

Bumble bees selectively use native and exotic species to maintain nutritional intake across highly variable and invaded local floral resource pools

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Abstract. 1. Changes to plant community composition after invasion are well documented but how these shifts directly affect higher trophic levels is still poorly understood. One potentially important factor is the change in nutritional availability after an invasion. Shifts in nutrient availability could affect the nutrient intake of organisms that live in invaded habitats, causing reduced fecundity and survival.

2. The effects of the interaction among nutrient availability, selection, and diet on nutrient intake of a native bumble bee were examined. No nutritional differences were found between exotic and native pollen or collected and non-collected pollen in protein or amino acid content, suggesting that differences in nutrient intake from random are based on selection.

3. Nutrient intake was simulated when pollen was selected randomly across all available plant species and when selection was restricted to native plants only or exotic plants only using a permutation model and compared with observed collection. The results suggest that pollen collection is non-random and that selecting only native or exotic plants cannot provide the protein or amino acid intake observed.

4. These results may help to explain why the responses of native bees to exotic plants are so variable. If the exotic plants in a community can supply the necessary nutrients, bees may readily incorporate them into their diets, but if not, exotic plants may be avoided.

Key words. Amino acid, exotic plant, invasive plant, pollinators, protein.

Introduction

Exotic species can homogenise plant communities by competitively displacing native plants (Hejda *et al.*, 2009) and have been linked to changes in community composition of some animal species (Fiedler *et al.*, 2012). Although losses in plant diversity appear to contribute to declines in animal diversity (Grundel *et al.*, 2010; Borer *et al.*, 2012; Dinnage *et al.*, 2012), individual species responses to plant loss are highly variable, with some species and groups declining with decreasing plant diversity and others having improved or neutral responses to changes in plant diversity in disturbed areas (Rode *et al.*, 2006;

Johnstone *et al.*, 2010; Holmquist *et al.*, 2013). The variability in animal responses to losses in plant diversity makes it difficult to understand and predict the effects of species losses on higher trophic levels.

One potentially important factor that has received little attention in animal declines is how losses in plant diversity affect the nutritional intake of organisms at the next trophic level. Changes in nutrition can decrease fecundity and survival rates (Bishop *et al.*, 2009) while also increasing the risk of life-threatening infections (Plowright *et al.*, 2008), all of which can cause significant demographic shifts in individual animal species. Micronutrient deficiency has also been linked to changes in community composition of arthropods in grasslands (Joern *et al.*, 2012), which further suggests that nutrition might play an important role in explaining patterns of survival and diversity in disturbed habitats. There has been little work exploring how exotic species might alter nutritional availability of plant communities or how exotics might be affecting the diet of animal species. Nutritional

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intake of animals is a product of both nutritional availability (i.e. the amount of resources available, such as proteins, carbohydrates, and micronutrients) and diet (i.e. the amount of each item consumed), both of which could be highly impacted by the spread of exotic species.

Understanding how nutritional availability and diet are affected by the spread of exotic species is particularly important for species that are functionally important for maintaining plant reproduction and diversity, such as pollinators (Ashman *et al.*, 2004). In general, exotic plants were found to both increase and decrease bee abundance or diversity in different systems (Stout & Morales, 2009), but few studies have examined the direct role of non-native plants on native pollinator diet (but see Tepedino *et al.*, 2008; Williams *et al.*, 2011). Bees are known to require adequate pollen nutrition for full larval development (Brodschneider & Craillshheim, 2010) and pollen of poor quality can affect the size, maturation, survival and development of hypopharyngeal glands in honey bees and thus diets with inadequate nutrition can have significant demographic consequences (Herbert *et al.*, 1970; Peng & Jay, 1976; Cane & Roulston, 2002; Di Pasquale *et al.*, 2013). However, bees as a whole do not consistently forage on the richest pollen sources for proteins or amino acid, but instead collect uniquely to meet the nutritional needs of each species (Roulston *et al.*, 2000; Weiner *et al.*, 2010), which could suggest that the spread of exotic species and losses of plant diversity may differentially affect species' ability to obtain necessary nutrients. By contrast, bees are also known to be highly selective of pollen resources, which might limit the nutritional effects of exotic species if they are simply not selected, or selected to provide a given component of the diet.

