



Nutritional profiling of common eastern North American pollen species with implications for bee diet and pollinator health

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Abstract – Urbanization, agriculture, and climate change have known negative impacts on global bee populations, but malnutrition as a result of disrupted or altered floral resources also hinders bee survivorship. Hence, understanding the nutritional content of pollen—the main source of protein, minerals, and lipids required by bees—is paramount to capture the requirements of a balanced diet to support bee health. Here, we characterize the non-esterified fatty acid (NEFA) and amino acid (AA) content of 22 pollen species from 11 families commonly found in eastern North America, to assess their nutritional profile and importance to bee diet. We found that total NEFA content was highly variable across pollen families and genera, but essential fatty acids omega-6 and omega-3 were the most abundant across the majority of pollen species. Total AA content was also diverse across pollen families and genera, and the presence of ten essential amino acids was detected in all pollen species (except for methionine in *Rhus glabra*). P:L ratios ranged broadly from 2.9 in *Trifolium* sp. to 74.4 in *Prunus* sp., but omega-6:3 ratios were generally below one for the majority (73%) of studied pollen species. Phylogenetic comparisons detected a significant negative correlation between essential AA against total NEFA and total omega content, suggesting potential trade-offs with lipid and essential AA in pollen nutrition. Our findings suggest that multiple pollen species have the potential to be considered a valuable source of protein and lipid, and that a diversity of flora is essential to meet diverse bee diet and nutritional needs.

pollen nutrition / amino acids / fatty acids / P:L ratio / omega-6:3 / bee nutrition / pollinator diet / bee health

1. INTRODUCTION

Herbivorous animals rely on plants to meet their nutritional needs, often as a mutualistic relationship; a prominent example is plant-pollinator interactions. Plants use various non-nutritional characteristics such as floral features, scent, and color to attract various insect pollinators that in turn provide crucial pollination services via the transfer of pollen to plant conspecifics, improving plant fitness and aiding in plant reproduction (van der Kooi et al. 2019,

2020). As a reward, insect pollinators feed on flower nectar which provide the main source of sugars and carbohydrates, and pollen as the main source of protein, amino acids, lipids, vitamins, and other micronutrients essential for larval and adult pollinator development and reproduction (Nicholls and de Ibarra 2017; Ruedenauer et al. 2019; Thakur and Nanda 2020). As a result, co-evolutionary processes in plant-pollinator interactions have resulted in rapid angiosperm diversification promoting various plant morphologies designed to attract pollinators (Lunau 2004; Johnson and Anderson 2010), while pollinators evolve adaptations to enhance foraging and extraction of nectar and pollen (Anderson and Johnson 2008). For example, the long-tongued

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fly (*Prosoeca ganglbaueri*) evolved a long proboscis to better feed on *Zaluzianskya microsiphon*, a flower that hides nectar at the tip of a long corolla (Anderson and Johnson 2008). Several bee species evolved floral sonication (i.e., “buzz” pollination) required to obtain the rewards concealed within the plant’s anthers or corollas (Cardinal et al. 2018). These co-evolutionary processes establish that pollinators rely on plants, and evolve alongside them in order to obtain their dietary requirements.

Bees (Anthophila) include many economically important species that provide essential pollination services, but disruptions or limitations in their floral resources appear to incite a nutritional stress via nutritional deficiencies which may be one of the top causes of current global bee population declines (Goulson et al. 2015). Malnutrition in bees has been shown to cause deficiencies in memory and learning (Arien et al. 2015, 2018; Bennett et al. 2022), larval and brood development (Schofield and Mattila 2015; Lawson et al. 2017, 2020; Arien et al. 2020), adult sexual reproduction (Czekońska et al. 2015; Ruedenauer et al. 2020), and immunity (Foley et al. 2012). Because of variable nutritional profiles across plant species, even within the same family or genus, bees forage either on a diversity of plants (e.g., generalists) or on a few plants with specific nutritional profiles (e.g., specialists). Furthermore, bee nutrition is not conserved across species, and even when located in the same area bees will display distinct foraging preferences based on their needs (Leonhardt and Blüthgen 2011). Social bees change foraging preferences depending on the nutritional needs of their colony (Zarchin et al. 2017), and are capable of differentiating plants based on the lipid and protein profiles in pollen (Vaudo et al. 2016; Ruedenauer et al. 2020, 2021). Because of their influence on bee fitness, lipids and proteins are the most researched components of pollen associated with bee foraging preference and overall bee nutrition.

Pollen contains lipids such as fats and oils in various concentrations depending on plant species, and serves important functional roles in bee development, reproduction, cognition, and

overall nutrition (Manning 2001; Arien et al. 2015, 2018; Vaudo et al. 2020). Lipids make up generally less than 10% of pollen dry weight, but range from 0.6 in eucalypts to 32% in canola (Nicolson 2011). Lipids can act as biochemical cues for pollinators to distinguish between high- and low-reward pollen. For example, it was observed that bumble bees differentiated pure pollen from pollen enriched with fatty acids, with a preference for pure pollen (Ruedenauer et al. 2020). Excessive lipid content in pollen can have detrimental effects on bees, such as impairment of associative learning and olfaction in honey bees (Arien et al. 2015, 2018; Bennett et al. 2022), and it is recognized that bees may preferentially forage for pollen with lower lipid concentrations. Lipids contain free fatty acids (i.e., non-esterified fatty acids; NEFA), some of which have antimicrobial activity which were found to inhibit growth of known honey bee bacterial and fungal pathogens such as American foulbrood (Feldlaufer et al. 1993) and chalkbrood (Ababouch et al. 1992). Polyunsaturated NEFA include omega-3 and omega-6, which are well explored in mammals as these constitute important essential fatty acids which contribute to key developmental roles such as brain development and cognition (Yehuda 2003) and fetal development (Shrestha et al. 2020). Despite the omega-6:3 ratio used as a common metric to determine well-balanced diets for mammalian studies, only a handful of studies explore this ratio in insects. Studies on honey bees suggest ratios around one or less are ideal, and imbalances in this ratio can lead to cognitive impairment (Arien et al. 2015, 2018; Bennett et al. 2022).

