect directly before ingesting or collecting pollen. The diverse and large number of odor receptors and glomeruli of bees may allow them to decipher the chemical composition of the pollenkitt (53). Bumble bees may ingest some pollen while foraging (potentially while grooming and packing pollen) or when returning to the hive, and the nutritional quality of the pollen then may influence sensory physiology via postingestive effects (54, 55). Further evidence for postingestive effects comes from our studies using modified pollen diets, in which *B. impatiens* workers most frequently ate pollen with high P:L ratios (5:1 and 10:1) but consumed more of the 1.6:1 and 5:1 diets than the 10:1 and 25:1 diets. Reduced consumption of diets with higher protein and/or lipid concentrations indicates postingestive effects, because eating less food of a higher concentration may result in the same quantity of nutrients ingested. Postingestive

behavioral responses to pollen quality may involve changes in chemo- or gustatory receptor sensitivity based on hemolymph concentration of nutrients after consumption (56). Finally, bumble bees may receive social information about pollen quality and availability when pollen is stored in pollen pots by returning foragers (27, 55). Regardless of whether foragers use pre- or postingestive effects to evaluate pollen nutritional quality, there is ample evidence that bumble bees can learn to identify rewarding flowers after only a handful of foraging bouts, quickly establishing traplines to the highest-quality flowers (57–59). Overall, the most efficient way for the colony to regulate nutritional intake would be for the foragers themselves to assess and compare nutrient ratios among available types of pollen.

Although the nutritional requirements of bees vary throughout their lifetime and differ substantially between developing brood and adult foragers (9, 42), the foraging preferences of *B. impatiens* workers in our study remained consistent in the presence and absence of their mother colony. In our experiment involving the foraging of host-plant pollen, workers collected pollen for their colonies, which had queens and developing larvae. In the caged feeding assay, workers were kept separate from brood, but they still exhibited preferences for the same plant species. Here, they may have had social influence on each other's feeding behavior; however, the results were replicated across 30 cages, and the group preference was clearly consistent. Thus, foraging preferences and the nutritional needs of foragers may be closely matched to the nutritional needs of larvae, although additional studies are needed to examine comprehensively the foraging preferences of colonies with and without brood. Furthermore, although we demonstrated that *B. impatiens* foragers were consistently attracted to their preferred P:L ratio, ∼5–10:1 P:L (Figs. 1, 2 *B* and *C*, and 3*A*), increasing the concentrations of protein and lipids had different effects on feeding behavior (Fig. 4). Additional studies are needed to determine if these ratios are optimal to support bumble bee fitness (6, 42, 43). Indeed, in studies using the geometric framework for nutrition approach (6, 60, 61), bees can regulate their protein and carbohydrate intake (42, 43), and other arthropod species can regulate their protein and lipid intake to support fitness (37–39).

The results of these studies can readily be applied to the management and conservation of pollinator populations. Bumble bees are critical pollinators of many agricultural crops (62). As generalist foragers, covering large geographic ranges, supporting colonies over entire growing seasons, and populations of up to 500 workers, they forage on a wide array of plant species (63–68). Unfortunately, populations of approximately half of the bumble bee species in North America and Europe are declining (69–71). One of the key factors driving this decline (and the decline of other bee species) is loss of habitat and the associated loss of nutritional resources provided by a diversity of flowering plant species (72, 73). In depauperate landscapes, bees likely do not have access to the diversity of host-plant species needed to self-select their diet and balance their nutritional intake adequately. This lack of resources may reduce colony growth, health, and reproduction, negatively influencing long-term bee populations. Once we better understand pollen nutritional values across diverse, commercially available plant species, floral provisioning protocols could address nutritional shortcomings by restoring pollen sources that allow bees to balance macro- and micronutrients and phytochemical pollen components that differ among plant species (1). By selecting plant species that better satisfy the nutritional requirements of pollinators, the effectiveness of these management and conservation schemes can undoubtedly be greatly improved and optimized.

Conclusion

In this study, a generalist pollinator species discriminated among host-plant species according to nutritional quality. Notably, the preference of *B. impatiens* for P:L ratios remained remarkably stable across different conditions: (*i*) foraging among host-plant species, pollen isolated from flower species, and nutritionally modified single-source pollen and (*ii*) foraging in the presence or absence of a colony with developing brood. These results suggest that bees may consistently navigate through a variety of environmental influences to find optimal pollen resources. Furthermore, optimizing the P:L intake may improve the fitness of *B. impatiens*, as is the case in other insect species (6, 37–39), although additional research must examine effects on individual, larval, and colony health and productivity. The species-specific nutritional needs and preferences of bees should be considered when designing protocols and policies for conservation and management of bee populations.

