

ORIGINAL ARTICLE

Editors' Choice

Macronutrient composition in pollen affects development and survival in wild bees

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Abstract

Pollinators rely on diverse floral resources to meet their nutritional needs, as pollen macronutrient composition varies significantly among plant species, influencing bee development and survival. In this study, we collected freshly laid eggs of the small carpenter bee (*Ceratina calcarata*) (Hymenoptera: Apidae) Robertson, 1900 from artificial nest substrates and reared them on diets formulated with black poplar (*Populus nigra*) (Malpighiales: Salicaceae) Linnaeus, 1753 and dandelion (*Taraxacum officinale*) (Asterales: Asteraceae) F.H. Wigg, 1780 pollen, while closely monitoring developmental metrics, lipid content and survival. We also conducted macronutrient analyses on both pollen types to quantify protein, amino acids, fatty acids and sugars. While dandelion pollen is abundant and carbohydrate-rich, it contained lower levels of protein, essential amino acids and several fatty acids in our analyses, a nutrient profile that corresponded with slower larval development in our study. In contrast, black poplar pollen, despite being primarily wind-dispersed, had higher concentrations of protein, essential amino acids and fatty acids in our analyses and supported better developmental performance. Bees reared on black poplar pollen exhibited faster maturation, larger head widths and greater body mass, highlighting the possible role of protein availability in development. Survival analysis further revealed that protein-rich diets supported higher brood viability, whereas bees fed dandelion pollen experienced delayed development and reduced body sizes. Bees on 50:50 mixed diets displayed intermediate development times and improved survival, highlighting the benefits of dietary diversity in mitigating nutritional stress. These findings emphasize the importance of arboreal pollen as a possible nutritional resource and the potential to incorporate tree pollen sources into conservation planning. Given the increasing pressures of habitat loss and climate change, ensuring access to nutritionally diverse pollen sources may be useful for sustaining pollinator populations and promoting ecosystem resilience.

KEYWORDS

amino acids, bee health, larval development, nutritional ecology, pollinator diet, wind-pollinated trees

INTRODUCTION

Bees are important pollinators in terrestrial ecosystems, facilitating the reproduction of over 85% of flowering plants, including numerous economically important crops and diverse wild plant species (Ollerton, 2021). Their evolutionary success and adaptive foraging behaviours are intrinsically linked to their nutritional ecology, which has been shaped by coevolutionary interactions with floral resources (Janz & Nylin, 2008; Johnson, 2010; Vaudo et al., 2024). This coevolution has influenced bees' dietary specialization and generalization strategies, directly impacting their nutritional acquisition, reproductive success and survival (Filipiak & Filipiak, 2020; Vaudo et al., 2015).

Pollen is the primary nutritional resource for bees, supplying proteins, amino acids, carbohydrates and lipids that are essential for larval development, adult energy maintenance, immune function and overwintering survival (Di Pasquale et al., 2013; Filipiak & Filipiak, 2020). Nectar primarily provides carbohydrates, fuelling adult metabolic processes, flight and thermoregulation (Filipiak & Filipiak, 2020). Additionally, a minority of bee species utilize specialized floral oils, further diversifying their nutritional resources (Manning, 2001; van der Kooi et al., 2021).

Amino acids, particularly essential amino acids, are fundamental nutritional components required by bees for proper larval development, adult growth, reproductive success and overall colony health (De Groot, 1953; Weeks et al., 2018). Bees must obtain 10 essential amino acids: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine from pollen, as these amino acids cannot be synthesized *de novo* and must therefore be supplied by dietary intake (De Groot, 1953). Recent research further demonstrates that deficiencies or imbalances in essential amino acids can adversely affect bee longevity, fecundity and resilience to environmental stressors, showcasing their part in bee nutritional ecology (Filipiak & Filipiak, 2020; Hendriksma et al., 2019; Jeannerod et al., 2022). Consequently, variation in essential amino acid content can influence bee's nutritional intake and may contribute to foraging decisions, although responses are species and context dependent (Hendriksma et al., 2019; Vaudo et al., 2016).

Lipids, in particular, have become a necessary and influential macronutrient that significantly influences bee physiology (Vaudo et al., 2020). Essential fatty acids, notably omega-3 (α -linolenic acid) and omega-6 (linoleic acid), are fundamental in shaping bee cognitive performance, immunity and overall metabolic efficiency (Arien et al., 2018). Recent studies reinforce that an imbalanced omega-6:3 fatty acid ratio can impair cognitive functions such as learning and memory in honey bees (Arien et al., 2020). Omega-3 fatty acid deficiencies specifically diminish associative learning capacities, negatively affecting foraging efficiency, colony productivity and survival (Arien et al., 2015; Wright et al., 2018). While prior work emphasizes the importance of omega-6:3 balance for bee cognition, our study assessed overall fatty acid composition rather than specific ratio effects.

In social bee colonies, such as honey bees (*Apis mellifera*), nurse bees process pollen by combining it with glandular secretions to

produce nutrient-rich 'bee bread', which is fed directly to larvae (Crailsheim, 1992; Crailsheim et al., 1992). This processing modifies the biochemical composition of pollen, including shifts in lipid and fatty acid profiles, which can influence larval nutrition (Leonhardt et al., 2022). Although adult nurse bees themselves require relatively lower lipid reserves, the nutritional composition of brood food has been linked to larger growth, immunity and overall developmental condition (Leonhardt et al., 2022; Wright et al., 2018). In contrast, solitary subsocial bees such as the small carpenter bee *Ceratina calcarata* provision pollen directly into brood cells, placing greater initial importance on the pollen's intrinsic nutritional quality, particularly lipid content and fatty acid ratios (Lawson et al., 2020; Shell & Rehan, 2018).

Pollen nutritional quality varies widely among plant species, with some abundant floral resources providing relatively low protein or imbalanced macronutrient profiles despite high availability. For example, blueberry (*Vaccinium corymbosum*) pollen contains comparatively low crude protein (Graham et al., 2023), illustrating that floral abundance does not necessarily indicate nutritional suitability for bee larvae. Blueberry is a well-studied case because it is economically important and widely visited by bees, yet its pollen is nutritionally limiting, and environmental stressors such as extreme heat can further reduce its nutritional value (Walters et al., 2025). Dandelion fits into this broader pattern, as it is highly abundant early in the season but offers relatively low protein compared to other pollen types (Stephen et al., 2024). Together, these examples highlight why examining nutritionally distinct pollen sources is essential for understanding how diet shapes larval development, foraging decisions and adult condition in wild bees (Jones & Rader, 2022).

Seasonality also significantly shapes the nutritional landscape available to pollinators (Harris et al., 2024). Wild bee species differ widely in their phenology, and because most solitary bees are active only for a short portion of the year and their emergence is closely synchronized with floral availability, the nutritional value of floral resources must be considered in relation to when each species is active (Forrest & Thomson, 2011). Early-season arboreal pollen sources such as willow (*Salix* spp.), maple (*Acer* spp.) and poplar (*Populus* spp.) dominate the earliest spring bloom and often represent the primary foraging resources available to spring-emerging bees (Rehan & Richards, 2010; Timberlake et al., 2019). In contrast, bee species active in late summer and early fall rely on asters and goldenrods (Asteraceae), and many of these late-season bees are well adapted to forage during this period rather than nutritionally stressed (Smart et al., 2019). Mid-season floral diversity is typically high, meaning that resource limitation is generally most pronounced for early-spring bees whose foraging options are tightly constrained by plant phenology (Forrest & Thomson, 2011). Thus, understanding seasonal variation in floral resource availability and nutritional quality is essential for interpreting diet effects in wild bees and for designing conservation and restoration strategies that support species with different activity periods (Splitt et al., 2021; Timberlake et al., 2019).