Protein is another major component of pollen that is critical in bee development and nutrition and often representative of overall pollen nutritional quality (Cook et al. 2003). Bee foraging preferences have been linked to the protein content of pollen. For instance, bumble bees will preferentially forage on invasive plants with more protein-rich pollen versus native species (Russo et al. 2019), as well as show an overall preference for Fabaceae pollen species as these plants generally have protein-rich pollen (Vaudo et al. 2020). However, more important than

protein content is the free amino acid composition which is considered the main determinant of bee health, despite amino acids constituting about 10% of the total protein content in pollen (approximately 23% of pollen dry weight is protein; Komosinska-Vassev et al. 2015; Jeannerod et al. 2022). Amino acids (AA) may be non-essential and synthesized within the body, or essential and need to be obtained from the diet. There are 10 essential amino acids for bees based on honey bee diet requirements and include the following: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (de Groot 1953). Pollen may be protein-rich but if it lacks essential amino acids, its nutritional value is considered poor (Cook et al. 2003). Finally, the protein to lipid (P:L) ratio is also a useful metric to determine nutritional quality of pollen and commonly used in bee nutritional studies to assess species-specific pollen preferences (Vaudo et al. 2020). For instance, bumble bees prefer more protein-rich plants, with P:L ratios averaging at 4:1 in field tests, whereas honey bees prefer a more balanced P:L nutrition, with ratios ranging from 1:1 to 2:1 (Vaudo et al. 2018). Conversely, the solitary horned-faced mason bee, *Osmia cornifrons*, P:L preference is around 2.9 and to meet its requirements will forage on low P:L pollen belonging to Rosaceae and higher P:L pollen belonging to Fabaceae (Vaudo et al. 2020).

There is a growing interest to improve pollinator health in order to combat the negative effects of climate change, agricultural practices, and urbanization. Thus, knowledge of pollen nutritional profiles is critical to illuminate key floral resources in different landscapes for bees. Most bee nutrition research is focused on commercially relevant and managed species such as honey bees and bumble bees, and on the study of nutritional content of bee-collected pollen (Taha et al. 2019; Ruedenauer et al. 2019). However, bees manipulate and add other salivary compounds (e.g., regurgitated nectar) to pollen which alters the pollen's nutrient composition, consequently not giving a clear synopsis of the original pollen nutrient composition prior to bee handling (Roulston and Cane 2000; Ruedenauer

et al. 2019). Therefore, hand-collected pollen offers a more practical approach to exploring pollen nutrition to determine its nutritional value for bees (Vanderplanck et al. 2014). Here, we aim to (1) profile non-essential and essential NEFA and AA content of 22 eastern North American pollen species commonly used as food sources by bees, (2) determine if NEFA and AA content is linked to pollen species' phylogenetic signal, and (3) determine the nutritional value of these pollen profiles for bees.

2. MATERIALS AND METHODS

2.1. Sampling

Flowers were collected from May to August 2017 and 2018 in Durham, NH (USA), to obtain pollen samples. Collection of pollen was adapted for each flower according to pollen characteristics and flower morphology to optimize pollen extraction following a modified protocol in Nicolson and Human (2012). Pollen was hand-collected from freshly collected flowers that were brought back to the lab in plastic bags, or from air-dried flowers. Flowers were either brushed or slowly tapped upside down on new, clean sheets of white paper. Frass and debris were removed from pollen using a sieve (45 μ m; Hogentogler & Co, Columbia) or forceps. The pollen was weighed in grams and stored at -80°C before analysis. In total, pollen was collected from 22 wild plant species that encompass 19 genera and 11 families (Table 1).

2.2. Pollen analyses

Pollen samples were subsequently analyzed for their non-esterified fatty acid (NEFA) and amino acid (AA) content at the Mayo Clinic in Rochester, MN. Each pollen sample was prepared by adding 1 \times phosphate-buffered saline (PBS) to the pollen. The mixture was sonicated and vortexed prior to preparing for NEFA and AA measurements.

Table I Protein to lipid (P:L) and omega-6:3 ratios of pollen species ($N=22$) sampled in this study. Lipid includes all non-esterified fatty acids, and protein includes all essential and non-essential amino acids and metabolites

Family	Genus	Species	Total protein (nmols/mg of pollen)	Total lipid (nmols/mg of pollen)	P:L	Omega- 6:3
Adoxaceae	Viburnum	<i>Viburnum opulus</i>	83.86	8.74	9.59	1.15
		<i>Viburnum prunifolium</i>	56.02	3.10	18.07	1.01
Anacardiaceae	Rhus	<i>Rhus glabra</i>	51.69	1.55	33.35	0.83
		<i>Rhus typhina</i>	165.84	3.84	43.19	0.43
Apiaceae	<i>Daucus</i>	<i>Daucus carota</i>	149.15	14.64	10.19	0.62
Asteraceae	<i>Coreopsis</i>	<i>Coreopsis lanceolata</i>	103.69	10.67	9.72	1.25
	<i>Leucanthemum</i>	<i>Leucanthemum vulgare</i>	196.20	6.47	30.32	1.67
	<i>Rudbeckia</i>	<i>Rudbeckia hirta</i>	128.10	5.80	22.09	1.19
	<i>Taraxacum</i>	<i>Taraxacum officinale</i>	129.94	39.55	3.29	0.11
	<i>Vernonia</i>	<i>Vernonia noveboracensis</i>	247.30	19.31	12.81	0.33
Cornaceae	<i>Cornus</i>	<i>Cornus sericea</i>	180.52	9.30	19.41	0.12
Ericaceae	<i>Vaccinium</i>	<i>Vaccinium corymbosum</i>	183.5	2.55	71.96	0.70
Fabaceae	<i>Lotus</i>	<i>Lotus corniculatus</i>	280.01	4.29	65.27	0.63
	<i>Securigera</i>	<i>Securigera varia</i>	314.30	10.85	28.97	0.61
	<i>Trifolium</i>	<i>Trifolium sp.</i>	101.44	39.14	2.59	0.11
Oleaceae	<i>Syringa</i>	<i>Syringa vulgaris</i>	223.44	3.42	65.33	0.96
Pinaceae	<i>Pinus</i>	<i>Pinus resinosa</i>	120.44	3.40	35.42	2.03
Plantaginaceae	<i>Plantago</i>	<i>Plantago lanceolata</i>	79.79	3.34	23.89	0.31
Rosaceae	<i>Prunus</i>	<i>Prunus sp.</i>	160.05	2.15	74.44	0.46
	<i>Rosa</i>	<i>Rosa rugosa</i>	115.86	10.52	11.01	0.23
		<i>Rosa sp.</i>	43.21	10.05	4.30	0.11
	<i>Rubus</i>	<i>Rubus idaeus</i>	132.19	26.91	4.91	0.46

The concentration of 12 NEFA was quantified against a standard curve on a Thermo Quantum Ultra triple quadrupole coupled with a Waters' Acquity liquid chromatography system (Milford, USA) as previously described (Persson et al. 2010). In brief, a 10–15 mg pollen solution was spiked with an internal standard solution containing isotopes prior to extraction. The extracts were dried down and brought up in running buffer prior to liquid chromatography-mass spectrometry (LC/MS) analysis. Data acquisition was performed under negative electrospray ionization conditions. The total amount of free fatty acids, the overall NEFA composition, and the proportion

of essential NEFA (omega-3 (linolenic acid) and omega-6 (linoleic acid)) were calculated per pollen species (in nmols/mg of pollen).