Materials and Methods

Pollen Nutritional Analysis. We collected pollen from 16 individual plants of seven perennial pollinator host-plant species native to Pennsylvania (Table 1). The individual plants were reared in pots outdoors and were used to study bumble bee foraging preferences (40). When not in use for collecting foraging data, the plants were stored in outdoor field cages to exclude any floral visitors and to allow efficient pollen collection. Fresh pollen was collected from the plant species by gently brushing the pollen off the flowers into a glass container. Because *S. hebecarpa* has poricidal anthers, we collected whole anthers into a glass container and vortexed them to release the pollen. All pollen was stored at −20 °C until analysis or use in experiments. Pollen was dried for ∼24 h at 36 °C for analysis. To analyze the protein, lipid, and carbohydrate concentrations of pollen, we divided the pollen into three 1-mg replicates for protein analysis and three 1-mg replicates for lipid and carbohydrate analysis. We analyzed the protein concentration of pollen using the Bradford assay and lipid and carbohydrate concentrations using an assay modified from Van Handel and Day (74). See *SI Pollen Nutritional Analysis* for specific protocols. Pollen concentrations of protein, lipids, or carbohydrate are reported as micrograms of nutrient per milligram of pollen, and subsequent P:C and P:L ratios were determined for each plant species (Table 1).

Assessing Host-Plant Pollen-Foraging Preferences. Using the community visitation rate data we collected in 2013 (40), we correlated pollen-foraging rates of *B. impatiens* to different host-plant species to measures of pollen nutritional quality. In a controlled foraging arena or hoop house (11 × 6.1 × 3.05 m), we confined two *B. impatiens* colonies to perennial host-plant species (three or four individuals of eight species) (Table 1). On 18 separate days over the course of 5 wk, we continuously [from 0930 to 1315 Daylight Saving Time (DST)] recorded the frequency (visits per minute) with which bumble bee foragers collected pollen from each plant species. Note that colonies were allowed to acclimate to flowers and learn pollen-handling techniques 3 d before data collection (75); on each day of data collection, colony entrances were opened at 0930 DST, ensuring that foragers were active only when all species' flowers were presenting pollen and an observer was present. We also measured the number of workers in each colony each week to standardize the data for the bumble bee foraging population. On each day of data collection we measured the area of floral display of flowers presenting pollen of each plant species to standardize the foraging data for the potential influence of relative floral patch size, difference in number of flowers (single vs. composite flowers), and amount of pollen per plant species on foraging behavior. Therefore, the community visitation rate is presented as a single metric: the number of visits·min^{-1}·cm^{-2} floral area per 100 bees. Two species that were used in the study, *Monarda fistulosa* and *Pycnanthemum tenuifolium*, were predominately nectar rewarding, were almost never visited for pollen collection by the bumble bees (40), and did not produce enough pollen for nutritional analysis; therefore these species were excluded. For a full, detailed discussion of the methodology, please see Vaudo et al. (40).

Because the nutritional components of pollen are not independent of one another, we conducted a multiple regression analysis of nutrient concentration (protein, lipid, carbohydrate, the interaction of protein and carbohydrate, and the interaction of protein and lipid) and visitation rate. We followed with regression analysis to determine if the pollen P:C ratio [considered an essential nutritional ratio in arthropod herbivore foraging behavior and nutrient regulation (6)] influences the visitation rate. The interaction of protein and lipid was significant; therefore we conducted nonlinear regression of the P:L ratio of pollen and average visitation rates to each plant species. We also performed the analysis with log-transformed P:L ratios, which did not influence the results; therefore we reported actual P:L values.

Isolated Pollen-Feeding Preference Assay. We confined *B. impatiens* workers to cages to assess their preferences for pollen collected from different plant species in the absence of other floral cues. We purchased four research colonies of

B. impatiens from Koppert Biological Systems. Each colony contained ~100 workers and the natal queen. Three *B. impatiens* workers from the same mother colony were placed in 6.6 × 8.3 × 9.5 cm Plexiglas cages. Pollen was presented to bees in small plastic thimbles; 0.02 g of pollen was placed in each thimble, and four drops of water were added to prevent the pollen from being spilled by the bees. The bees were kept in a dark room at ~30 °C and ~30% humidity.

In each trial, we used four pollen species. We presented each cage of three bees with a choice between two host-plant pollen species and monitored six cages per trial so that each trial comprised all pairwise combinations of the four pollen species (Fig. 2*A*). Because we had limited amounts of fresh-collected pollen from six plant species, we were able to test only four pollen species in each trial for a total of five trials. In each trial, we assigned each of the four pollen species a nutritional rank based on P:L ratios, with the highest ratio considered "1" and the lowest "4" (nutritional rank was based on the host-plant foraging preferences assessed earlier, in which the highest P:L ratio was most attractive). We used this nutritional rank for our statistical analyses. Thus, we tested if the bees could differentiate and choose between pollen types based on nutritional value, not simply between species. The nutritional ranks and plant species used were 1, *S. hebecarpa* (trials 1–5); 2, *T. ohiensis* (trials 1–5); 3, *Eutrochium purpureum* (trials 1and 2) and *Echinacea purpurea* (trials 3–5); and 4, *Eupatorium perfoliatum* (trials 1 and 2), *E. purpureum* (trials 3 and 4), and *Symphyotrichum novae-angliae* (trial 5).