Historically, arboreal pollen from some wind-pollinated trees such as poplars (*Populus* spp.) was overlooked due to presumed lower accessibility and sampling difficulties compared to herbaceous species,

although their nutritional value may indeed be significant (Bentrup et al., 2021; Westreich et al., 2023). Recent work has shown that bees do make use of these pollen sources, suggesting that wind-pollinated trees can contribute nutritionally even if they are not traditionally regarded as pollinator plants (Ghosh et al., 2023; Westreich et al., 2023). In addition to wind-pollinated taxa, flowering trees such as apple, pear, redbud and serviceberry also provide high-quality pollen resources and are routinely visited by bees in temperate systems (Ostaff et al., 2015; Wu et al., 2021).

Ceratina calcarata is particularly suitable as a model organism for nutritional ecology studies due to its dietary flexibility, broad floral preferences and ease of rearing under controlled laboratory conditions (Lawson et al., 2017; Shell & Rehan, 2018). This species emerges in the spring and remains active through the summer and into early fall due to its extended provisioning period, allowing individuals to encounter a wide range of floral resources and thus reflect nutritional demands across seasonally changing environments (Rehan & Richards, 2010; Tucker & Rehan, 2016).

Despite increased attention toward the nutritional ecology of flagship bee species such as honey bees and bumble bees, there remains a significant knowledge gap regarding the nutritional ecology of solitary, non-corbiculate wild bees like *C. calcarata*. Lawson et al. (2020) provided insight into how pollen diet composition influences *C. calcarata* development. Using clover and rose pollen, they showed that larvae raised on diets with higher clover content developed faster and emerged as larger adults, whereas those raised on diets dominated by rose pollen were smaller and developed more slowly. Our study complements this work by shifting the focus to wild pollen sources that bees encounter early in the season and by characterizing their naturally occurring macronutrient composition to evaluate how ecologically relevant diets shape larval physiology. This research explicitly evaluates how pollen macronutrients, particularly proteins, lipids and fatty acid ratios, influence solitary bee survival, larval development and adult body size. We specifically quantify the nutritional profiles of two ecologically relevant pollen sources, black poplar (*Populus nigra*) and dandelion (*Taraxacum officinale*) and assess the physiological consequences of consuming these pollens individually and as mixed diets.

Given that some early-spring tree pollens used by bees, such as willow and maple, can contain relatively higher protein levels compared to other early-season floral resources, we hypothesize that bees fed exclusively on black poplar pollen will exhibit improved physiological and developmental outcomes compared to bees reared exclusively on dandelion pollen (Cook et al., 2003; Roulston & Cane, 2000). Additionally, acknowledging the nutritional complementarity commonly observed in polyfloral diets, we predict intermediate physiological and developmental outcomes for bees reared on mixed pollen diets, particularly 50:50 blends of poplar and dandelion (Eckhardt et al., 2014; Vaudo et al., 2016). Intermediate performance is anticipated because dandelion pollen is documented as a nutritionally suboptimal resource, whereas black poplar provides a comparatively richer nutrient profile (Stephen et al., 2024). Dandelion pollen is especially controversial in pollinator nutrition because, despite being widely accessible during

periods of scarcity, its low protein content, poor amino acid composition and spiny exine structure may limit its nutritional value, digestibility and larval growth potential (Anderegg et al., 2021; Fan et al., 2018; Peng et al., 1985). Ultimately, this study provides a focused assessment of how variation in naturally occurring pollen sources influences larval development, survival and adult body size in *C. calcarata*, contributing to a broader understanding of nutritional ecology in early-season solitary bees.

MATERIALS AND METHODS

Nest collection, dissection and brood extraction

Raspberry canes (*Rubus idaeus*) were zip-tied to bamboo stakes and placed at several sampling sites around York University in Toronto, Canada, during May 2024 to facilitate colonization by *C. calcarata* for subsequent laboratory rearing experiments. Nest collections occurred throughout June and early July. Collected stems were carefully split lengthwise to expose internal nest structures and brood cells. Each brood cell was recorded following the developmental stage classifications (egg, larva, pupa) established by Rehan and Richards (2010). To ensure developmental consistency and experimental control, only freshly laid eggs were selected for subsequent rearing experiments. Brood extraction was performed under sterile conditions using forceps, and eggs were randomly assigned to experimental diet treatments to ensure unbiased distribution across dietary groups.

Pollen diet preparation and larval rearing

Dandelion (*T. officinale*) and black poplar (*P. nigra*) pollen were sourced from Stallergenes Greer Laboratories (Charlotte, NC, USA). Five artificial pollen diets were formulated to represent a range of macronutrient compositions, consisting of *Taraxacum*-to-*Populus* pollen weight ratios of 100:0 ($n = 10$), 75:25 ($n = 12$), 50:50 ($n = 15$), 25:75 ($n = 12$) and 0:100 ($n = 15$), alongside a control group of naturally provisioned pollen ($n = 23$). Each pollen mixture was homogenized with an equal proportion (1:1) of sugar-water solution (prepared with lab-grade sucrose dissolved in double-distilled water) to mimic natural nectar-pollen textures used by *C. calcarata* females when provisioning larvae (Lawson et al., 2017, 2020). This step was applied only to the artificial diets; naturally collected provisions were left unaltered. Standardized 4-g pollen patties were prepared and then subdivided into precisely weighed 15 mg (± 2 mg) pollen balls to simulate typical maternal provisioning in nature (Lawson et al., 2017). Pollen balls were individually weighed using a high-precision analytical balance (Mettler) and stored at 4°C in airtight containers to prevent moisture loss prior to use. For the control diet treatment, pollen balls naturally provisioned by maternal bees in collected nests were carefully extracted, individually weighed (15 ± 2 mg) and transferred directly into wells to serve as a baseline comparison for artificially prepared diets.

Larvae were placed individually into sterile 48-well culture plates and incubated at a constant 25°C using a dark Percival incubator. Brood development was monitored and recorded every 2 days, categorizing 19 developmental stages according to established protocols (Rehan & Richards, 2010). Upon emergence into adulthood, bees were flash-frozen at −80°C for later measurements. Mature adult bees were assessed under a calibrated microscope to measure head width (mm) using an ocular micrometre, and their sex was confirmed based on abdominal segment counts (females possess six segments; males possess seven; Rehan & Richards, 2010). Adult body mass was determined using an analytical balance (precision ±0.01 mg).

Macronutrient analysis of pollen

The macronutrient compositions of 100% dandelion and 100% black poplar pollen diets were analysed to quantify protein, carbohydrate (sugar), amino acids and fatty acids. To standardize dry weight, pollen samples were dried for 4 days prior to analyses. Sugar content was assessed by suspending pollen samples in 200-μL distilled water, centrifuging at 2500 rpm for 5 min and measuring soluble sugars in the supernatant using a handheld refractometer, expressed as degrees Brix (°Bx). Protein content was determined through nitrogen combustion analysis with thermal conductivity detection (Carl-Erba nitrogen analyser), with total protein calculated using a standard nitrogen-to-protein conversion factor of 6.25 (Roulston & Cane, 2000).