This is the first work to include plant species that are available but not collected, a factor that has limited inference in previous studies by providing a biased understanding of how diet and nutrient availability interact to affect nutrient uptake. Plant species that are available but not collected offer valuable information on how bees form preferences and how changes in availability will affect nutrition. Using a species of native bumble bee (*Bombus vosnesenskii*), we examine how diet and nutrition are affected by the presence of exotic plant species. Bumble bees have previously been observed collecting pollens containing higher protein and amino acids than those collected by honey bees when foraging within the same environment, which may make them especially selective for plants with high nutrient availability (Leonhardt & Blüthgen, 2012). First, we determine whether the nutrient availability differs when plants are grouped based on whether they are native and exotic species or species that are collected or not. These groupings provide general insight into whether nutrient availability of plant species differs predictably based on whether they are native or collected. Next, we estimate the nutrient intake for bees if chosen randomly from available plant species using a permutation procedure and compare it with observed nutrient intake. Observed pollen collection is assumed to be representative of an acceptable nutrient level, given that bumble bees are generalist foragers and strong fliers and are thus able to select and acquire acceptable nutrients even in harsh environments. Lastly, we repeat the permutation procedure to determine how nutrient intake might change if bees only selected from the

set of native or exotic plant species. Exotic plant species were previously found to have a variety of pollination systems, from self-pollination to obligate dependence on animal pollinators (Harmon-Threatt *et al.*, 2009), and are thus not expected to vary from native species in nutritional rewards. Due to the importance of adequate pollen nutrition for larval development, bees were expected to selectively collect plant species of higher protein content and to limit variability of nutritional intake by collecting both native and exotic species non-randomly.

Materials and methods

Site description

During 2009, five grassland sites of 1 ha each were chosen in Briones East Bay Regional Park and Mount Diablo State Park in Contra Costa County, California. All sites were >1 km apart to limit overlap in bees foraging across multiple sites. Each of the five sites was visited bi-weekly from mid-May to late July (herein referred to as 'site-dates') for a total of five rounds of sampling to check for presence of the bumble bee, *Bombus vosnesenskii* Radoszkowskii. If bees were found, bees, plants and pollen were sampled as detailed in the following section. This resulted in 14 site-dates across the five sites and five sampling rounds. *Bombus vosnesenskii* has a wide distribution and was previously observed collecting from a large diversity of plant species (Thorp *et al.*, 1983), thus making it a good candidate for examining how dietary preferences and nutrient composition interact to affect nutritional intake.

Bee, flower, and pollen sampling

Sampling was conducted between 08.00 and 13.00 hours, the peak of activity in this system, on a single day during the sample period. To limit differences between sites due to temporal turnover, all sites were sampled within 3 days during a sampling period. Bumble bee visits to flowers were observed along 500-m transect walks through the site to determine which plant species were visited by *B. vosnesenskii* and whether pollen and/or nectar were collected during floral visits. Pollen or nectar collection was determined based on whether probing or grooming behaviors were observed. Approximately 20 bees were captured and released in each site after removing a single pollen load for pollen collection analysis. Removal of the pollen load also helped to prevent recapture of the same individual. Each pollen load was homogenised and subsampled, and 300 pollen grains were identified to plant species by comparison with a reference collection of available pollens.

Site-level floral diversity was determined using a block quadrat sampling method in which the number of flowers blooming in each site was counted in 50 m² quadrats placed evenly across a site. These data were used to determine the relative floral abundance of each plant species. Plant species that occurred within the site but outside the quadrats were also recorded and assigned an abundance of 0.5. None of these species were found in significant abundance in pollen loads. After floral and bee sampling was completed, flowers of all blooming species were

collected from field sites and placed in water in the laboratory. After at least 24 h, each flower was vibrated with a 512 Hz tuning fork (Kearns & Inouye, 1993) to collect pollen into Petri dishes. After collection, Petri dishes were placed in a drying oven for at least 48 h at 40 °C. Samples were stored in a –20 °C freezer for subsequent processing. Storage at –20 °C was previously found to preserve pollen adequately for later protein analysis, even after long-term storage (>12 years) (Roulston *et al.*, 2000). During 2010, flowers for which insufficient pollen was collected for nutritional analysis in 2009 (see following section) were re-collected in the same sites previously sampled. Four plant species whose abundances were too low in either year to adequately collect pollen for nutritional analysis were excluded from abundance and statistical analysis.

Pollen quality

Dried pollen samples were hand-cleaned with forceps under a microscope to remove all plant and insect debris. Percentage carbon and nitrogen content of pollen was determined by combusting approximately five samples of each plant species weighing between 1 and 5 mg in a Carla Erba Elantech elemental analyzer (CE Instruments, Lakewood, New Jersey). Acetiniilide was used as a standard and was run every 10 samples to ensure the calibration of the machine. Previous work on the combustion of pollen found that the percentage of nitrogen is highly correlated with protein measured using other analysis techniques and it is therefore considered an appropriate method to estimate the amount of protein available in pollens (Roulston *et al.*, 2000). Additionally, percentage nitrogen in pollen has low variability within a species and is not likely to change from year to year, making it a reliable metric for multi-year collections.