Amino acids were measured by LC/MS as previously described (Lanza et al. 2010). Briefly, isotope internal standards were added to 2 mg pollen solutions before deproteinization with cold methanol. The supernatant was immediately derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate according to Waters' MassTrak™ kit. A 10-point calibration standard curve underwent a similar derivatization procedure after the addition of internal standards. Both derivatized standards and samples were analyzed on a

Thermo Quantum Ultra triple quadrupole mass spectrometer coupled with a Waters Acquity liquid chromatography system. Data acquisition was done using a select ion monitor (SRM). Concentrations (in nmols/mg of pollen) of 42 analytes in each sample were calculated against the respective calibration curves.

The total amount of amino acids, the proportion of essential amino acids (arginine, histidine, iso-leucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, valine), and the amino acid composition were calculated per pollen species. The percent composition of each essential amino acid relative to the percent composition required in the honey bee diet, as determined by de Groot (1953), was also calculated and illustrated with a heat map. P:L ratios were calculated using the total amino acid and metabolite content (nmols/mg of pollen) per pollen species, divided by the total NEFA content per pollen species (nmols/mg of pollen). We also calculated omega-6:3 ratios for each pollen species to determine if our pollen species may have inhibitory effects on bee cognition, as omega-6:3 ratios were found to impact honey bee learning (Arien et al. 2015, 2018; Bennett et al. 2022).

2.3. Statistical analyses

Differences in the total NEFA, total AA, total essential amino acid (EAA), and total non-essential amino acid (NEAA) among the different pollen species were visualized using non-metric multi-dimensional scaling (NMDS) using Bray–Curtis dissimilarity. Data was initially transformed using the “total” method from the *decostand* function within the R package *vegan* (Oksanen et al. 2017), to convert data into relative total amounts per species. Next, we assessed if nutritional content (total AA, total EAA, total NEAA, total NEFA, P:L ratio, and omega-6:3 ratio) was significantly different among families that had at least 2 or more species (Asteraceae, Adoxaceae, Fabaceae, Rosaceae, and Anacardaceae). We used Bartlett’s test and Shapiro–Wilks test, *bartlett.test*, and *shapiro.test* functions in base R, respectively, to test for

violations of group variance and normal distribution. Variance of data was equal among families but was not normally distributed; hence, we used Kruskal–Wallis test to determine if nutritional content varied by family using the *kruskal.test* method in base R. All R analyses were done using R version 4.2.2 (R Core Team 2022).

Phylogenetic generalized least squares (PGLS) using the R package *nlme* (Pinheiro and Bates 2000; Pinheiro et al. 2023) analysis was performed on the pollen for (1) total NEFA vs. total AA, (2) total NEFA vs. total EAA, and (3) total NEFA vs. total NEAA and (4) total omega vs. total EAA calculated per pollen species to assess if there is an evolutionary correlation between total NEFA and different amino acid compositions. To generate the tree topology, complete or partial ribulose-1,5-bisphosphate carboxylase large subunit (*rbcL*) sequences from chloroplast were obtained from NCBI Genbank for each of the species. Three of our plant species were not identified to the species level (*Trifolium* sp., *Prunus* sp., and *Rosa* sp.); hence, we used *rbcL* sequences for *Trifolium repens* (MF135470.1), *Prunus serotina* (NC_036133.1), and *Rosa multiflora* (NC_039989.1) to generate our tree as these plants’ distributions fall within North America. The remaining *rbcL* accession for the remaining plants is as follows: *Viburnum opulus* (HQ591775.1), *V. prunifolium* (HQ591756.1), *Rhus glabra* (KX397919.1), *R. typhina* (GU935441.1), *Daucus carota* (NC_008325.1), *Coreopsis lanceolata* (MF135394.1), *Leucanthemum vulgare* (NC_047460.1), *Rudbeckia hirta* (AY215173.1), *Taraxacum officinale* (NC_030772.1), *Vernonia noveboracensis* (MF349587.1), *Cornus sericea* (AY725857.1), *Vaccinium corymbosum* (NC_068711.1), *Lotus corniculatus* (U74213.1), *Securigera varia* (NC_054256.1), *Syringa vulgaris* (NC_036987.1), *Pinus resinosa* (AB063384.1), *Plantago lanceolata* (NC_068049.1), *Rosa rugosa* (NC_044094.1), and *Rubus idaeus* (U06825.1). The conifer *Pinus resinosa* is a wind-pollinated plant but was included in this study as bees were found to also forage on wind-pollinated species (Saunders 2017; Cannizzaro et al. 2022). To generate a phylogeny, *ultrafast IQ-TREE*

(version 1.6.12) with 1000 bootstrap replicates was used to generate a maximum likelihood tree (Nguyen et al. 2015; Hoang et al. 2017). The resulting tree was constrained to match known angiosperm phylogeny using Mesquite version 3.70 (Maddison and Maddison 2021) to follow more closely the angiosperm phylogeny depicted in Soltis et al. (2011).

Finally, we describe each pollen species as either “native” or “introduced” to eastern North America to assess whether nutritional content significantly differs depending on pollen endemicity. To do this, plant endemicity was obtained using the United States Department of Agriculture (USDA) PLANTS database (<http://plants.usda.gov>; USDA and NRCS 2023). For pollen described to the genus level, we used the same species-level information that was used to obtain rbcL data which includes *Trifolium repens*, *Rosa multiflora*, and *Prunus serotina*. Three plants lacked distinct endemicity information from the PLANTS database, and instead, we used literature to determine endemicity for *Taraxacum officinale* (Stewart-Wade et al. 2002), *Viburnum opulus* (Česonienė et al. 2010), and *Rubus idaeus* (Fernald 1919). Tests of normality using Shapiro-Wilks test for total EAA, total NEAA, total AA, total NEFA, P:L, and omega-6:3 against endemicity were violated. Hence, Mann-Whitney-Wilcoxon test using the *wilcox.test* method in base R was used to determine if nutritional content significantly differed between native and introduced pollen species.

3. RESULTS

3.1. Pollen composition of NEFA and AA

NEFA content in each species ranged from 7 to 10 fatty acids with a total amount ranging from 1.55 to 39.55 nmols/mg of pollen in *Rhus glabra* and *Taraxacum officinale*, respectively (Table S1). The omega 3 docosahexaenoic acid (DHA) was not identified in any species. Arachidonic, elaidic, and eicosapentaenoic (EPA) acids were also rarely detected in any of the species and represented only 1.7% of the total NEFA content

across all species. Six NEFA were consistently identified across species which include the essential NEFA (linoleic and linolenic acid), oleic, palmitic, palmitoleic, and stearic acids (Figure 1). Among these, linolenic (omega-3, 44.3%), palmitic (27.7%), and linoleic (omega-6, 13.9%) acids were the top three most abundant fatty acids represented. The essential NEFA linoleic acid ranged from 0.19 to 4.44 nmols/mg of pollen in *Rhus glabra* and *Coreopsis lanceolata*, respectively. The essential NEFA linolenic acid ranged from 0.23 to 29.98 nmols/mg of pollen in *Rhus glabra* and *Taraxacum officinale*, respectively.