Amino acid and fatty acid analyses followed the protocol described by Stephen et al. (2024). Briefly, pollen samples were mixed with phosphate-buffered saline, homogenized via sonication and vortexed. For non-esterified fatty acid (NEFA) analysis, a 10 mg aliquot was spiked with isotopic internal standards, extracted, dried and reconstituted before analysis. Quantification of 12 NEFAs was performed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) on a Thermo Quantum Ultra triple-quadrupole mass spectrometer interfaced with a Waters Acquity system, using negative electrospray ionization in selected-reaction-monitoring (SRM) mode. For AA profiling, 2 mg of pollen was treated with isotopic internal standards and subjected to protein precipitation using chilled methanol. The supernatant was derivatized using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC), following the Waters MassTrak™ protocol, and analysed using LC–MS/MS in SRM mode. A 10-point calibration curve was constructed using derivatized standards. Quantified analytes were expressed in nmol/mg pollen.

Lipid extraction and quantification

Total lipid content in adult bees was measured using a modified vanillin–phosphoric acid colorimetric assay (Toth & Robinson, 2005). Briefly, bees were air-dried under a fume hood for 3 days and homogenized in 5 mL of chloroform: methanol (2:1, v/v) using a sterile pestle to break down tissue and release lipids. Lipids were extracted for at least 12 h at room temperature. Extracts were centrifuged

at 14,000 rpm for 10 min (Eppendorf 5425), and the clear supernatant was transferred to clean, pre-weighed glass tubes. After adding petroleum ether to 2-mL total volume, solvents were evaporated overnight, and lipid mass was calculated gravimetrically.

For colorimetric analysis, lipids were resuspended in 200 μL of concentrated sulphuric acid, heated, cooled and reacted with 4 mL of vanillin–phosphoric acid reagent. Absorbance was measured at 525 nm using a BioTek Synergy 2 microplate reader. A cholesterol standard curve (1 mg/mL in petroleum ether) was used for quantification. Lipid content was normalized to dry bee mass for all samples.

Statistical analyses

All statistical analyses were conducted in R version 4.3.1 (R Core Team, 2022). Data were first assessed for normality and homogeneity of variance using Shapiro–Wilk tests and Levene's tests, respectively (using the car package in R; Fox & Weisberg, 2019). Parametric data (head width, body mass and lipid content) were analysed using one-way analysis of variance (ANOVAs), followed by Tukey's post hoc tests. Non-parametric data, including development duration and sugar concentration, were analysed with Kruskal–Wallis tests followed by Dunn's multiple comparison tests with Benjamini–Hochberg *p*-value correction (using the FSA package; Ogle et al., 2021). Kaplan–Meier survival analyses were performed to evaluate diet effects on survival, and diet groups were compared using log-rank tests (survival package; Therneau & Grambsch, 2001). Macronutrient differences between pollen types (e.g., protein, amino acids, fatty acids, sugar) were analysed using independent-sample *t*-tests for normally distributed data or Wilcoxon rank-sum tests for non-normal data. All statistical significance was assessed at $\alpha = 0.05$, and assumptions were verified prior to model selection.

RESULTS

Macronutrient composition

The macronutrient profiles of black poplar and dandelion pollen revealed significant differences in protein, sugar, amino acid and fatty acid content (Figure 1, Table 1). All values represent pollen only measurements expressed as per mg of dry pollen. Black poplar pollen contained substantially higher sugar (9.6 mg) compared to dandelion pollen (4.3 mg), as well as markedly higher protein concentrations (52.8 vs. 5.6 nmols/mg, respectively).

Amino acid analysis indicated higher concentrations of several essential amino acids in black poplar pollen compared to dandelion pollen. Specifically, arginine (17.3 nmols/mg), histidine (10.6 nmols/mg), leucine (4.6 nmols/mg), isoleucine (3.7 nmols/mg), lysine (3.1 nmols/mg) and phenylalanine (1.3 nmols/mg) were notably more abundant in black poplar pollen (all amino acids *p* = 0.071; Table 1). Fatty acid profiling revealed higher levels of essential fatty acids, especially linolenic acid (16.6 nmols/mg) and linoleic acid (3.1 nmols/

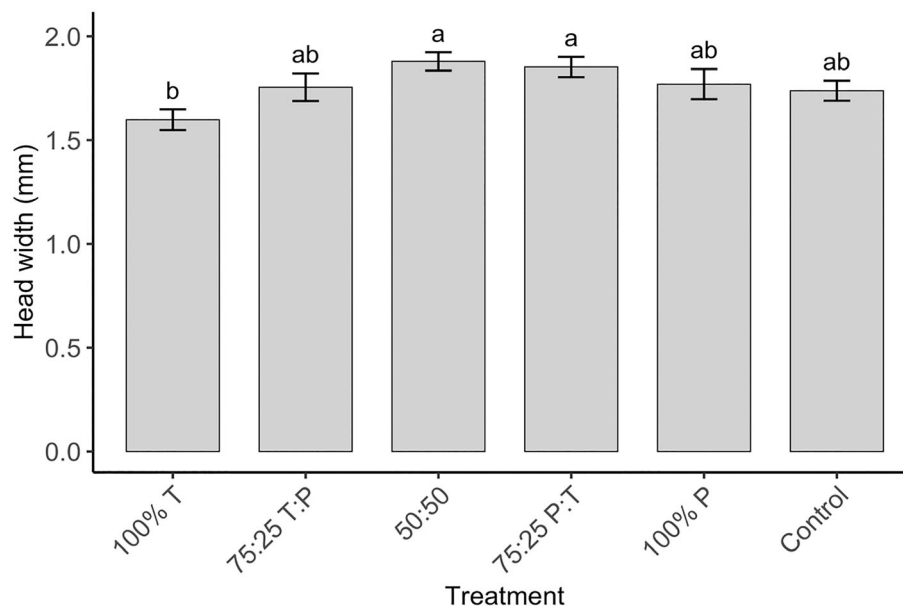


FIGURE 1 Mean head width (mm) of *Ceratina calcarata* reared on different pollen diet treatments. Treatments are abbreviated as follows: '100% T' (representing 100% *Taraxacum officinale*), '75:25 T:P' (75% *T. officinale*, 25% *Populus nigra*), '50:50', '75:25 P:T' (75% *P. nigra*, 25% *T. officinale*), '100% P' (100% *P. nigra*) and 'Control'. Letters above bars indicate groupings from post hoc comparisons, with bars sharing the same letter not differing significantly. Data from both sexes were combined because no sex differences were detected.

TABLE 1 Macronutrient composition of *Populus nigra* (Black Poplar) and *Taraxacum officinale* (dandelion) pollen.