Pollen for each plant species was also analysed for composition of 19 of the 20 amino acids found in proteins (at the UC Davis Molecular Structure Facility). Tryptophan could not be reliably quantified due to high levels of sugar in the pollen samples and was removed from the analysis. Samples were first processed using a sodium citrate-based hydrolysis analyser (Hitachi L-8800 analyzer, Tokyo, Japan). A second analysis was used to determine cysteine and methionine using performic acid, because these amino acids are destroyed in the hydrolysis process. To ensure that amino acid composition was not influenced by collection year, pollen samples (one each) of four plant species collected in 2009 and 2010 were compared. No significant differences were found between the eight samples collected in different years when compared using the multiple-response permutation procedure (MRPP – described in the following section) grouped by year and paired by species.

Analysis

To assess whether the protein content differed between collected pollens and non-collected pollens and between exotic and native plant species, *t*-tests were conducted on the mean percentage nitrogen found using combustion analysis for each plant species. A species was considered collected if the relative abundance of pollens collected was greater than 5% at

any point during the sampling period. The 5% cutoff point was based on observation data from a previous study in which plant species that were only visited for nectar consistently accounted for less than 5% of the total abundance of pollen (A. N. Harmon-Threatt, unpublished). The MRPP was used to compare factors that contain multiple non-independent measures (McCune & Grace, 2002) such as amino acid composition. MRPP is a non-parametric method to test for differences among two or more predetermined groups. Using a pairwise Euclidean distance matrix of the entire dataset, MRPP calculates mean within-group distance (δ) for the observed groups and then permutes group membership and pairwise distance and recalculates δ for each permutation. Significance of the observed δ is determined by the percentage of permuted δ values that are less than the observed. MRPP is considered a superior test to MANOVA for community ecological data (McCune & Grace, 2002). The effect size of MRPP is described by the chance-corrected within-group agreement (*A*). This analysis was first performed on all 18 amino acids and then separately on just the essential amino acids identified by DeGroot (1954) for honeybee larval development and previously used for bumble bee pollen analysis (Hanley *et al.*, 2008). We expected variation in the essential amino acids to have a greater effect on bees and to be more detectable than variation across all amino acids. Only results for essential amino acids are reported here as they were not found to differ from all amino acids. All MRPP analyses were conducted in R 2.15.1 with the VEGAN package. Previous work by Weiner *et al.* (2010) found that nutrient content is conserved within families; however, a phylogenetically constrained analysis is not possible when sampling is restricted only to plants available to a single bee species, as done here.

Observed and estimated nutrient intake

To determine the amount of protein and amino acid collected by the bees from a site, the relative abundance of each plant species found in an individual pollen load *i* at site *j* was multiplied by the known protein or amino acid (see Table 1) content for that plant species and summed across all plant species (eqn 1). This provides reliable estimates of the protein and amino acid collected from a site based on the amount of pollen collected and the total nutrition available within those plant pollens.

$$\begin{aligned} \text{nutrient collected}_j &= \sum_{i=1}^n \text{relative abundance of pollen collected}_i \\ & * \text{measured protein or amino acid}_i \end{aligned} \quad (1)$$

A permutation method was used to estimate the possible nutrient collection from a site if pollen was chosen by bees randomly from a site. To simulate random collection, observed values of pollen collection were selected randomly from the full dataset. The randomly selected values were then associated with available plant species from a site-date and rescaled to ensure the relative abundances of pollen collected summed

Table 1. Nutrient availability for each plant species.

Origin	% nitrogen	Threonine	Valine	Methionine	Isoleucine	Phenylalanine	Histidine	Lysine	Arginine
Native									
<i>Brodiaea elegans</i>	5.91	3.45	4.13	1.56	3.16	2.36	1.27	4.59	2.48
<i>Clarkia purpurea</i>	3.10	1.85	2.10	0.71	1.67	1.34	0.88	2.31	1.28
<i>Eriogonum</i> spp.	3.49	1.52	1.67	0.59	1.33	1.11	0.87	2.22	1.01
<i>Eschscholzia californica</i>	6.20	3.70	4.27	1.55	3.40	2.70	1.55	4.89	2.54
<i>Holocarpha heermannii</i>	4.46	1.97	2.00	0.73	1.77	1.44	1.88	3.35	1.27
<i>Lupinus bicolor</i>	7.16	4.70	5.65	1.40	4.47	3.58	1.91	6.50	3.62
<i>Lupinus microcarpus</i>	7.95	4.46	5.49	1.83	4.37	3.35	2.02	6.35	3.34
<i>Mimulus guttatus</i>	5.77	3.69	4.42	1.58	3.39	2.63	1.80	4.83	2.73
Exotic									
<i>Brassica nigra</i>	6.45	3.37	3.43	1.25	2.66	2.08	1.26	4.95	2.30
<i>Carduus pycnocephalus</i>	3.92	2.00	2.20	0.84	1.66	1.33	1.34	3.26	1.29
<i>Centaurea solistitalis</i>	5.30	2.98	3.25	1.31	2.46	2.00	1.91	4.20	1.82
<i>Silybum marianum</i>	4.67	2.36	2.39	0.95	1.82	1.45	1.70	3.29	1.45
<i>Trifolium oliganthum</i>	5.72	3.24	3.83	1.28	3.02	2.36	1.28	4.28	2.34
<i>Vicia villosa</i>	6.11	3.22	3.99	1.11	3.05	2.57	1.35	4.51	2.48