We used two behavioral metrics to assess the bees' pollen preferences. We continuously monitored the bees for 3 h and counted the number of times the bees fed from each of the pollen species with their proboscis or mandibles, (feeding events). Second, we measured the amount of each pollen species consumed (pollen consumed), by determining the difference between the starting (0.02 g) and end weight (weight after drying) of each pollen sample. To determine if bumble bees consistently chose the pollen with the higher P:L ratio in each paired choice, independent of plant species, we assigned each cage/replicate of the study ($n = 30$ cages) a category of "win," "tie," or "loss" based on whether the pollen with the higher P:L ratio received more feeding events or was consumed in greater quantity. We used a χ^2 test to analyze if the frequency with which the pollen with the higher P:L ratio received more feeding events or was consumed in greater quantity (i.e., won) was greater than random choice (Fig. S1). We also conducted independent *t* tests to compare average feeding events and pollen consumed for each paired choice between nutritional ranks (i.e., in each cage) (Fig. 2*B* and Fig. S2*A*). Finally, to determine relative preferences between nutritional ranks for both feeding events and pollen consumed, we used ANOVA with pollen nutritional rank 1–4 as the independent variable and trial as the blocking variable. Post hoc pairwise analyses were used to determine differences between individual pollen nutritional ranks.

Modified Pollen-Feeding Preference Assay. In the modified pollen-feeding preference experiment, we manipulated homogenized HB multifloral pollen (obtained from Brushy Mountain Bee Farm) to a range of different protein and lipid concentrations and P:L ratios. Because honey bees collect pollen from multiple floral sources, and the pollen was trapped as corbiculate pollen balls, we first lightly ground ~20 g of the pollen with a mortar and pestle to break the balls apart, sifted it through a strainer, and then stirred it to create a homogenous mix. We repeatedly tested the protein and lipid concentrations of the pollen mix (see *Pollen Nutritional Analysis* above and *SI Pollen Nutritional Analysis*), averaging a P:L ratio of 1.6:1 (Fig. 3, diet HB).

We then added purified casein from bovine milk (Sigma-Aldrich) as a protein source to create diets with P:L ratios of 5:1, 10:1, and 25:1 (Fig. 3, diets 2–4), which are above the range of P:L ratios of fresh pollen we observed in the previous experiments (Table 1). These ratios were used to test the findings that bumble bees prefer pollen with higher P:L ratios. We then added the same protein amounts to three more diets, also adding canola oil (which contains bees' essential omega-3 and omega-6 fatty acids) to maintain the same 1.6 P:L ratio as the HB pollen (Fig. 3, diets 5–7). These diets were used to test the hypothesis that bumble bees prefer higher total nutrient concentrations.

We then followed the experimental protocol of the isolated pollen-feeding assay, providing caged bees all 21 paired choices of the seven different diets and recording feeding events and pollen consumed over four trials (see Fig. 2*A* and Fig. S3 for the design). We provided 0.03 g of each diet plus four drops of water in the feeding thimbles and repeated the assay four times with four different *B. impatiens* colonies. We analyzed the response variables "feeding events" and "pollen consumed" between all diets with ANCOVA using colony/trial and the mean weight of the three bees in each cage as a covariate (to control for intercage variation; larger bees tend to eat more) and post hoc pairwise analyses to determine differences between individual diets. Because the nutrient concentration appeared to affect bumble bee consumption of each diet significantly, we used regression analyses of protein and lipid concentrations to determine the influence of absolute nutrient concentration on pollen consumed. All data were analyzed with JMP Pro-12.1.0 software (SAS Institute 2015).

ACKNOWLEDGMENTS. We thank the J.F.T. and C.M.G. laboratories for helpful discussions and critical insight in the preparation of this manuscript; and Heike Betz, Bekki Waskovich, Edwin Hochstedt, Victoria Bolden, and Liam Farrell for assistance in experiment preparation and data collection. This work was supported by a North American Pollinator Protection Campaign Bee Health Improvement Project Grant (to A.D.V.); USDA Agriculture and Food Research Initiative (AFRI) National Institute of Food and Agriculture (NIFA) Grant 2009-05207 (to C.M.G.); USDA AFRI NIFA Predoctoral Fellowships Grant 2014-02219 (to A.D.V.); and generous funding from an anonymous donation to the Center for Pollinator Research at Pennsylvania State University.