Group	Macronutrient	<i>T. officinale</i>	<i>P. nigra</i>	fold_diff	log2_fold_diff
Carbohydrate (mg)	Sugar (sucrose)	4.30	9.55	2.22	1.15
Essential amino acid (nmols/mg)	Arginine	0.12	17.33	144.42	7.17
	Histidine	0.89	10.61	11.92	3.58
	Isoleucine	0.05	3.74	74.8	6.22
	Leucine	0.12	4.63	38.58	5.27
	Lysine	0.10	3.11	31.10	4.96
	Methionine	0.01	0.35	35	5.13
	Phenylalanine	0.07	1.26	18	4.17
	Threonine	0.07	7.65	109.29	6.77
	Tryptophan	0.03	1.61	53.67	5.75
	Valine	0.13	7.40	56.92	5.83
	Linoleic	6.23	3.11	0.50	-1.00
Essential fatty acid (nmols/mg)	Linolenic	23.52	16.58	0.70	-0.50
Non-esterified fatty acid (nmols/mg)	Arachidonic	0.00	0.00	N/A	N/A
	Myristic	0.04	0.08	2	1
	Oleic	0.95	0.17	0.18	-2.48
	Palmitoleic	0.00	0.00	N/A	N/A
	Palmitic	8.38	6.86	0.82	-0.29
	Palmitoleic	0.04	0.06	1.5	0.58
Protein (nmols/mg)	Protein	5.56	52.81	9.50	3.25

Note: All values are reported per mg of dry pollen, except carbohydrate, which reflects mg sucrose-equivalent per mg pollen based on refractometer readings. 'fold_diff' represents the ratio of black poplar to dandelion for each nutrient, while 'log₂ fold_diff' is the base-2 logarithm of that ratio and is included to facilitate comparison across nutrients with large differences in magnitude. Groups include essential amino acids (nmols/mg), essential fatty acids (nmols/mg), non-essential fatty acids (NEFAs) (nmols/mg), protein and carbohydrate (mg).

Abbreviation: N/A, not applicable.

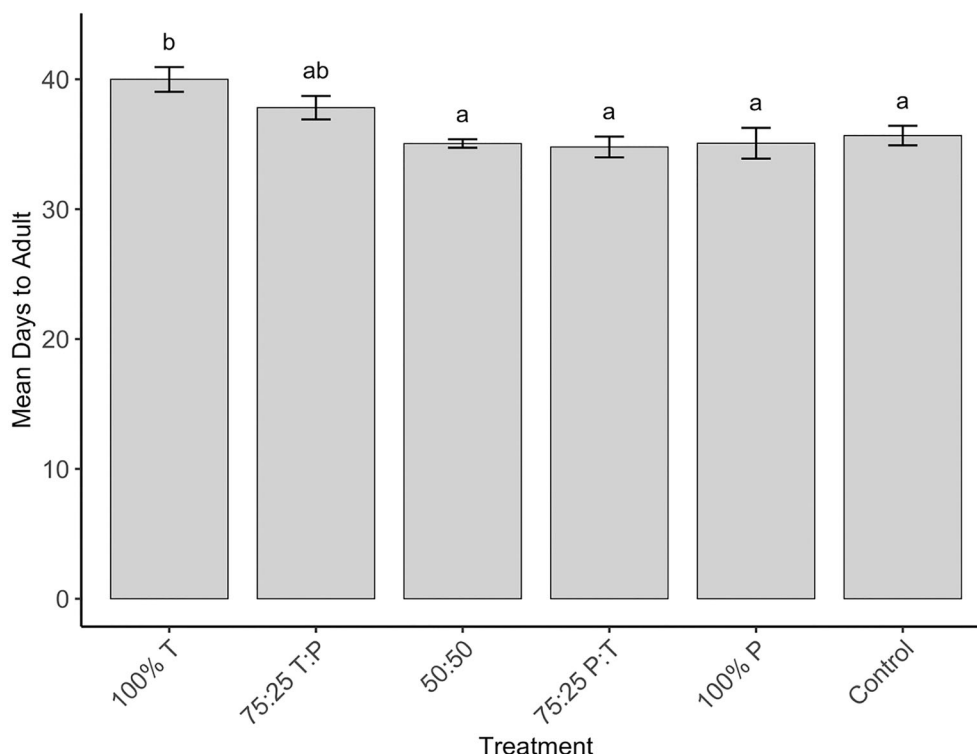


FIGURE 2 Mean days to adult for *Ceratina calcarata* reared on different pollen diet treatments. Treatments are abbreviated as follows: '100% T' (representing 100% *Taraxacum officinale*), '75:25 T:P' (75% *T. officinale*, 25% *Populus nigra*), '50:50', '75:25 P:T' (75% *P. nigra*, 25% *T. officinale*), '100% P' (100% *P. nigra*) and 'Control'. Letters above bars indicate groupings from post hoc comparisons, with bars sharing the same letter not differing significantly.

mg), in black poplar pollen compared to dandelion pollen (23.5 and 6.2 nmols/mg, respectively).

Body size and developmental rates

A total of 215 bee eggs were collected and reared across six dietary treatments: control ($n = 23$), 50:50 mixed pollen ($n = 15$), 75:25 black poplar: dandelion ($n = 15$), 100% black poplar ($n = 12$), 25:75 black poplar: dandelion ($n = 12$) and 100% dandelion ($n = 10$). Of these, 87 bees successfully emerged as adults.

Diet significantly affected bee head width ($F = 2.8$, $df = 5,80$, $p = 0.024$; Figure 1). Bees raised on the 50:50 mixed diet had significantly wider heads compared to those on the 100% dandelion diet (mean difference = 0.28 mm, Tukey honestly significant difference [HSD], $p = 0.017$). Bees fed the 75:25 black poplar: dandelion diet also had significantly larger head widths than bees on the 100% dandelion diet (mean difference = 0.25 mm, $p = 0.042$). No other pairwise comparisons were statistically significant (all $p > 0.05$; Table S1). Mean head width was not significantly different between males (1.77 mm) and females (1.78 mm; $p > 0.05$).

Developmental time varied significantly among dietary treatments ($F = 4.8$, $df = 5,78$, $p < 0.001$; Figure 2). Bees raised on the 75:25 black poplar:dandelion diet developed fastest (mean = 34.8 days), closely followed by those fed 50:50 (mean = 35.1 days) and 100% black poplar

diets (mean = 35.1 days). Bees raised on 100% dandelion diets exhibited the slowest development (mean = 40.0 days), significantly slower compared to bees on 100% black poplar (mean difference = 4.9 days; $p = 0.009$), 50:50 diets (mean difference = 4.9 days; $p = 0.004$) and 75:25 black poplar:dandelion diets (mean difference = 5.2 days; $p = 0.002$). Control bees (mean = 35.7 days) also matured significantly faster than those fed exclusively dandelion pollen (mean difference = 4.3 days; $p = 0.009$; Table S2).

Survivorship, body mass and lipid content

Kaplan–Meier survival analysis indicated significant differences among diet treatments (log-rank test, $p = 0.002$; Figure 3). Bees fed exclusively dandelion pollen exhibited greater survival and prolonged developmental durations during larval stages, indicative of delayed maturation. Conversely, bees fed 100% black poplar and mixed diets matured faster but showed comparatively lower overall survival probabilities. Control and mixed pollen diet treatments showed intermediate survival durations.