to 1. For each permutation, eqn 1 was used to determine the amount of nutrient collected when the relative pollen abundances changed. This was permuted 999 times to produce a robust estimate of the mean nutrient collected from a site. The observed value of nutrient collected at that site-date was added as the 1000th value to ensure it was represented in the dataset. A paired Wilcoxon test was then used to compare the simulated protein values and the observed protein values. MRPP was used to determine whether the mean amino acid content differed between groups.

To determine if the nutrient collection differs when bee selection is restricted to either native or exotic species, the procedure was repeated, but the species available for selection were limited to only native or exotic species blooming at a site-date. This simulates how nutrient intake is affected if pollen availability or preference changes, to limit the simultaneous use of both types of resources. For example, if bees opt to use only native species after an environment is invaded or if exotic species become so dominant that they are the only species available and are incorporated into diets, either of these could change nutrient intake.

Results

A total of 232 bees were captured throughout the experiment. Bees for which more than 5% of the pollen counted was from plants not occurring at the site were removed, leaving 217 pollen loads for analysis. Only one plant species (*Eschscholzia californica*) was available across all sites throughout the duration of sampling, but it had a wide range in percentage collected (4–99%; see Table 2). Mean floral richness across sites was 5 ± 1.36 species with a range between three and seven species blooming on a given site-date. Sixty-one percent of all individual bees caught carried more than one pollen species and almost 70% of individuals carried pollen from an exotic plant species. This supports the fact that *B. vosnesenskii* is a generalist forager and uses exotic plants in its diet. However, within a site-date, bees collected most of their pollen from native plant species

Table 2. Range of pollen available and collected for each plant species.

Plant species	Plant family	% collected (range)	% available (range)
Native			
<i>Brodiaea elegans</i>	Asparagaceae	0	0.5–1.0
<i>Clarkia purpurea</i>	Onagraceae	0	0.3–3.2
<i>Eriogonum</i> spp.	Polygonaceae	20.9*	0.1–0.5
<i>Eschscholzia californica</i>	Papaveraceae	22.9–99.8	0.1–47.2
<i>Holocarpha heermannii</i>	Asteraceae	5.4*	22.4
<i>Lupinus bicolor</i>	Fabaceae	0	0.3–49.6
<i>Lupinus microcarpus</i>	Fabaceae	0–43.9	0.6–8.0
<i>Mimulus guttatus</i>	Phrymaceae	0–24.2	2.0–8.5
Exotic			
<i>Brassica nigra</i>	Brassicaceae	0–0.1	3.3–72.4
<i>Centaurea solistitalis</i>	Asteraceae	0–3.3	0.3–68.3
<i>Carduus pycnocephalus</i>	Asteraceae	2.6–49.9	0.0–74.8
<i>Silybum marianum</i>	Asteraceae	3.3*	0.6
<i>Trifolium oliganthum</i>	Fabaceae	4.2–14.0	0.1–84.6
<i>Vicia villosa</i>	Fabaceae	0–88.2	0.0–75.1

*represents the plants only found in one site-date.

and only had more exotic than native pollen in three of the 15 cases (see Fig. 1).

Pollen of native plant species did not differ from exotic pollens with regard to protein or amino acid composition [$t_{(12)} = -0.1694$, $p = 0.868$ and $A = -0.035$, $p = 0.682$, respectively; Fig. 2a], suggesting that exotic plant species are not consistently different in terms of nutrition from native plants. Similarly, pollens of collected and non-collected species did not differ in amino acid or protein content [$t_{(12)} = -0.959$, $p = 0.357$ and $A = 0.014$, $p = 0.308$, respectively; see Fig. 2b). Despite there being no difference in nutrient content based on origin or collection, simulated random protein intake was significantly less than observed protein collection across site-dates ($W_{15} = 115$, $p = 0.000$; see Fig. 3a). The simulated random acid intake was also significantly different from the observed amino acid composition for collected pollen (MRPP, $p = 0.015$,

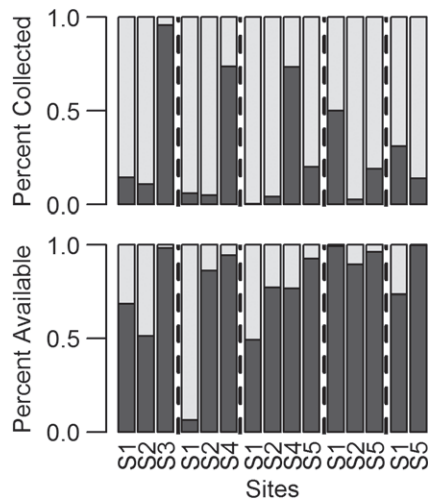


Fig. 1. Proportion of native and exotic pollens collected in pollen loads and available at a site during a sampling period. Native is shown in light grey and exotic in dark grey. Dotted lines separate sampling periods.