1. Vaudo AD, Tooker JF, Grozinger CM, Patch HM (2015) Bee nutrition and floral resource restoration. *Curr Opin Insect Sci* 10:133–141.
2. Willmer P (2011) *Pollination and Floral Ecology* (Princeton Univ Press, Princeton, NJ).
3. Brunet J, Thairu MW, Henss JM, Link RI, Kluever JA (2015) The effects of flower, floral display, and reward sizes on bumblebee foraging behavior when pollen is the reward and plants are dichogamous. *Int J Plant Sci* 176(9):811–819.
4. Ollerton J, Erenler H, Edwards M, Crockett R (2014) Pollinator declines. Extinctions of aculeate pollinators in Britain and the role of large-scale agricultural changes. *Science* 346(6215):1360–1362.
5. Goulson D, Nicholls E, Botías C, Rotheray EL (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 347(6229):1255957.
6. Behmer ST (2009) Insect herbivore nutrient regulation. *Annu Rev Entomol* 54: 165–187.
7. Behmer ST, Joern A (2008) Coexisting generalist herbivores occupy unique nutritional feeding niches. *Proc Natl Acad Sci USA* 105(6):1977–1982.
8. Dussutour A, Simpson SJ (2009) Communal nutrition in ants. *Curr Biol* 19(9):740–744.
9. Brodschneider R, Crailsheim K (2010) Nutrition and health in honey bees. *Apidologie (Celle)* 41(3):278–294.
10. Roulston TH, Cane JH (2000) Pollen nutritional content and digestibility for animals. *Plant Syst Evol* 222(1):187–209.
11. Haydak MH (1970) Honey bee nutrition. *Annu Rev Entomol* 15(1):143–156.
12. Génissel A, Aupinel P, Bressac C, Tasei JN, Chevrier C (2002) Influence of pollen origin on performance of *Bombus terrestris* micro-colonies. *Entomol Exp Appl* 104(2-3): 329–336.
13. Human H, Nicolson SW, Strauss K, Pirk CWW, Dietemann V (2007) Influence of pollen quality on ovarian development in honeybee workers (*Apis mellifera scutellata*). *J Insect Physiol* 53(7):649–655.
14. Tasei J-N, Aupinel P (2008) Nutritive value of 15 single pollens and pollen mixes tested on larvae produced by bumblebee workers (*Bombus terrestris*, Hymenoptera: Apidae). *Apidologie (Celle)* 39(4):397–409.
15. Li C, Xu B, Wang Y, Feng Q, Yang W (2012) Effects of dietary crude protein levels on development, antioxidant status, and total midgut protease activity of honey bee (*Apis mellifera ligustica*). *Apidologie (Celle)* 43(5):576–586.
16. Alaux C, Ducloz F, Crauser D, Le Conte Y (2010) Diet effects on honeybee immunocompetence. *Biol Lett* 6(4):562–565.
17. LeBlanc BW, Davis OK, Boue S, DeLucca A, Deeby T (2009) Antioxidant activity of Sonoran Desert bee pollen. *Food Chem* 115(4):1299–1305.
18. Roulston TH, Cane JH, Buchmann SL (2000) What governs protein content of pollen: Pollinator preferences, pollen-pistil interactions, or phylogeny? *Ecol Monogr* 70(4): 617–643.
19. Di Pasquale G, et al. (2013) Influence of pollen nutrition on honey bee health: Do pollen quality and diversity matter? *PLoS One* 8(8):e72016.
20. Cardoza YJ, Harris GK, Grozinger CM (2012) Effects of soil quality enhancement on pollinator-plant interactions. *Psyche* 2012:581458.
21. Vanderplanck M, et al. (2014) How does pollen chemistry impact development and feeding behaviour of polylectic bees? *PLoS One* 9(1):e86209.
22. Moerman R, et al. (2016) Growth rate of bumblebee larvae is related to pollen amino acids. *J Econ Entomol* 109(1):25–30.
23. Canavoso LE, Jouni ZE, Karnas KJ, Pennington JE, Wells MA (2001) Fat metabolism in insects. *Annu Rev Nutr* 21:23–46.
24. Arien Y, Dag A, Zarchin S, Masci T, Shafir S (2015) Omega-3 deficiency impairs honey bee learning. *Proc Natl Acad Sci USA* 112(51):15761–15766.
25. Leonhardt SD, Blüthgen N (2011) The same, but different: Pollen foraging in honeybee and bumblebee colonies. *Apidologie (Celle)* 43(4):449–464.
26. Ruedenauer FA, Spaethe J, Leonhardt SD (2015) How to know which food is good for you: Bumblebees use taste to discriminate between different concentrations of food differing in nutrient content. *J Exp Biol* 218(Pt 14):2233–2240.
27. Kitaoka TK, Nieh JC (2008) Bumble bee pollen foraging regulation: Role of pollen quality, storage levels, and odor. *Behav Ecol Sociobiol* 63(4):501–510.
28. Konzmann S, Lunau K (2014) Divergent rules for pollen and nectar foraging bumblebees–a laboratory study with artificial flowers offering diluted nectar substitute and pollen surrogate. *PLoS One* 9(3):e91900.
29. van der Moezel PG, Delfs JC, Pate JS, Loneragan WA, Bell DT (1987) Pollen selection by honeybees in shrublands of the Northern Sandplains of Western Australia. *J Apic Res* 26(4):224–232.