Pollen of each species was composed of 26 to 34 AA and metabolites with a total amount ranging from 43.2 to 314.3 nmols/mg of pollen in *Rosa* sp. and *Securigera varia*, respectively (Table S2). All ten EAA were identified in each genus, and across all species except for *Rhus glabra* which had no detectable levels of methionine. EAA levels varied from 1.3 to 44.4 nmols/mg of pollen in *Rosa* sp. and *Syringa vulgaris*, respectively (Figure 2A). The most common EAA was histidine (36.4%) followed by arginine (19.1%), and the least common EAA was methionine (0.84%). Among the six species with EAA content $\leq 20\%$ (*Rudbeckia hirta*, *Taraxacum officinale*, *Leucanthemum vulgare*, *Pinus resinosa*, *Coreopsis lanceolata*, and *Syringa vulgaris*), four of them belonged to Asteraceae, but they were particularly high in only one amino acid—histidine.

The NEAA content ranged from 41.9 to 303.5 nmols/mg of pollen in *Rosa* sp. and *Securigera varia*, respectively (Figure 2B). Among the top three pollen species with NEAA content > 200 nmols/mg of pollen, one belonged to Asteraceae (*Veronica noveboracensis*) and two belonged to Fabaceae (*Lotus corniculatus* and *Securigera varia*). The NEAA anserine, carnosine, cystathionine 2, hydroxylysine 1, and hydroxylysines 2 were not detected in any of the pollen species. The most common NEAA was proline (60.3%) followed by asparagine (11.6%), and the least common NEAA were allo-isoleucine (0.01%) and alpha-amino-N-butyric-acid (0.01%).

The P:L ratios ranged from 2.9 to 74.44 in *Trifolium* sp. and *Prunus* sp., respectively, with

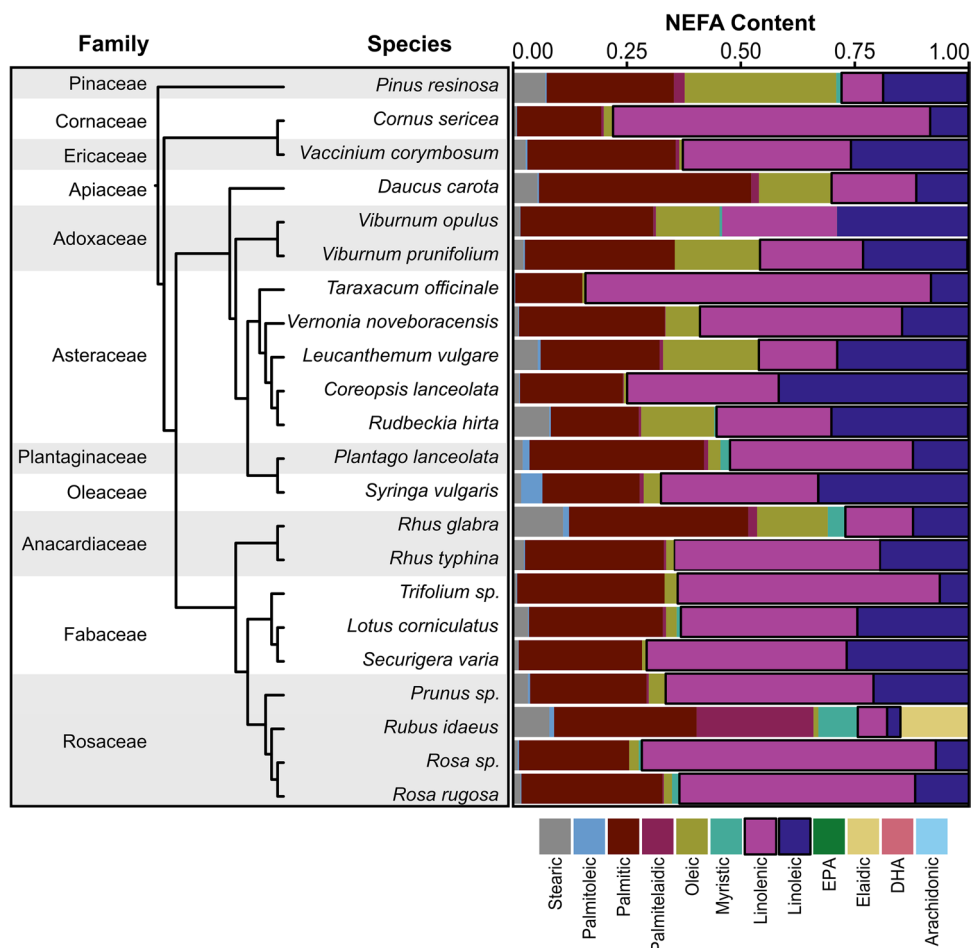


Figure 1. Non-esterified fatty acid (NEFA) content across species. Barplot distribution of essential amino acids in each pollen species, ordered by phylogeny with tree shown. Essential NEFA omega-3 (linolenic acid) and omega-6 (linoleic acid) are bolded in black.

a mean P:L of 27.3 ± 23.2 across pollen species (Table I). Pollen species exceeding P:L ratios > 60 belonged to a mix of families (Fabaceae *Lotus corniculatus*, Oleaceae *Syringa vulgaris*, Ericaceae *Vaccinium corymbosum*, and Rosaceae *Prunus* sp.). Omega-6:3 ratios ranged from 0.11 to 2.03, with a mean of 0.70 ± 0.52 across pollen species (Table I). Six species had omega-6:3 ratios greater than one and primarily belonged to Adoxaceae (*Viburnum* spp.) and Asteraceae families (*Rudbeckia hirta*, *Coreopsis lanceolata*, and *Leucanthemum vulgare*), but only the conifer *Pinus resinosa* had a ratio above two.