$A_{14} = 0.098$). Together, these results suggest that, despite plants not having different nutritional components, they are selected non-randomly by bumble bees, which causes differences in nutritional intake.

Only the nine site-dates with more than one available native plant species were used to estimate protein intake if bees restricted selection to native species alone. The estimated native-only protein intake was not significantly different from the observed protein intake when paired by site-dates ($W_9 = 50$, $p = 0.5995$; see Fig. 3b). The same simulated dataset found significant differences in essential amino acid intake between estimated native-only collection and observed values (MRPP, $A_8 = 0.104$, $p = 0.015$). This suggests that the random collection by bumble bees could yield similar protein intake but not essential amino acid composition.

Fourteen site-dates with multiple exotic plant species were used to estimate how selection of only exotic plant species would affect nutrient intake. Estimates of protein intake when only exotic species were selected were significantly lower

than observed values of protein intake ($W_{14} = 116$, $p = 0.000$; see Fig. 3c). However, essential amino acid intake did not differ between mean values and observed values when models only used exotic species to determine the mean ($A = 0.0875$, $p = 0.185$). Similar to native-only selection, this suggests that exotic plants can provide one component of nutrient intake, essential amino acid content, but not both essential amino acid content and protein. Therefore, native and exotic pollens are both necessary to provide the observed nutrient intake for bumble bees, which cannot be achieved with only native or exotic plants.

Discussion

Changes in nutritional intake are known to affect survival and development, but little work has examined whether nutrient availability or nutrient intake by organisms is altered following a disturbance, such as invasion. The spread of exotic species could significantly alter nutrient availability and potentially nutrient intake if they vary significantly from native species in nutrient content and are incorporated into diets. However, our results found no observed difference between native and exotic species with regard to protein or amino acid content, which suggests that if exotics were incorporated into diets, individuals would not differ significantly in nutrient intake. Despite this lack of difference in nutrient content, bumble bees still collected far fewer exotic than native pollens and seemed to prefer native plant species in most cases. Interestingly, no differences were observed in nutrient content between collected and non-collected species. This result counters previous work on bumble bees, which found a preference for high-protein plant species under controlled foraging conditions (Genissel *et al.*, 2002). Thus, our findings highlight the importance of field-based studies on collection and sampling of the entire plant community in understanding foraging preferences and nutrition. Furthermore, these results suggest that factors such as floral morphology (Heinrich, 1976), nectar quality (Somme *et al.*, 2015) and memory (Toda *et al.*, 2009), among many others, which were previously found to be important for selection, likely work in conjunction with pollen nutrition to influence

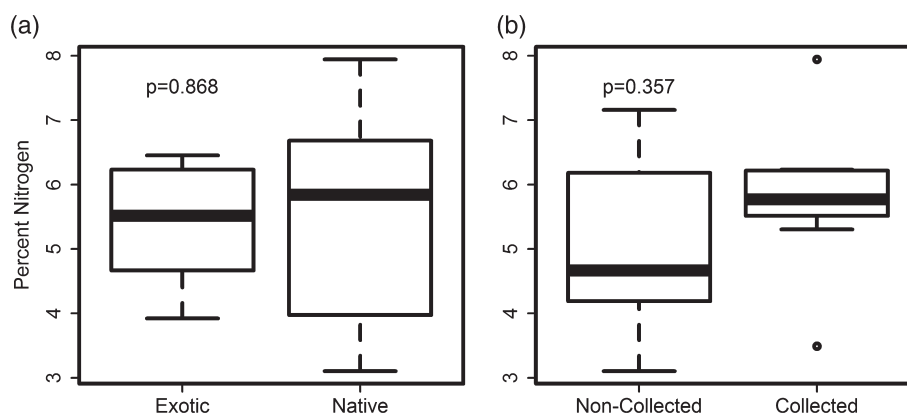


Fig. 2. Boxplot of percentage nitrogen when compared between: (a) native and exotic plant species; and (b) collected and non-collected species. The box represents the first and third quartiles, while the whiskers include the 95% CI. Any points falling outside the 95% interval are represented as points.