30. Pernal S, Currie R (2001) The influence of pollen quality on foraging behavior in honeybees (*Apis mellifera* L.). *Behav Ecol Sociobiol* 51(1):53–68.
31. Hanley ME, Franco M, Pichon S, Darvill B, Goulson D (2008) Breeding system, pollinator choice and variation in pollen quality in British herbaceous plants. *Funct Ecol* 22(4): 592–598.
32. Somme L, et al. (2014) Pollen and nectar quality drive the major and minor floral choices of bumble bees. *Apidologie (Celle)* 46(1):92–106.
33. Dobson HEM (1987) Role of flower and pollen aromas in host-plant recognition by solitary bees. *Oecologia* 72(4):618–623.
34. Dobson HEM, Bergström G (2000) The ecology and evolution of pollen odors. *Plant Syst Evol* 222(1-4):63–87.
35. Pacini E, Hesse M (2005) Pollenkitt—Its composition, forms and functions. *Flora* 200(5):399–415.
36. Piskorski R, Kroder S, Dorn S (2011) Can pollen headspace volatiles and pollenkitt lipids serve as reliable chemical cues for bee pollinators? *Chem Biodivers* 8(4):577–586.
37. Mayntz D, Raubenheimer D, Salomon M, Toft S, Simpson SJ (2005) Nutrient-specific foraging in invertebrate predators. *Science* 307(5706):111–113.
38. Raubenheimer D, Mayntz D, Simpson SJ, Tøft S (2007) Nutrient-specific compensation following diapause in a predator: Implications for intraguild predation. *Ecology* 88(10):2598–2608.
39. Jensen K, Mayntz D, Toft S, Raubenheimer D, Simpson SJ (2011) Nutrient regulation in a predator, the wolf spider *Pardosa prativaga*. *Anim Behav* 81(5):993–999.
40. Vaudo AD, Patch HM, Mortensen DA, Grozinger CM, Tooker JF (2014) Bumble bees exhibit daily behavioral patterns in pollen foraging. *Arthropod-Plant Interact* 8(4): 273–283.
41. Altaye SZ, Pirk CWW, Crewe RM, Nicolson SW (2010) Convergence of carbohydrate-biased intake targets in caged worker honeybees fed different protein sources. *J Exp Biol* 213(Pt 19):3311–3318.
42. Paoli PP, et al. (2014) Nutritional balance of essential amino acids and carbohydrates of the adult worker honeybee depends on age. *Amino Acids* 46(6):1449–1458.
43. Stabler D, Paoli PP, Nicolson SW, Wright GA (2015) Nutrient balancing of the adult worker bumblebee (*Bombus terrestris*) depends on the dietary source of essential amino acids. *J Exp Biol* 218(Pt 5):793–802.
44. Stockhoff BA (1993) Ontogenetic change in dietary selection for protein and lipid by gypsy moth larvae. *J Insect Physiol* 39(8):677–686.
45. Cook SM, Awmack CS, Murray DA, Williams IH (2003) Are honey bees' foraging preferences affected by pollen amino acid composition? *Ecol Entomol* 28(5):622–627.
46. Weiner CN, Hilpert A, Werner M, Linsenmair KE, Blüthgen N (2010) Pollen amino acids and flower specialisation in solitary bees. *Apidologie (Celle)* 41(4):476–487.
47. Hendriksma HP, Shafir S (2016) Honey bee foragers balance colony nutritional deficiencies. *Behav Ecol Sociobiol* 70(4):509–517.
48. Linander N, Hempel de Ibarra N, Laska M (2012) Olfactory detectability of L-amino acids in the European honeybee (*Apis mellifera*). *Chem Senses* 37(7):631–638.
49. Cook SM, et al. (2005) Could learning of pollen odours by honey bees (*Apis mellifera*) play a role in their foraging behaviour? *Physiol Entomol* 30(2):164–174.
50. Eckhardt M, Haider M, Dorn S, Müller A (2014) Pollen mixing in pollen generalist solitary bees: A possible strategy to complement or mitigate unfavourable pollen properties? *J Anim Ecol* 83(3):588–597.
51. Müller A, Kuhlmann M (2008) Pollen hosts of western palaearctic bees of the genus *Colletes* (Hymenoptera: Colletidae): The Asteraceae paradox. *Biol J Linn Soc Lond* 95(4):719–733.
52. Ruedenauer FA, Spaethe J, Leonhardt SD (May 13, 2016) Hungry for quality—Individual bumblebees forage flexibly to collect high-quality pollen. *Behav Ecol Sociobiol*, 10.1007/s00265-016-2129-8.
53. Robertson HM, Wanner KW (2006) The chemoreceptor superfamily in the honey bee, *Apis mellifera*: Expansion of the odorant, but not gustatory, receptor family. *Genome Res* 16(11):1395–1403.
54. Simpson SJ, Simpson CL (1992) Mechanisms controlling modulation by haemolymph amino acids of gustatory responsiveness in the locust. *J Exp Biol* 168(1):269–287.
55. Dornhaus A, Chittka L (2005) Bumble bees (*Bombus terrestris*) store both food and information in honeypots. *Behav Ecol* 16(3):661–666.
56. Abisgold JD, Simpson SJ (1988) The effect of dietary protein levels and haemolymph composition on the sensitivity of the maxillary palp chemoreceptors of locusts. *J Exp Biol* 135(1):215–229.
57. Lihoreau M, et al. (2013) Unravelling the mechanisms of trapline foraging in bees. *Commun Integr Biol* 6(1):e22701.
58. Ohashi K, Thomson JD (2009) Trapline foraging by pollinators: Its ontogeny, economics and possible consequences for plants. *Ann Bot (Lond)* 103(9):1365–1378.
59. Raine NE, Chittka L (2012) No trade-off between learning speed and associative flexibility in bumblebees: A reversal learning test with multiple colonies. *PLoS One* 7(9):e45096.
60. Simpson SJ, Raubenheimer D (1993) A multi-level analysis of feeding behaviour: The geometry of nutritional decisions. *Philos T R Soc B* 342(1302):381–402.
61. Raubenheimer D, Simpson SJ (1999) Integrating nutrition: A geometrical approach. *Entomol Exp Appl* 91(1):67–82.
62. Velthuis HHW, van Doorn A (2006) A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie (Celle)* 37(4):421–451.
63. Robertson C (1928) *Flowers and Insects* (Science Press, Lancaster, PA).
64. Szabo TI, Pengelly DH (1973) The over-wintering and emergence of *Bombus* (*Pyrobombus*) *impatiens* (Cresson) (Hymenoptera: Apidae) in southern Ontario. *Insectes Soc* 20(2): 125–132.
65. Cnaani J, Schmid-Hempel R, Schmidt JO (2002) Colony development, larval development and worker reproduction in *Bombus impatiens* Cresson. *Insectes Soc* 49(2): 164–170.
66. Rasmont P, Coppee A, Michez D, De Meulemeester T (2008) An overview of the *Bombus terrestris* (L. 1758) subspecies (Hymenoptera: Apidae). *Ann Soc Entomol Fr* 44(2):243–250.
67. Lozier JD, Strange JP, Stewart IJ, Cameron SA (2011) Patterns of range-wide genetic variation in six North American bumble bee (Apidae: *Bombus*) species. *Mol Ecol* 20(23):4870–4888.
68. Amsalem E, Grozinger CM, Padilla M, Hefetz A (2015) The physiological and genomic bases of bumble bee social behaviour. *Advances in Insect Physiology*, eds Zayed A, Kent CF (Academic, New York), Vol 48, pp 37–93.
69. Goulson D, Lye GC, Darvill B (2008) Decline and conservation of bumble bees. *Annu Rev Entomol* 53(1):191–208.
70. Cameron SA, et al. (2011) Patterns of widespread decline in North American bumble bees. *Proc Natl Acad Sci USA* 108(2):662–667.
71. Potts S, et al. (2015) *Status and Trends of European Pollinators: Key Findings of the STEP Project* (Pensoft Publishers, Sofia, Bulgaria), pp 1–72.
72. Carvell C, et al. (2006) Declines in forage availability for bumblebees at a national scale. *Biol Conserv* 132(4):481–489.
73. Potts SG, et al. (2010) Global pollinator declines: Trends, impacts and drivers. *Trends Ecol Evol* 25(6):345–353.
74. Van Handel E, Day JF (1988) Assay of lipids, glycogen and sugars in individual mosquitoes: Correlations with wing length in field-collected *Aedes vexans*. *J Am Mosq Control Assoc* 4(4):549–550.
75. Raine NE, Chittka L (2007) Pollen foraging: Learning a complex motor skill by bumblebees (*Bombus terrestris*). *Naturwissenschaften* 94(6):459–464.