3.2. Nutrition content by family and phylogeny

Analysis of total NEFA content showed that pollen species did not form distinct groups by family (Figure 3A), whereas family groups were more distinguishable when assessing total AA content (Figure 3B) and total NEAA (Figure 3D). When assessing total EAA content, only pollen species belonging to Asteraceae formed a group that was separate from the remaining families (Figure 3C). Furthermore, there was no significant difference in average nutritional content across families with

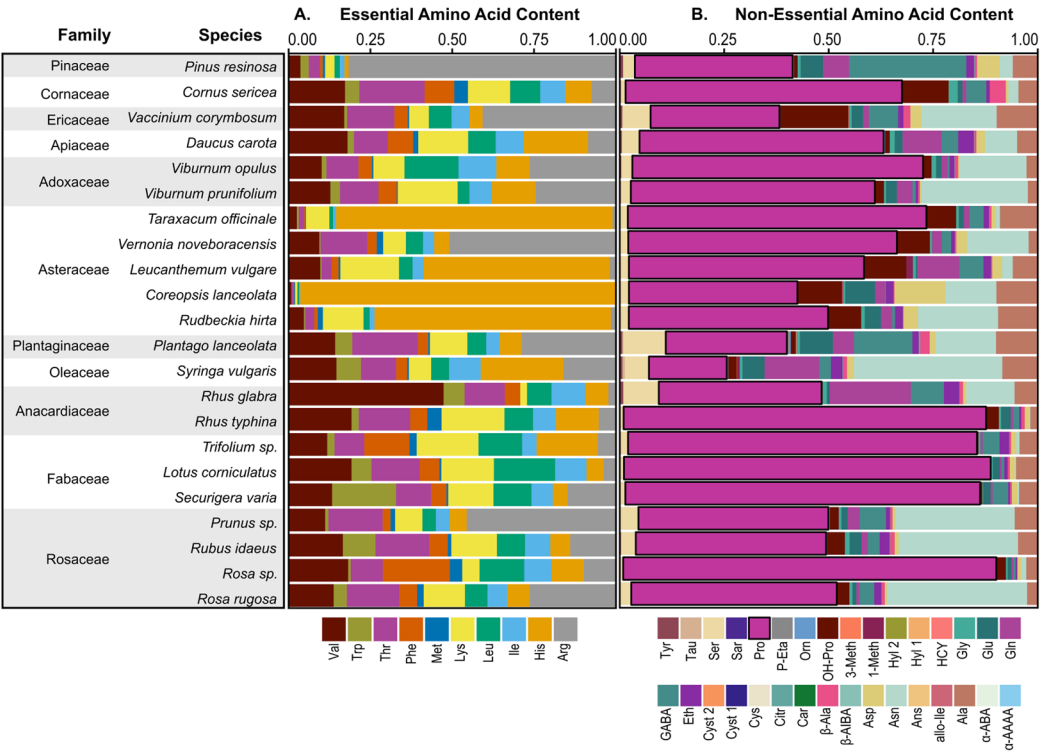


Figure 2. Amino acid content across species. Barplot distributions of (A) essential amino acids and (B) non-essential amino acids in each pollen species (proline highlighted in black). Species are ordered by phylogeny (refer to Figure 1 for tree).

two or more genera. When considering phylogenetic relationship among pollen species, total NEFA correlated significantly, positively with total AA ($t = 17.8$, $df = 22$, $P < 0.001$, Figure 4A), negatively with total EAA ($t = -16.3$, $df = 22$, $P < 0.001$, Figure 4B), and positively with total NEAA ($t = 17.8$, $df = 22$, $P < 0.001$, Figure 4C). When analyzing just total essential NEFA content (omega-3 and omega-6) against total EAA content, the correlation was also significant ($t = -18.6$, $df = 22$, Figure 4D).

3.3. Pollen nutrition related to honey bee requirements

Most pollen species were dominant in a single amino acid (Figure 5, Table S3). Among the pollen species with > 20% EAA content overall, they

were disproportionately dominated by one amino acid, either histidine or arginine. Pollen species that had $\leq 20\%$ difference between the maximum and minimum EAA proportion appeared more balanced in their overall EAA composition, and include *Daucus carota*, *Cornus sericea*, *Lotus corniculatus*, *Securigera varia*, *Trifolium* sp., *Rosa* sp., *Rubus idaeus*, *Viburnum prunifolium*, and *Rhus typhina*. These species also more closely matched the EAA proportions when compared to optimal honey bee diet requirements (Figure S1).

3.4. Native vs. introduced pollen species

Out of 22 pollen species, 12 were categorized as introduced (54.5%) and 10 were native to North America (45.5%) (Table S4). Most families with multiple genera included both

native and introduced pollen species, except Fabaceae which were all introduced species. Mean total lipid content of introduced pollen species was 14.83 ± 13.1 nmols/mg of pollen, compared to 6.17 ± 5.5 nmols/mg of pollen in native pollen. On average, native pollen species had 139.72 ± 60.4 nmols/mg of pollen total protein content (4.3% total protein content) compared to 154.12 ± 83.2 nmols/mg of pollen in introduced pollen species (4.75% total protein content). Native species had a mean omega-6:3 of 0.84 ± 0.56 , which was slightly greater than introduced species at 0.58 ± 0.48 . Average P:L was similar between native and introduced pollen species, with P:L ratios of 27.8 and 26.8, respectively. Only total lipid content significantly differed between native and introduced

pollen species with greater lipid content in introduced species (Mann-Whitney-Wilcoxon $W=93$, $P=0.03$), whereas nutritional content based on AA, P:L, and omega-6:3 did not differ depending on endemism.

4. DISCUSSION

We examined the pollen NEFA and AA content for 22 plant species across 11 plant families. The most abundant NEFA across all pollen species were the essential omega-3 and omega-6. Among the AA explored, all ten EAA were identified across all pollen species (except for *Rhus glabra* which lacked methionine), with some pollen species dominated by a single amino acid