Bee body mass was significantly influenced by diet ($F = 4.9$, $df = 5,80$, $p < 0.001$; Figure 4). Mean body mass was 13.3 mg for males and 12.3 mg for females, although sex was not a significant factor in this model. Bees fed the 25:75 black poplar: dandelion diet were significantly heavier than bees fed 100% dandelion diets (mean difference = 6.3 mg;

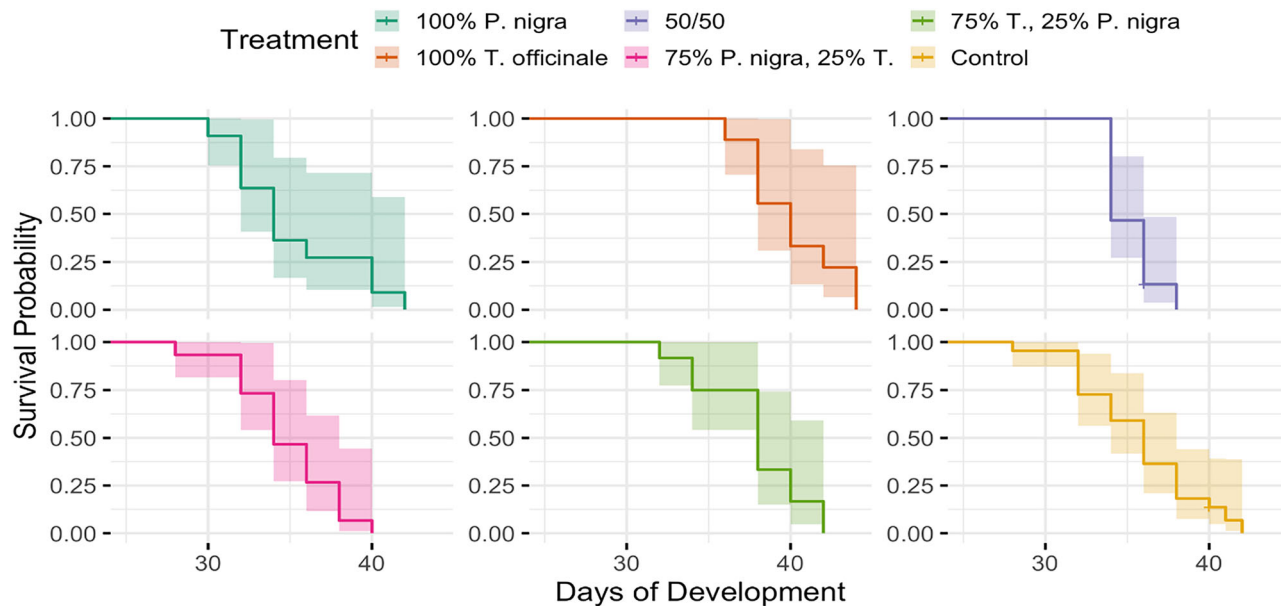


FIGURE 3 Kaplan–Meier survival curves showing the probability of reaching adulthood over days of development for *Ceratina calcarata* reared on six different pollen diet treatments. Each panel corresponds to one of the following treatments: ‘100% T’ (representing 100% *Taraxacum officinale*), ‘75:25 T:P’ (75% *T. officinale*, 25% *Populus nigra*), ‘50:50’, ‘75:25 P:T’ (75% *P. nigra*, 25% *T. officinale*), ‘100% P’ (100% *P. nigra*) and ‘Control’. Shaded areas represent the 95% confidence intervals for each survival curve.

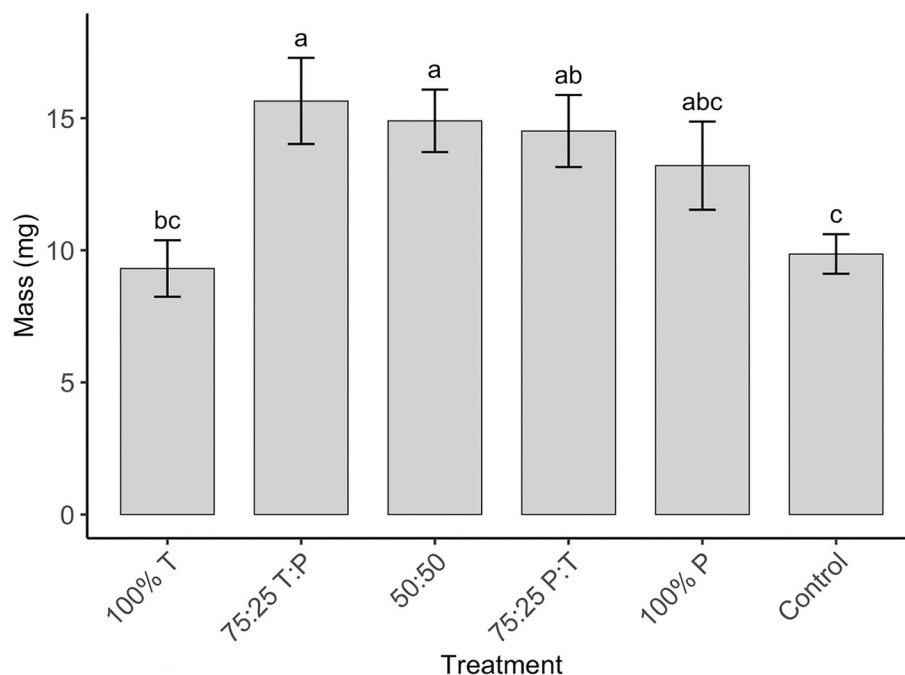


FIGURE 4 Mean body mass (mg) of *Ceratina calcarata* reared on different pollen diet treatments. Treatments are abbreviated as follows: ‘100% T’ (100% *Taraxacum officinale*), ‘75:25 T:P’ (75% *T. officinale*, 25% *Populus nigra*), ‘50:50’, ‘75:25 P:T’ (75% *P. nigra*, 25% *T. officinale*), ‘100% P’ (100% *P. nigra*) and ‘Control’. Data from both sexes were combined because no sex differences were observed. Letters above bars indicate groupings from post hoc comparisons, with bars sharing the same letter not differing significantly.

Tukey HSD, $p = 0.025$). The 50:50 diet also resulted in significantly greater body mass compared to bees consuming only dandelion pollen (mean difference = 5.6 mg; $p = 0.047$). Control bees showed significantly lower body mass than bees fed the 25:75 diet (mean

difference = 5.8 mg; $p = 0.010$), 50:50 diet (mean difference = 5.0 mg; $p = 0.020$) and 75:25 diet (mean difference = 4.7 mg; $p = 0.039$). All other comparisons were not statistically significant (Table S3).