Supporting Information

Vaudo et al. 10.1073/pnas.1606101113

SI Pollen Nutritional Analysis

We analyzed the protein concentration of pollen using the Bradford assay. To prepare the samples for analysis, we dried the pollen for 24 h at 36 °C. Then we divided the pollen into three 1-mg replications for each individual plant species in 1.5-mL Eppendorf microcentrifuge tubes (Eppendorf North America). To facilitate breaking of the pollen wall, three drops of 0.1-M NaOH were added to each sample, and samples then were ground with a microcentrifuge pestle. After the sample was ground, 0.1-M NaOH was added to a total volume of 1.5 mL, and the sample was then vortexed. All samples were allowed to sit for 24 h and were centrifuged at 2,000 × *g* for 30 seconds to precipitate all debris/solids. We conducted the Bradford assay with the Bio-Rad Protein Assay Kit microassay 300-μL microplate protocol using bovine γ-globulin as the protein standard (Bio-Rad Laboratories). Because of the high protein concentration of the pollen, we diluted 50 μL of each replicate into 100 μL 0.1-M NaOH in each well of a BD Falcon 300-μL sterile non-tissue culture–treated 96-well plate (BD). Absorbance readings at 595 nm were measured using a SpectraMax 190 spectrophotometer (Molecular Devices), and protein concentrations were calculated using simple linear regression analysis from the protein standards using SoftMax Pro v.4.0 software (Molecular Devices).