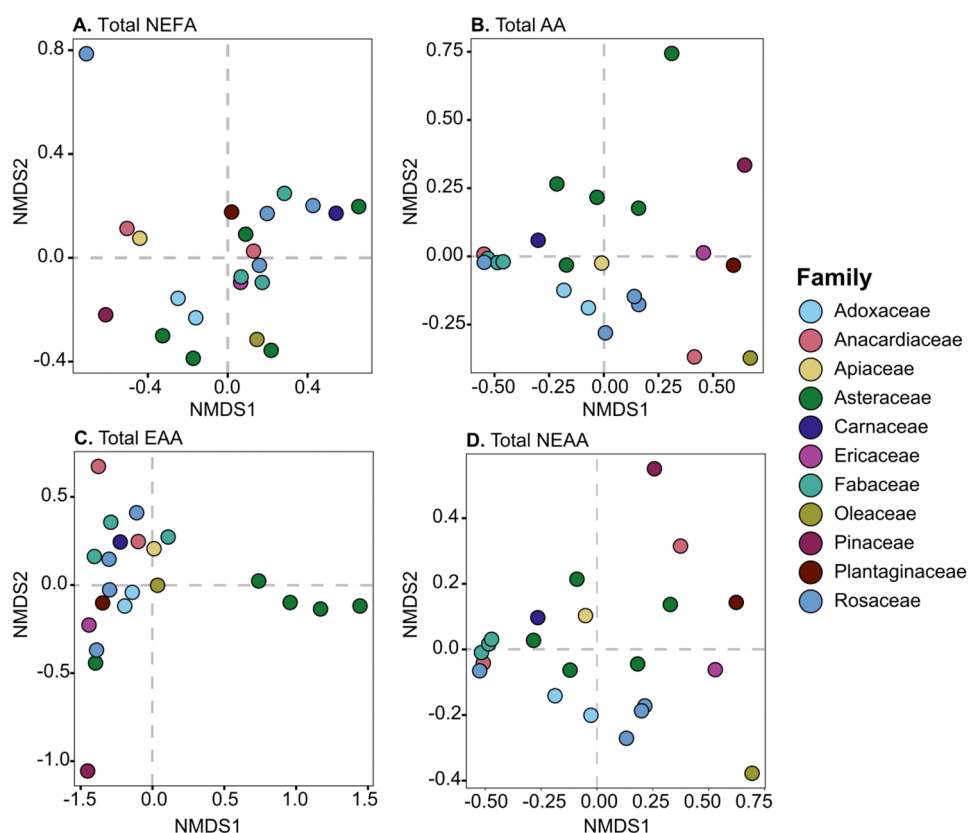


Figure 3. Nonmetric dimensional scale (NMDS) plots. **A** Total non-esterified fatty acid (NEFA) content, **B** total amino acid (AA) content (essential + non-essential amino acids), **C** total essential amino acid (EAA) content, and **D** total non-essential amino acid (NEAA) content.

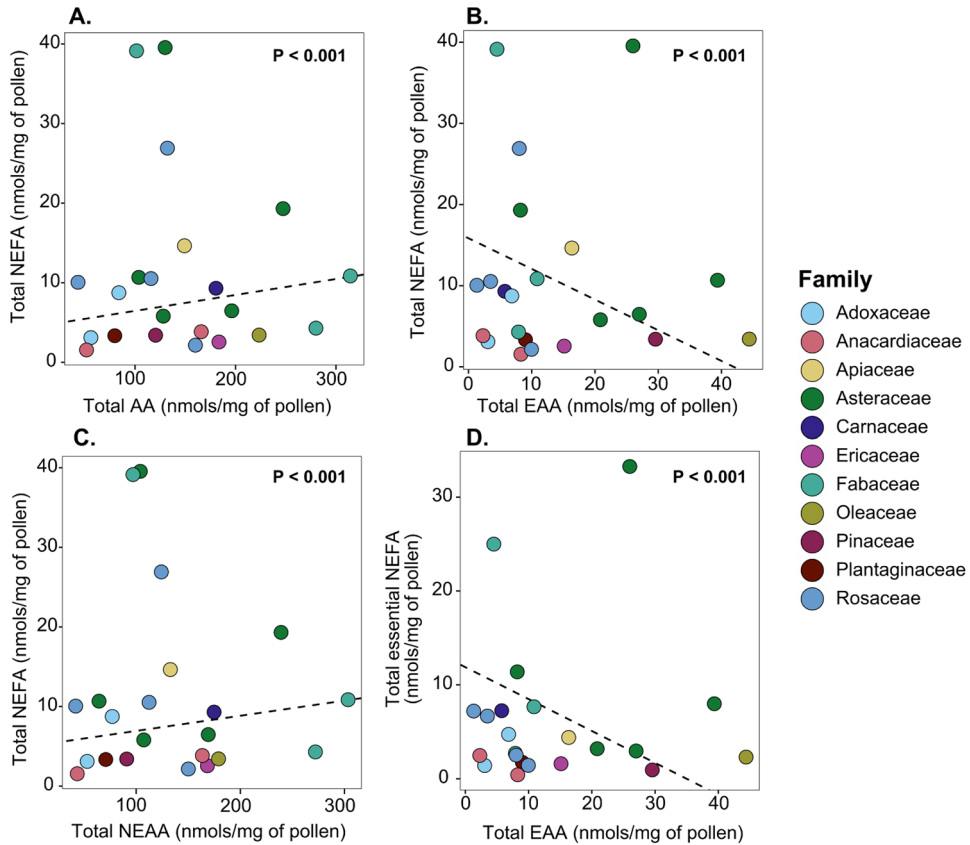


Figure 4. Phylogenetic generalized least square (PGLS) plots (nmols/mg of pollen). PGLS fitted using a maximum likelihood model with a Brownian correlation, denoting relationships between lipid and protein content for (A) total non-esterified fatty acids (NEFA) against total amino acids (AA), (B) total NEFA against total essential amino acids (EAA), (C) total NEFA against total NEAA, and (D) total essential NEFA (omega-6; linoleic acid and omega-3; linolenic acid) against total EAA.

which was typically histidine or arginine. We also found that total EAA content was significantly negatively correlated with total NEFA or total omega content, suggesting potential trade-offs between lipids and proteins in pollen nutrition. Overall, NEFA and AA content was highly variable across pollen families and genera, indicating an importance in diverse floral resources to compensate for variable bee nutrition requirements and to ensure bees obtain a balanced diet.

Across all species, the composition of NEFA was mainly driven by a richness of linolenic (omega-3), linoleic (omega-6), palmitic, palmitoleic, stearic, and oleic acids which serve important roles in bee nutrition and development

(Manning 2001). The essential fatty acids omega-3 and omega-6 were generally the two most abundant fatty acids found across all species, and their concentrations are known to impact pollinator physiology (Arien et al. 2015, 2018; Bennett et al. 2022). Larval provisions of the stingless bee *Tetragonula carbonaria* that foraged in floral rich environments (e.g., forests) had reduced omega-6 and increased omega-3 content, thus low omega-6:3 ratios (Trinkl et al. 2020). While fatty acids are important in bee diet, specific omega-6:3 ratios and/or excessive amounts of omega-6 can be detrimental to bee development and cognition. For instance, honey bees (*Apis mellifera*) that fed on diets higher