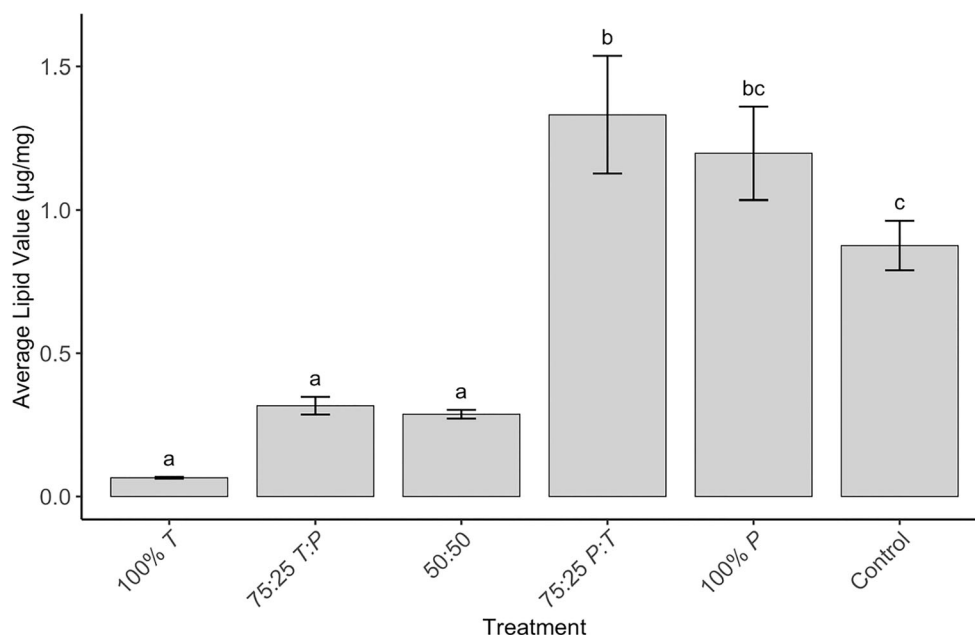


FIGURE 5 Mean lipid content (µg/mg) of *Ceratina calcarata* reared on different pollen diet treatments. Treatments are abbreviated as follows: '100% T' (100% *Taraxacum officinale*), '75:25 T:P' (75% *T. officinale*, 25% *Populus nigra*), '50:50', '75:25 P:T' (75% *P. nigra*, 25% *T. officinale*), '100% P' (100% *P. nigra*) and 'Control'. Letters above bars indicate groupings from post hoc comparisons, with bars sharing the same letter not differing significantly.

Lipid content also varied significantly across dietary treatments ($F = 9.2$, $df = 10,94$, $p < 0.001$; Figure 5). Bees fed the 75:25 black poplar: dandelion diet had the highest lipid levels (mean = 1.33 µg), significantly higher compared to those consuming 100% dandelion (0.07 µg; $p < 0.001$), 50:50 diets (0.29 µg; $p < 0.001$) and 25:75 diets (0.32 µg; $p < 0.001$). Bees fed exclusively black poplar pollen also had significantly higher lipid levels than those fed exclusively dandelion (mean difference = 1.13 µg; $p < 0.001$), 50:50 diets (mean difference = 0.91 µg; $p = 0.001$) and 25:75 diets (mean difference = 0.88 µg; $p = 0.003$). Control bees exhibited intermediate lipid content (0.88 µg), significantly higher than bees consuming 100% dandelion pollen (mean difference = 0.81 µg; $p = 0.003$) but lower than bees consuming the 75:25 diet (mean difference = 0.46 µg; $p = 0.219$, not significant, Table S4).

DISCUSSION

Here we profiled the nutritional composition of black poplar and dandelion pollen and subsequently fed bees diets formulated from these sources in order to assess their impact on development, body size, lipid reserves and overall survival to adulthood. We began by quantifying key macronutrients, fatty acids and essential amino acids in each pollen type and found that black poplar pollen contains significantly higher levels of protein, sugar and essential amino acids such as arginine, leucine, isoleucine, lysine and histidine compared to dandelion pollen. These nutrient differences provided the basis for our feeding trials, in which bees were reared on either a monofloral diet of black poplar pollen, a monofloral diet of dandelion pollen or on mixed diets with varying proportions of the two pollen types. Our results showed

that bees consuming the protein-rich black poplar diet developed more rapidly, attained larger body sizes and accumulated greater lipid reserves than bees fed solely on dandelion pollen. While these results are consistent with the elevated protein and amino acid content of black poplar pollen, additional factors such as fatty acid composition or variation in pollen consumption may also contribute to these outcomes and should be explored in future work. In contrast, bees reared on dandelion pollen showed prolonged developmental periods and reduced body mass, indicating that the lower protein content and less favourable amino acid profile may contribute to limited growth and overall survival. Bees fed mixed diets exhibited intermediate outcomes, suggesting that dietary mixing can partially mitigate the limitations of a single-source, low-quality pollen diet. Overall, these findings show that natural variation in pollen quality can influence larval development, body size, lipid reserves and survival in *C. calcarata*. Our results demonstrate that some early-season pollens provide stronger nutritional support than others, with black poplar outperforming dandelion in this study. Rather than identifying a single pollen source as essential, these outcomes highlight the value of ensuring access to nutritionally rich early-season flowers when designing conservation and restoration strategies.

Macronutrient composition, development and bee nutrition

The nutritional quality of pollen profoundly influences the development, health and survival of bees (Roulston & Cane, 2000; Vaudo et al., 2015). In our study, we observed substantial differences in the

macronutrient composition of black poplar pollen compared to dandelion pollen, emphasizing the role that dietary macronutrients play in bee life history traits. Specifically, the higher protein and sugar content of black poplar pollen aligns with previous research demonstrating considerable nutritional variation among pollen sources (Di Pasquale et al., 2013; Filipiak & Filipiak, 2020; Vaudo et al., 2015). Such differences likely underpin the variations we noted in developmental rates, body mass and lipid reserves.

Protein-rich pollen sources have been associated with enhanced larval growth, survival and subsequent adult fitness in bees (Hendriksma et al., 2019; Vaudo et al., 2015). Honey bees (*A. mellifera*) that consume protein-deficient diets experience delayed brood development, reduced worker performance and elevated mortality (Brodschneider & Crailsheim, 2010; Di Pasquale et al., 2013). Consistent with these findings, we observed substantial developmental delays and smaller adult size in bees reared on protein-deficient dandelion pollen compared to the protein-rich black poplar pollen. These results suggest that pollen protein content and sugar availability contribute to larval growth and developmental rates, which aligns with previous work demonstrating that adequate protein intake is essential for brood development (Brodschneider & Crailsheim, 2010; Vaudo et al., 2015). While protein and sugars likely played major roles in the outcomes observed here, the two pollen types also differed in fatty acid composition, indicating that multiple nutrients, rather than protein alone, probably contributed to the developmental patterns we recorded.

This link between dietary macronutrients and developmental outcomes may be explained by conserved molecular pathways such as the Target of Rapamycin (TOR) signalling pathway, a central regulator of cell growth, nutrient sensing and metabolism (Delescluse et al., 2024; Obata & Miura, 2024). High-protein and amino acid-rich diets, such as black poplar pollen, are likely to stimulate TOR activation, promoting faster larval growth, earlier pupation and accelerated adult eclosion (Honda et al., 2011; Ma et al., 2016). In contrast, bees fed low-protein diets such as dandelion pollen may experience reduced TOR signalling, resembling a caloric restriction-like state, resulting in delayed development and prolonged time to adulthood (Gallinetti et al., 2013; Obata & Miura, 2024).