Pollen lipid and carbohydrate concentrations were determined using a protocol modified from Van Handel and Day (74). To prepare the samples for analysis, we divided dried pollen into three 1-g replications for each individual plant species in 1.5-mL Eppendorf microcentrifuge tubes. We added 0.2 mL 2% (wt/vol) sodium sulfate to each tube and homogenized the samples with a microcentrifuge pestle. We washed each sample into a glass tube with 1.6 mL chloroform/methanol (1:1 vol:vol) and centrifuged the samples at 2,175 × *g* for 5 min, separating all solids, including the indigestible pollen exines and intines (including cellulose), from the lipid and sugar extract. We transferred the supernatant to a clean glass tube, added 600 μL deionized water, and centrifuged the sample at 2,175 × *g* for 5 min. We separated the top carbohydrate/water/methanol fraction for sugar analysis and used the remaining chloroform fraction for lipid analysis. For carbohydrate analysis, we heated each sample at 100 °C to evaporate the solvent to ∼100 μL. We added anthrone/sulfuric acid reagent to equal 5 mL and heated the samples at 100 °C for 17 min. Each sample was removed from the heat and allowed to cool. We used two technical replications for each biological replication and measured absorbance at 625 nm using a SpectraMax 190 spectrophotometer (Molecular Devices). Carbohydrate concentrations were calculated using simple linear regression analysis from anhydrous glucose standards using SoftMax Pro v.4.0 software (Molecular Devices). The lipid/chloroform fraction was heated at 100 °C to evaporate the solvent. We added 0.2 mL sulfuric acid to the sample, heated the sample at 100 °C for 10 min, added vanillin/phosphoric acid reagent to equal 5 mL, removed the sample from the heat, and allowed it to cool. We used two technical replications for each biological replication and measured absorbance at 525 nm using a SpectraMax 190 spectrophotometer. Lipid concentrations were calculated using simple linear regression analysis from soybean-based vegetable oil (Crisco; The J. M. Smucker Company) standards using SoftMax Pro v.4.0 software. Pollen concentrations of protein, carbohydrate, or lipids is reported as micrograms of nutrient per milligram of pollen, and subsequent P:C and P:L ratios were determined for each plant species (Table 1). All nutritional values were within the range expected for pollen (10).

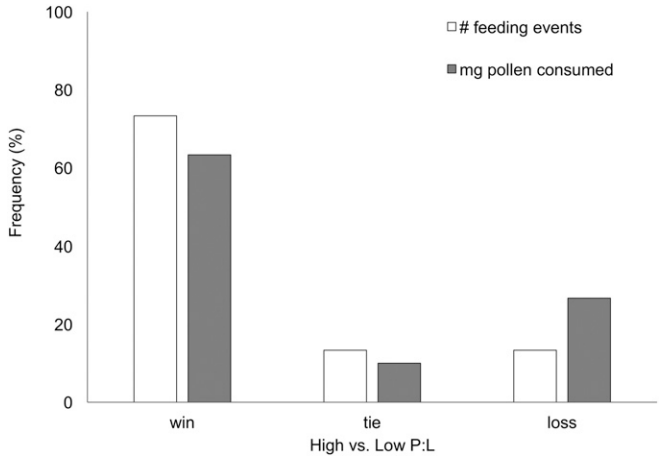


Fig. S1. In the isolated pollen-feeding assay, the frequency with which the pollen with the higher P:L ratio was preferentially selected by the bees relative to the pollen with the lower P:L pollen. For each cage ($n = 30$ paired comparisons), we assessed whether the pollen with the higher P:L ratio was preferred (as assessed by the number of feeding events or amounts consumed) ("won") relative to the pollen with the lower P:L ratio, whether the two pollens were equally preferred ("tied"), or whether the pollen with the higher P:L ratio was less preferred ("lost") than the pollen with the lower ratio. The pollen with the higher P:L ratio in each paired choice, independent of plant species, was preferred more frequently when assessed by both the number of feeding events: that is, attractiveness (73%, $\chi^2_{(2,\ n\ =\ 30)} = 20.03$, $P < 0.01$) and milligrams of pollen consumed (63%, $\chi^2_{(2,\ n\ =\ 30)} = 13.6$, $P = 0.011$).