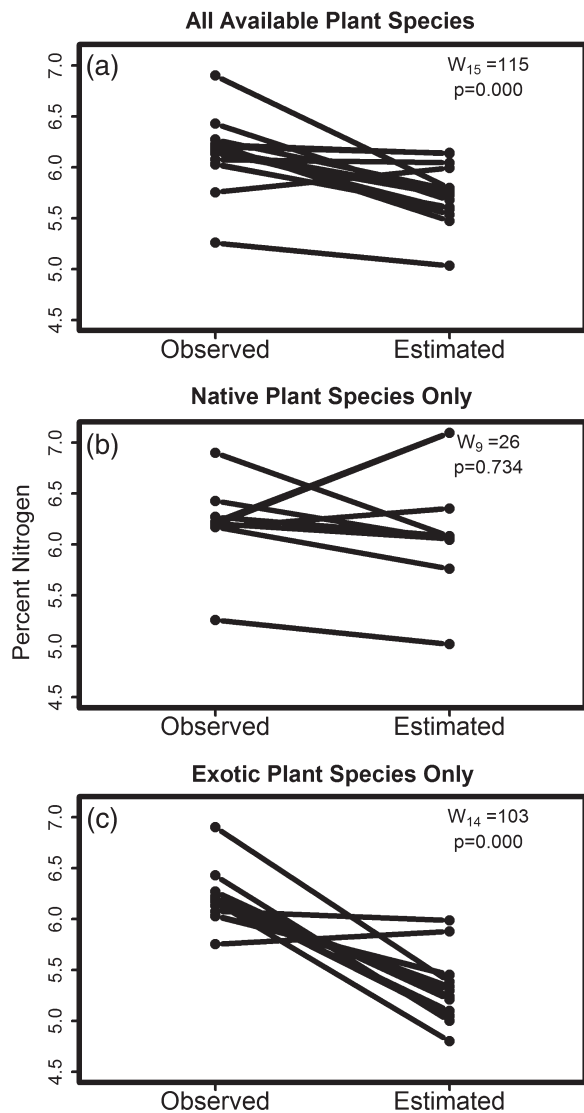


Fig. 3. Paired comparison of collected and estimated nutrients for each site-date. (a) All available plant species; (b) exotic plant species only; (c) native plant species only.

bumble bee selection. One potential limitation of the analyses is a lack of comparisons that control for phylogeny due to low species richness in the communities observed. Previous work on pollen nutrition found that protein and amino acid content are phylogenetically conserved (Weiner *et al.*, 2010), which may explain why nutrient content did not differ when species were grouped based on origin or collection.

Despite finding no significant differences in nutrient availability between collected and non-collected species, nutrient intake was significantly different for both protein and amino acids when compared to a simulated random collection. Although this was expected, this result shows the importance of non-random selection for controlling nutrient intake of larvae as nutrient availability changes. Non-random selection has also been observed for nectar collection in honey bees to maintain

consistent C:N ratios despite different protein consumptions (Altaye *et al.*, 2010). The importance of non-random selection is further emphasised by the significant differences between observed amino acid content and simulated amino acid intake for selection of only native plants, and between observed protein content and simulated protein intake for selection of only exotic plants. While previous work has found that bumble bees and honey bees seek high-quality sources with high amino acid content (Cook *et al.*, 2003; Somme *et al.*, 2015), little work has attempted to understand how changes in community might affect selection, nutrient intake, and availability. Neither the observed amino acid nor protein intake is possible if selection is limited to only native or exotic plant species, suggesting that both native and exotics are fully incorporated into the bees' diets and are both necessary to provide the nutrient intake observed. Assuming that the observed nutrient intake represents the necessary intake for survival to maturity, the selection of exotic and native species allows this species of bumble bee to persist in the highly invaded landscape. Non-random selection has been observed in many insect species that appear to be highly selective to maintain necessary nutrient intake, as changes in nutrients can have significant effects on life span, fecundity, and growth (Lee *et al.*, 2008; Harrison *et al.*, 2014). These results suggest that the foraging choices of *B. vosnesenskii* are not arbitrary and that generalised foraging allows this species to maintain similar nutritional intake across local floral communities that change extensively across space or time. Notably, the difference in amino acid content between observed and simulated collection was driven by the amino acid histidine, which was more abundant in exotic plant species than in native species. If histidine was previously limited, the addition of exotic species might help to increase the availability of this essential amino acid. However, it seems more likely that the observed level of histidine is higher than the historic level and the differences in amino acids are driven by bees incorporating more of the exotic species in their diets. Historic data on pollen collection, which can be obtained from museum samples (Scheper *et al.*, 2014), would help to determine the effect of exotic plants on nutritional intake. Future studies on the development of bees with increased levels of histidine or other amino acids could help to illuminate how changes in diet by exotic species affect survival.