Branched-chain amino acids (BCAAs), which include leucine, isoleucine and valine, are among the most effective activators of the TOR pathway and are regulators of larval development and metabolic homeostasis (Honda et al., 2011; Ma et al., 2016). Although our study did not specifically quantify BCAA concentrations in the pollen types tested, prior research demonstrates that BCAA abundance can significantly influence larval growth rates and developmental timing in insects given their essential roles in protein synthesis, tissue formation and metabolic regulation during development (Tomberlin et al., 2023). Accordingly, differences in BCAA profiles between black poplar and dandelion pollen may partially explain the observed differences in developmental rates. Future research incorporating direct BCAA quantification would further clarify the specific macronutrient mechanisms underlying bee development and fitness outcomes observed in this study.

Moreover, proteins and amino acids are especially imperative for larval bee growth, immune function and metabolic processes (De Groot, 1953; Hendriksma et al., 2014). Black poplar pollen contained higher levels of essential amino acids, including arginine, leucine, isoleucine, lysine and histidine, likely providing improved nutrition that enhanced larval growth rates and adult body size. Bees exhibit selective preferences for pollen rich in these essential amino acids, such as isoleucine and leucine, which are necessary for muscle development, metabolism and reproductive success (Hendriksma et al., 2014; Leonhardt et al., 2011). Consequently, the marked disparity between amino acid content in black poplar and dandelion pollen closely corresponds to the observed differences in developmental duration and adult morphology.

Influence of pollen quality on body size and fitness

Body size is a pivotal trait that influences bee survival, foraging efficiency and reproductive success (Bosch & Vicens, 2006; Goulson et al., 2015). Our results demonstrated that bees fed black poplar pollen or pollen mixtures (50:50 and 75:25 black poplar: dandelion) developed larger head widths and greater body mass than bees reared on dandelion pollen alone. These findings echo previous work demonstrating the importance of protein and lipid availability in shaping adult body size (Hendriksma & Shafir, 2016; Vaudo et al., 2015). Larger bees typically exhibit enhanced flight performance, more efficient foraging and improved thermoregulatory capacity (Glass & Harrison, 2022). Therefore, the pronounced body size advantage observed in bees consuming protein-rich diets, such as black poplar pollen, may have substantial ecological implications by potentially boosting reproductive output and colony success (Brasil et al., 2023; Ghramh & Khan, 2023).

Enhanced body mass often correlates with higher lipid storage, which is useful for long-term survival, especially during periods of seasonal food scarcity (Vaudo et al., 2016; Verrier et al., 2024). Ample lipid reserves can improve bees' resilience to environmental stressors and aid in energy-intensive tasks such as foraging (Deeter et al., 2023). In our study, the notable increase in lipid content in bees fed black poplar pollen underlines the importance of diets that supply not only high protein levels but also balanced macronutrients. By contrast, the smaller body sizes and potentially reduced lipid reserves in dandelion-fed bees may limit their foraging range and efficiency.

Beyond immediate survival and energy considerations, larger body size can also confer advantages related to competitive foraging and broader habitat utilization. Bees with larger body sizes often have higher flight capacity and can explore a wider range of floral resources, which may facilitate improved bee colony resource intake (Greenleaf et al., 2007). Additionally, body size can influence thermoregulatory strategies, a factor especially relevant in cooler climates or early-spring conditions when resource availability is low (Heinrich, 1979). In contrast, smaller body sizes of bees derived from protein-deficient diets may face greater challenges under such conditions, potentially exhibiting reduced tolerance to temperature

fluctuations and increased susceptibility to competition from other pollinators (Vanderplanck et al., 2019). These findings collectively illustrate how pollen quality, by modulating body size and lipid reserves, can cascade through multiple facets of bee ecology and life history.

Survivorship, developmental trade-offs and dietary mixing

Our survivorship analyses revealed distinct nutritional trade-offs linked to pollen quality. Bees that consumed protein-poor dandelion pollen exhibited significantly prolonged developmental periods, echoing studies showing that nutritional stress can delay insect larval maturation (Di Pasquale et al., 2013; Naug, 2009; Vanderplanck et al., 2020). While these extended larval durations may serve as compensatory mechanisms enabling bees to extract more nutrients from suboptimal pollen, they can also increase vulnerability to parasitism and environmental stress (Alaux et al., 2010; Blanckenhorn, 2000; Schmid-Hempel, 2005). In contrast, although bees reared on black poplar pollen developed faster, rapid maturation can sometimes entail its own costs, such as reduced survivorship later in life, highlighting the complex trade-offs that govern bee fitness.

Mixed pollen diets (e.g., 50:50 and 75:25 black poplar: dandelion) led to improved body size, lipid reserves and developmental rates relative to monofloral diets. This observation supports a growing body of evidence indicating that bees generally perform optimally when their diets are diverse (Eckhardt et al., 2014; Filipiak, 2018; Hendriksma et al., 2014). Polyfloral pollen diets have consistently been associated with enhanced survival, stronger pathogen resistance and elevated colony productivity (Filipiak et al., 2017; Topal et al., 2022). Our data align with these findings by demonstrating that partial inclusion of protein-rich black poplar pollen in the diet can mitigate the developmental delays and lower body mass characteristic of dandelion-only diets. Moreover, this dietary mixing strategy likely broadens the range of essential amino acids and micronutrients available to bees, helping them balance their nutrient intake in the face of fluctuating floral resources.

Importantly, the benefits of dietary mixing may extend beyond immediate gains in growth and survivorship to influence long-term bee colony resilience. Bees that have access to a wider array of pollen sources may better regulate nutrient intake according to their changing physiological requirements (Wright et al., 2018). Such flexibility could enhance collective disease resistance, improve reproductive outcomes and facilitate adaptation to variable environmental conditions (Hendriksma et al., 2019). By buffering against the risks associated with single-source pollen diets, such as nutrient imbalances or the potential for exposure to localized pesticides, mixed diets may be an important factor in stabilizing bee populations over multiple generations. These findings highlight the adaptive significance of maintaining floral diversity, especially in landscapes where seasonal or anthropogenic changes can abruptly alter the availability of high-quality pollen sources.

Ecological and conservation implications of tree pollen

Pollinator conservation initiatives have traditionally centred on promoting floral diversity of insect-pollinated plants; it can be argued that they often disregard wind-pollinated trees due to presumed nutritional limitations. However, a growing body of evidence points to the ecological necessity of tree pollen and emphasizes the ecological role of arboreal pollen, particularly in early spring, when herbaceous plants have not yet begun flowering (Ghosh et al., 2023; Ostaff et al., 2015; Splitt et al., 2021). Our results highlight that wind-pollinated trees can supply nutritionally valuable pollen during seasonal bottlenecks. Doing so could bolster pollinator health by buffering seasonal pollen deficits and mitigating the adverse impacts of phenological mismatches driven by climate change (Eckhardt et al., 2014; Splitt et al., 2021). Additionally, our results support the idea that wind-pollinated trees, including *Populus* species, contribute meaningfully to the nutritional landscape of bees in temperate regions (Ostaff et al., 2015; Westreich et al., 2023).

Moreover, incorporating tree pollen sources into restoration strategies can provide a consistent supply of essential nutrients, thereby supporting broader pollinator resilience. Such actions may be particularly beneficial in regions where herbaceous plants alone cannot meet the nutritional demands of early-emerging bee species.