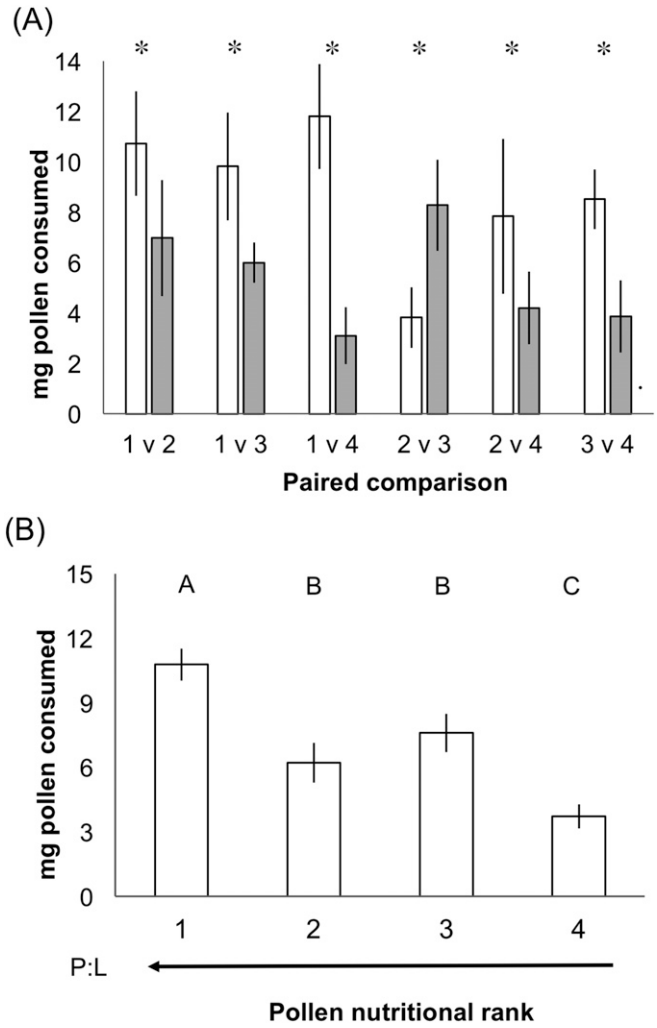


Fig. S2. *B. impatiens* consumption of pollen species based on nutritional rank in the isolated pollen-feeding preference assay. (*A*) Results of pairwise choice tests according to nutritional rank. Asterisks indicate significant differences within each pair ($P < 0.05$). (*B*) Average pollen consumed independent of paired comparison across trials. In each trial of the experiment, separate cages of three bees were presented with one of the six possible pairs of four pollen types. Pollen nutritional rank represents pollen species ranked 1–4 (from highest to lowest) by P:L ratio. Data are shown as mean ± SEM. Bars labeled with different letters are statistically different ($P < 0.05$). The arrow under the *x* axis indicates increasing P:L ratio. The nutritional ranks and plant species used were 1, *S. hebecarpa* (trials 1–5); 2, *T. ohiensis* (trials 1–5); 3, *E. purpureum* (trials 1 and 2) and *E. purpurea* (trials 3–5); and 4, *E. perfoliatum* (trials 1 and 2), *E. purpureum* (trials 3 and 4), and *S. novae-angliae* (trial 5).

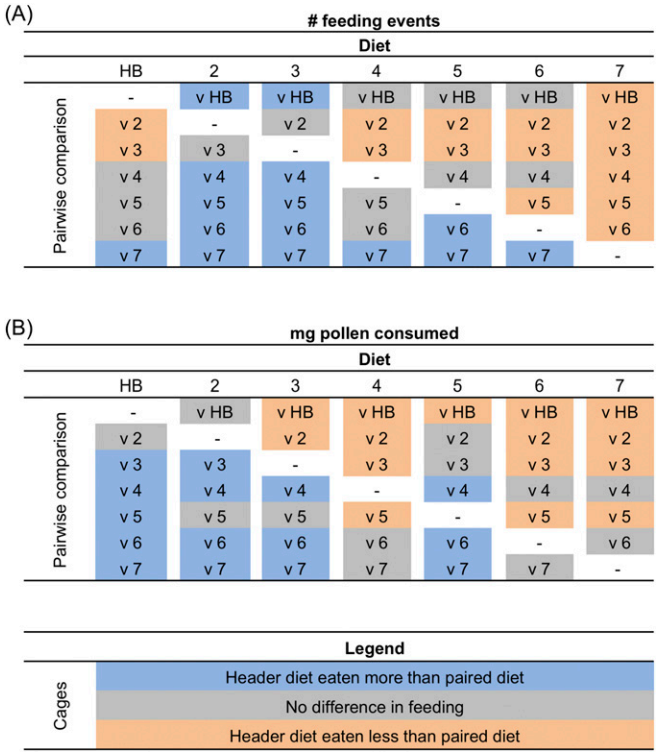


Fig. S3. Pairwise comparisons of feeding events (*A*) and consumption (*B*) of all pollen diet combinations in the modified pollen-feeding preference assay. Color coding indicates whether *B. impatiens* workers ate the diet in the column heading significantly more (blue), similarly (gray), or less (orange) than the diet type named in the cell ($P < 0.05$). Cells indicate the comparison of the header diet versus the paired diet. Diets are identified in *Materials and Methods*, *Modified Pollen-Feeding Preference Assay*, and in Fig. 3.