As generalist foragers, bumble bees are expected to be able to identify high-quality resources, as their typically broad ranges would expose them to extremely variable plant communities. While we did not examine oligolectic or specialist bees, it is likely that these groups would be more affected by the spread of exotic species and that the variability in responses of bees to exotic species is caused by how the invader affects nutritional availability. Consequently, our findings should not be generalised to suggest that exotic species do not negatively affect bees. In fact, four species of specialist bees failed to reach maturity when reared on non-host pollens (Praz *et al.*, 2008), suggesting that some groups could be strongly affected by losses in plant diversity and unable to adapt to an invaded environment. Additionally, the communities sampled during this study maintained some mixture of native and exotic plants, but in areas that are more significantly homogenised by exotic species, the effects, even on generalist species, might be more

severe. Plant diversity is known to be critical to pollinator health and resilience to infections, which may be at risk as exotic species spread through communities (Di Pasquale *et al.*, 2013). Future studies on exotic plant species should consider whether the invaders are able to play a similar role to existing native species within consumer diets, as well as the specific impacts of the reduction in diversity on health. This information could also be used to develop a predictive framework for how bee communities will be affected along an invasion frontier.

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References

- Altaye, S.Z., Pirk, C.W.W., Crewe, R.M. & Nicolson, S.W. (2010) Convergence of carbohydrate-biased intake targets in caged worker honeybees fed different protein sources. *The Journal of Experimental Biology*, **213**, 3311–3318.
- Ashman, T.L., Knight, T.M., Steets, J.A., Amarasekare, P., Burd, M., Campbell, D.R. *et al.* (2004) Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology*, **85**, 2408–2421.
- Bishop, C.J., White, G.C., Freddy, D.J., Watkins, B.E. & Stephenson, T.R. (2009) Effect of enhanced nutrition on mule deer population rate of change. *Wildlife Monographs*, **172**, 1–28.
- Borer, E.T., Seabloom, E.W., Tilman, D. & Novotny, V. (2012) Plant diversity controls arthropod biomass and temporal stability. *Ecology Letters*, **15**, 1457–1464.
- Brodtschneider, R. & Craillshheim, K. (2010) Nutrition and health in honey bees. *Apidologie*, **41**, 278–294.
- Cane, J.H. & Roulston, T. (2002) The effect of pollen protein concentration on body size in the sweat bee *Lasioglossum zephyrum* (Hymenoptera: Apiformes). *Evolutionary Ecology*, **16**, 49–65.
- Cook, S.M., Awmack, C.S., Murray, D.A. & Williams, I.H. (2003) Are honey bees' foraging preferences affected by pollen amino acid composition? *Ecological Entomology*, **28**, 622–627.
- DeGroot, A. (1954) Qualitative amino acid requirements of the honeybee. *Acta Physiologica et Pharmacologica Neerlandica*, **3**, 433–434.
- Dinnage, R., Cadotte, M.W., Haddad, N.M., Crutsinger, G.M., Tilman, D. & Hooper, D. (2012) Diversity of plant evolutionary lineages promotes arthropod diversity. *Ecology Letters*, **15**, 1308–1317.
- Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L.P., Decourtye, A., Kretzschmar, A. *et al.* (2013) Influence of pollen nutrition on honey bee health: do pollen quality and diversity matter? *PLoS ONE*, **8**, e72016.
- Fiedler, A.K., Landis, D.A. & Arduser, M. (2012) Rapid shift in pollinator communities following invasive species removal. *Restoration Ecology*, **20**, 593–602.
- Genissel, A., Aupinel, P., Bressac, C., Tasei, J. & Chevrier, C. (2002) Influence of pollen origin on performance of *Bombus terrestris* micro-colonies. *Entomologia Experimentalis et Applicata*, **104**, 329–336.
- Grundel, R., Jean, R.P., Frohnapple, K.J., Glowacki, G.A., Scott, P.E. & Pavlovic, N.B. (2010) Floral and nesting resources, habitat structure, and fire influence bee distribution across an open-forest gradient. *Ecological Applications*, **20**, 1678–1692.
- Hanley, M.E., Franco, M., Pinchon, S., Darvill, B. & Goulson, D. (2008) Breeding system, pollinator choice and variation in pollen quality in British herbaceous plants. *Functional Ecology*, **22**, 592–598.
- Harmon-Threatt, A.N., Burns, J.H., Shemyakina, L.A. & Knight, T.M. (2009) Breeding system and pollination ecology of introduced plants compared to their native relatives. *American Journal of Botany*, **96**, 1544–1550.
- Harrison, S.J., Raubenheimer, D., Simpson, S.J., Godin, J.-G.J. & Bertram, S.M. (2014) Towards a synthesis of frameworks in nutritional ecology: interacting effects of protein, carbohydrate and phosphorus on field cricket fitness. *Proceedings Biological Sciences/The Royal Society*, **281**, 20140539.
- Heinrich, B. (1976) Resource partitioning among some eusocial insects: bumblebees. *Ecology*, **57**, 874–889.
- Hejda, M., Pyšek, P. & Jarošík, V. (2009) Impact of invasive plants on the species richness, diversity and composition of invaded communities. *Journal of Ecology*, **97**, 393–403.
- Herbert, E., Bickley, W. & Shimanuk, H. (1970) Brood-rearing capability of caged honeybees fed dandelion and mixed pollen diets. *Journal of Economic Entomology*, **63**, 215–218.
- Holmquist, J.G., Schmidt-Gengenbach, J. & Haultain, S.A. (2013) Effects of a long-term disturbance on arthropods and vegetation in subalpine wetlands: manifestations of pack stock grazing in early versus mid-season. *PLoS ONE*, **8**, e54109.
- Joern, A., Provin, T. & Behmer, S.T. (2012) Not just the usual suspects: insect herbivore populations and communities are associated with multiple plant nutrients. *Ecology*, **93**, 1002–1015.
- Johnstone, C.P., Reina, R.D. & Lill, A. (2010) Impact of anthropogenic habitat fragmentation on population health in a small, carnivorous marsupial. *Journal of Mammalogy*, **91**, 1332–1341.
- Kearns, C.A. & Inouye, D.W. (1993) *Techniques for Pollination Biologists*, 1st edn. University Press of Colorado, Niwot, Colorado.
- Lee, K.P., Simpson, S.J., Clissold, F.J., Brooks, R., Ballard, J.W.O., Taylor, P.W. *et al.* (2008) Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 2498–2503.
- Leonhardt, S.D. & Blüthgen, N. (2012) The same, but different: pollen foraging in honeybee and bumblebee colonies. *Apidologie*, **43**, 449–464.
- McCune, B. & Grace, J.B. (2002) *Analysis of Ecological Communities*. MjM Software Design, Gleneden Beach, Oregon.
- Peng, Y. & Jay, S.C.S. (1976) Effect of diet on queen rearing by caged worker honeybees. *Canadian Journal of Zoology*, **54**, 1156–1160.
- Plowright, R.K., Field, H.E., Smith, C., Divljan, A., Palmer, C., Tabor, G. *et al.* (2008) Reproduction and nutritional stress are risk factors for Hendra virus infection in little red flying foxes (*Pteropus scapulatus*). *Proceedings Biological Sciences/The Royal Society*, **275**, 861–869.
- Praz, C.J., Müller, A. & Dorn, S. (2008) Specialized bees fail to develop on non-host pollen: do plants chemically protect their pollen? *Ecology*, **89**, 795–804.
- Rode, K.D., Chapman, C.A., McDowell, L.R., Stickler, C. & Box, P.O. (2006) Correlates of population density across habitats and logging intensities nutritional in redtail monkeys (*Cercopithecus ascanius*) I. *Biotropica*, **38**, 625–634.