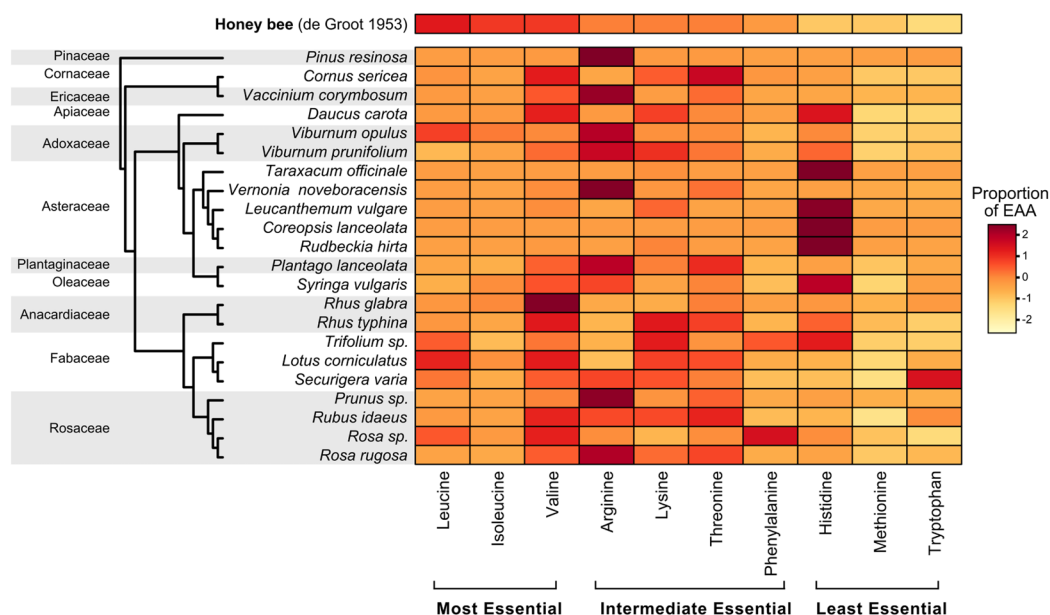


Figure 5. Heatmap of essential amino acid composition in plant pollen. Each color-coded square represents the percent total of each essential amino acid in each pollen species across all essential amino acids. We include the percent total of each essential amino acid required by honey bees across the total amount of essential amino acids (de Groot 1953). Essential amino acids are ordered from most to least essential according to honey bee requirements. Pollen species are ordered by phylogeny.

omega-6:3 ratios (i.e., excessive omega-6) exhibit reduced olfactory associative learning, whereas omega 6:3 ratios around one or less were found to not inhibit honey bee cognition (Arien et al. 2015, 2018; Bennett et al. 2022). Most of the pollen species across diverse families (72.7%) had omega-6:3 ratios less than one indicating suitability for (honey) bees, but further studies including other bee species are needed to validate this threshold. Additionally, while current studies on hand-collected and bee-collected pollen indicate minimal difference in fatty acid composition in social bees (Nicolson 2011; Nicolson & Human 2012; Arien et al. 2015), other studies are needed to determine if non-corbiculate bee-collected pollen have altered fatty acid compositions. Non-essential NEFA also serve important roles in bee physiology and cognition. The common eastern bumble bee, *Bombus impatiens*, had an increase in visual learning and survival when given sucrose solution with oleic acid (Muth et al. 2018). Certain NEFA such as myristic, stearic,

palmitoleic, arachidonic, and oleic acids (as well as the essential NEFA) act as antimicrobial agents against bee pathogens (Feldlaufer et al. 1993; Manning 2001).

Pollen provides a fundamental source of protein for bees, and a balanced diet consisting of diverse flora is even more crucial in generalist bees to acquire a complete nutritional profile (Trinkl et al. 2020). Overall, we found a broad range in protein content across pollen species. Pollen species can differ nutritionally regardless of similar overall protein content due to differences in EAA proportions (Standifer 1967). Amino acid and metabolite content varied greatly across pollen species, but it was evident that the NEAA proline was dominant across the majority of species. Free proline is found in high concentrations in insect tissue and also occurs in high amounts in nectar, suggesting that high plant production of proline serves as a reward for pollinators (Carter et al. 2006; Teulier et al. 2016). Past studies have documented the

importance of proline as the main energy source in blood-feeding insect flight (Bursell 1974), whereas carbohydrates and lipids are commonly used in other flying insects such as bees (Worm and Beenackers 1980). However, recent studies now find proline as another major energy substrate used in bee flight considering its high concentration in honey bee haemolymph (Teulier et al. 2016; Stec et al. 2021).

All ten EAA were identified in the all pollen species in our study, except *Rhus glabra* which lacked methionine. Methionine overall was the least abundant EAA across all pollen species, and is often found as a limited EAA in various pollen species that rely on insect pollinators such as honey bees (Taha et al. 2019; Jeannerod et al. 2022). The baseline EAA requirement for honey bees lists methionine among the least essential EAA along with histidine and tryptophan (de Groot 1953). In our study, lanceleaf tickseed (*Coreopsis lanceolata*) and common lilac (*Syringa vulgaris*) had the highest EAA content; however, *C. lanceolata* was imbalanced with majority of the content owing to histidine whereas *S. vulgaris* was more evenly balanced. Furthermore, four out of five Asteraceae plants had relatively high EAA content (> 20%), but again were dominated by histidine or arginine. Based on honey bee diet, we found that eight plants from diverse families had EAA proportions that more closely met the requirements for honey bee EAA needs, and they varied from low to high total lipid content. Our findings suggest that since protein content is highly diverse among pollen species, focusing only on protein or EAA content alone is not suitable to deduce the complete nutritional status of pollen species.

Based on our findings, pollen nutrition is variable across plant species, genera, and families, as well as endemism. As such, bees benefit from foraging on multiple resources in order to obtain a balanced diet and resources may be shifted with the introduction of novel plants. We found that only total NEFA content was significantly different between native and introduced pollen species, and that introduced species had on average greater total NEFA concentrations. While excessive amounts of lipids may

be detrimental to bees (Arien et al. 2015, 2018; Bennett et al. 2022), introduced plants which our results show have higher NEFA content and may offer diverse nutritional value attractive to native bees. A recent study found that an invasive plumeless thistle (*Carduus acanthoides*) had higher P:L ratios compared to other native Asteraceae (*Helianthus annuus*, *Gaillardia pulchella*, *Rudbeckia hirta*, and *Coreopsis tinctoria*), which attracted more pollinators (Russo et al. 2019). Global warming also impacts nutritional quality of pollen, and successfully established invasives likely already possess the desired traits to adapt to changing environments. Higher lipid content in introduced species may provide resilience against a warming climate. Indeed, a study examining the warming effects on the invasive plumeless thistle pollen found that lipid content increased in warmed thistle, but did not affect pollinator visitation rates (Russo et al. 2020). These findings may suggest an adaptive response of introduced plants whereby increased lipid content may increase tolerance to heat stress (Saidi et al. 2010), and offer a viable resource to bees as climate continues to warm.

De Groot (1953) determined an ideal breakdown of essential amino acids for a balanced honey bee diet. Based on just total EAA content, common lilac had the highest EAA concentrations with fairly evenly spread EAA concentrations, but when compared to honey bee composition requirements was not considered to provide a balanced nutrition. Crownvetch (*Securigera varia*) had the highest total AA content, and even with lower EAA concentrations, it did fit a more balanced EAA profile compared against honey bee requirements. However, a balanced bee diet must take into account lipid concentrations as several studies have demonstrated that fatty acid content needs to be present but not excessive for proper bee development (Arien et al. 2015, 2018; Bennett et al. 2022). Among the ideal pollen species based on EAA content, they ranged from high to low total lipid content with the majority of the ideal pollen species being introduced. While this study does not pinpoint a single pollen species as the most ideal based on protein and lipid content alone, eight species we identified

as most balanced according to EAA content also had omega-6:3 ratios less than one which should not negatively impact bee cognition (Vaudo et al. 2020), but had a highly variable P:L range. Since P:L ratios for bumble bees average 4:1 in the field, and ratios range from 1:1 to 2:1 preference for honey bees (Vaudo et al. 2018), according to our eight ideal pollen species, rose (*Rosa* sp.), clover (*Trifolium* sp.), and American red raspberry (*Rubus idaeus*) would be considered ideal for honey bees and bumble bees, with P:L ratios of 4.3, 2.59, and 4.91, respectively. Furthermore, all of Fabaceae (bird's-foot trefoil (*Lotus corniculatus*), crownvetch, and clover) were considered balanced according to EAA profiles and, interestingly, are also all introduced species. As such, both introduced and native pollen species with variable nutritional profiles can be considered ideal for bees, many of which will forage on multiple species in order to meet their dietary needs. This is evident in both social and solitary bees that forage on multiple plant resources. For instance, honey bee pollen provisions can range from 5 to 20 species (Avni et al. 2009), and in some instances may average 29 plant genera (Donkersley et al. 2017). Solitary species also produce highly diverse pollen provisions which, although may be dominated by a few plant genera, can still range from 40 to 65 genera depending on bee locality (Dew et al. 2020).

Currently, there is a lack of pollen nutrition studies that include phylogenies which may provide valuable insights into the chemical trade-offs and/or nutritional profiles across plant genera and families. For example, Solanaceae, despite having a wide floral variability from small to large flowers or relying on small insect to large mammal pollinators, maintained consistent protein-rich pollen. On the contrary, the species-rich and morphologically diverse plant families Fabaceae and Cactaceae had variable protein content (Roulston et al. 2000). We assessed phylogenetic correlations between total NEFA content and various AA content (e.g., total AA, NEAA, and EAA) which may indicate linked biochemical pathways between fatty and amino acids in pollen (Ruedenauer et al. 2019). We found that total NEFA content positively correlated with total AA or with total NEAA. Similar findings

were found in Ruedenauer et al. (2019) where the authors found that polypeptide and crude protein content was significantly, positively correlated with lipids. In contrast, we found that total NEFA was significantly, negatively correlated with total EAA content, and essential fatty acids were also negatively correlated with EAA. This finding depicts that pollen species with high amounts of essential proteins generally have lower levels of total lipids including essential NEFA, and that pollen species lineage emphasizes this trend. Ruedenauer et al. (2019) also found that free amino acid content negatively correlated with lipid content in bee-collected and hand-collected protein. However, results were insignificant and it is unclear if this pattern holds true when considering only free essential amino acids in their explored pollen species. We propose that a trade-off may occur in the production of essential fatty acids for lipids, and suggest future studies to explore this pattern across an expanded variety of pollen species and families.

While most studies focus on the social honey bee and bumble bee, about 85% of globally described bees are solitary species that pollinate a large number of plants and crops (Batra 1984), and also include important generalist and specialist species (Kleijn et al. 2015). A handful of manipulative laboratory studies have focused on optimal nutrient concentrations in pollen required for solitary bees, such as elemental concentrations needed for *Osmia bicornis* (Filipiak and Filipiak 2020), and protein, lipid, and sugar content for the small carpenter bee *Ceratina calcarata* (Lawson et al. 2020), but we still lack studies that explore nutritional profiles of diverse, foraged pollen from a variety of wild bees. Furthermore, nutritional studies focusing more on solitary bee species will be crucial to elucidate if dietary requirements differ among species that display differences in social complexity. Examination of pollen microbiomes would also enhance our understanding of pollen nutrition in bees, as various pollen-associated microbiota have been found to impact bee development. This is particularly important for specialist bees—most being solitary species—that specialize on monotypic or specific plant genera, or specific pollen types (Dharampal et al. 2019,

2020). For example, solitary mason bee *Osmia ribifloris* fed pollen provisions with natural pollen-associated microbes produced the fittest larvae overall as opposed to larvae fed pollen without microbiota (Dharampal et al. 2020). Pollen fatty acid content also correlated with certain pollen-associated bacteria which may be important in fatty acid provisioning in another mason bee, *Osmia bicornis* (Leonhardt et al. 2022). Adding additional plant characteristics into pollen nutritional studies can illuminate additional co-evolutionary processes affecting plant-pollinator networks. For instance, incorporating pollination syndrome (e.g., animal pollination, wind pollination, self-pollination) and dependence on a pollination method (e.g., high dependence on animal pollinators) has been shown to affect pollen protein content (Ruedenauer et al. 2019). Insect dependence on pollination significantly impacted pollen protein content more so than phylogenetic lineage, with plant species producing highly protein-rich pollen if they were highly dependent on insect pollination versus plants that could self-pollinate or wind pollinate (Ruedenauer et al. 2019). Flower morphology also influences nutritional content, such as longer styles requiring pollen to produce greater nutrient concentrations needed for increased pollen tube growth (Ruedenauer et al. 2019).

In conclusion, our findings suggest that pollen species between and within families harbor diverse nutritional profiles likely to meet the requirements of diverse pollinators. Both protein and lipid content is essential for the healthy development of bees, and most species will forage on a variety of plants in order to obtain a balanced diet or to maintain species-specific protein and lipid requirements. We found that P:L ratios were highly variable across pollen species but that omega-6:3 ratios were generally below one, a desirable amount for bees based on honey bee literature. Interestingly, we determined that total EAA content negatively correlated with total NEFA or with total essential NEFA content as a significant phylogenetic signal, suggesting potential trade-offs

between with lipid content and essential amino acids. We identify several pollen species with a balanced nutritional profile when compared to honey bee EAA requirements, but future studies are needed to determine a broader range of wild bee dietary needs.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at <https://doi.org/10.1007/s13592-023-01054-4>.

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AUTHOR CONTRIBUTION

SMR conceived and funded the study. KDC analyzed and visualized the data, and drafted the manuscript. All authors approved the final version of this manuscript.

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DATA AVAILABILITY

Requested code will be provided through contact with the corresponding author.

DECLARATIONS

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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