- Roulston, T., Cane, J.H. & Buchmann, S.L. (2000) What governs protein content of pollen: pollinator preferences, pollen-pistil interactions, or phylogeny? *Ecological Monographs*, **70**, 617–643.
- Scheper, J., Reemer, M., van Kats, R., Ozinga, W.A., van der Linden, G.T.J., Schaminée, J.H.J. *et al.* (2014) Museum specimens reveal loss of pollen host plants as key factor driving wild bee decline in The Netherlands. *Proceedings of the National Academy of Sciences*, **111**, 201412973.
- Somme, L., Vanderplanck, M., Michez, D., Lombaerde, I., Moerman, R., Wathelet, B. *et al.* (2015) Pollen and nectar quality drive the major and minor floral choices of bumble bees. *Apidologie*, **46**, 92–106.
- Stout, J.C. & Morales, C.L. (2009) Ecological impacts of invasive alien species on bees. *Apidologie*, **40**, 388–409.
- Tepedino, V., Bradley, B. & Griswold, T.L. (2008) Might flowers of invasive plants increase native bee carrying capacity? Intimations from Capitol Reef National Park, Utah. *Natural Areas Journal*, **28**, 44–50.
- Thorp, R.W., Horning, D.S. & Dunning, L.L. (1983) *Bumble Bees and Cuckoo Bumble Bees of California*, 1st edn. University of California Press, Berkeley, California.
- Toda, N.R.T., Song, J. & Nieh, J.C. (2009) Bumblebees exhibit the memory spacing effect. *Naturwissenschaften*, **96**, 1185–1191.
- Weiner, C.N., Hilpert, A., Werner, M., Linsenmair, K.E. & Bluthgen, N.B. (2010) Pollen amino acids and flower specialisation in solitary bees. *Apidologie*, **41**, 476–487.
- Williams, N.M., Cariveau, D., Winfree, R. & Kremen, C. (2011) Bees in disturbed habitats use, but do not prefer, alien plants. *Basic and Applied Ecology*, **12**, 332–341.

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