Future research directions

Future research should focus on identifying which early-season pollen sources bees rely on most frequently in natural habitats and on assessing the long-term fitness consequences of diets that include wind-pollinated pollen, examining how these resources influence reproduction, immunity and stress tolerance across development. Additionally, experimental evaluation of bee foraging preferences under field conditions would clarify how nutritional quality affects floral resource selection and pollination efficiency (Vaudo et al., 2020). Integrating molecular analyses to identify potential metabolic biomarkers related to dietary stress responses could enhance our understanding of nutritional ecology in bees (Amsalem & Hefetz, 2010; Brodschneider & Crailsheim, 2010; Wang et al., 2016). Further studies should also explore geographic and seasonal variation in pollen nutritional quality, particularly examining interactions between pollen chemistry, bee foraging behaviour and environmental stressors such as pesticides and pathogens (Arien et al., 2018; Ghosh et al., 2023). Such approaches would significantly inform conservation management strategies aimed at bolstering pollinator populations facing nutritional stress and habitat loss.

Our findings demonstrate the importance of pollen nutritional composition in shaping bee development, survival and overall fitness, with direct implications for conservation planning. Recognizing the previously undervalued nutritional value of wind-pollinated tree species such as black poplar broadens our perspective on bee nutritional ecology and provides tangible strategies for maintaining robust pollinator populations in a changing world.

AUTHOR CONTRIBUTIONS

Khara W. Stephen: Conceptualization; investigation; writing – original draft; methodology; visualization; formal analysis; data curation. **Sandra M. Rehan:** Conceptualization; funding acquisition; writing – review and editing; validation; project administration; supervision; resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in the supplementary material available on Dryad: <https://doi.org/10.5061/dryad.4mw6m90r7>.

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REFERENCES

- Alaux, C., Ducloz, F., Crauser, D. & Le Conte, Y. (2010) Diet effects on honeybee immunocompetence. *Biology Letters*, 6, 562–565. Available from: <https://doi.org/10.1098/rsbl.2009.0986>
- Amsalem, E. & Hefetz, A. (2010) The appeasement effect of sterility signaling in dominance contests among *Bombus terrestris* workers. *Behavioral Ecology and Sociobiology*, 64, 1685–1694. Available from: <https://doi.org/10.1007/s00265-010-0982-4>
- Anderegg, W.R.L., Abatzoglou, J.T., Anderegg, L.D.L., Bielory, L., Kinney, P.L. & Ziska, L. (2021) Anthropogenic climate change is worsening North American pollen seasons. *Proceedings of the National Academy of Sciences of the United States of America*, 118, e2013284118. Available from: <https://doi.org/10.1073/pnas.2013284118>
- Arien, Y., Dag, A. & Shafir, S. (2018) Omega-6:3 ratio more than absolute lipid level in diet affects associative learning in honey bees. *Frontiers in Psychology*, 9, 1001. Available from: <https://doi.org/10.3389/fpsyg.2018.01001>
- Arien, Y., Dag, A., Yona, S., Tietel, Z., Lapidot Cohen, T. & Shafir, S. (2020) Effect of diet lipids and omega-6:3 ratio on honey bee brood development, adult survival and body composition. *Journal of Insect Physiology*, 124, 104074. Available from: <https://doi.org/10.1016/j.jinsphys.2020.104074>
- Arien, Y., Dag, A., Zarchin, S., Masci, T. & Shafir, S. (2015) Omega-3 deficiency impairs honey bee learning. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 15761–15766. Available from: <https://doi.org/10.1073/pnas.1517375112>
- Bentrop, G., Hopwood, J., Adamson, N.L., Powers, R. & Vaughan, M. (2021) The role of temperate agroforestry practices in supporting pollinators. In: Udawatta, R.P. & Jose, S. (Eds.) *Agroforestry and ecosystem services*. Cham: Springer International Publishing, pp. 275–304. Available from: https://doi.org/10.1007/978-3-030-80060-4_11
- Blanckenhorn, W.U. (2000) The evolution of body size: what keeps organisms small? *The Quarterly Review of Biology*, 75, 385–407. Available from: <https://doi.org/10.1086/393620>
- Bosch, J. & Vicens, N. (2006) Relationship between body size, provisioning rate, longevity and reproductive success in females of the solitary bee *Osmia cornuta*. *Behavioral Ecology and Sociobiology*, 60, 26–33. Available from: <https://doi.org/10.1007/s00265-005-0134-4>
- Brodschneider, R. & Crailsheim, K. (2010) Nutrition and health in honey bees. *Apidologie*, 41, 278–294. Available from: <https://doi.org/10.1051/apido/2010012>
- Brasil, S.N.R., Khair, M.M.R., Ayers, A.C., Huisken, J.L. & Rehan, S.M. (2023) Urban landscapes affect wild bee maternal investment and body size. *Urban Ecosystems*, 26, 1319–1329. Available from: <https://doi.org/10.1007/s11252-023-01378-0>
- 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.
- Crailsheim, K. (1992) The flow of jelly within a honeybee colony. *Journal of Comparative Physiology B*, 162, 681–689. Available from: <https://doi.org/10.1007/BF00301617>
- Crailsheim, K., Schneider, L.H.W., Hrassnigg, N., Bühlmann, G., Brosch, U., Gmeinbauer, R. et al. (1992) Pollen consumption and utilization in worker honeybees (*Apis mellifera carnica*): dependence on individual age and function. *Journal of Insect Physiology*, 38, 409–419. Available from: [https://doi.org/10.1016/0022-1910\(92\)90117-V](https://doi.org/10.1016/0022-1910(92)90117-V)
- Deeter, M.E., Snyder, L.A., Meador, C. & Corby-Harris, V. (2023) Accelerated abdominal lipid depletion from pesticide treatment alters honey bee pollen foraging strategy, but not onset, in worker honey bees. *Journal of Experimental Biology*, 226, jeb245404. Available from: <https://doi.org/10.1242/jeb.245404>
- De Groot, A.P. (1953) Protein and amino acid requirements of the honey bee. *Physiologia Comparata et Oecologia*, 3, 1–83.
- Delescluse, J., Simonnet, M.M., Ziegler, A.B., Piffaretti, K., Alves, G., Grosjean, Y. et al. (2024) A LAT1-like amino acid transporter regulates neuronal activity in the drosophila mushroom bodies. *Cells*, 13(16), 1340. Available from: <https://doi.org/10.3390/cells13161340>
- 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. Available from: <https://doi.org/10.1371/journal.pone.0072016>
- 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? *Journal of Animal Ecology*, 83, 588–597. Available from: <https://doi.org/10.1111/1365-2656.12168>
- Fan, T., Park, J.H., Pham, Q.A., Tan, E.-L., Mundargi, R.C., Potroz, M.G. et al. (2018) Extraction of cage-like sporopollenin exine capsules from dandelion pollen grains. *Scientific Reports*, 8, 6565. Available from: <https://doi.org/10.1038/s41598-018-24336-9>
- Filipiak, M. (2018) A better understanding of bee nutritional ecology is needed to optimize conservation strategies for wild bees—the application of ecological stoichiometry. *Insects*, 9, 85. Available from: <https://doi.org/10.3390/insects9030085>
- Filipiak, M., Kuszewska, K., Asselman, M., Denisow, B., Stawiarz, E., Woyciechowski, M. et al. (2017) Ecological stoichiometry of the honeybee: pollen diversity and adequate species composition are needed to mitigate limitations imposed on the growth and development of bees by pollen quality. *PLoS One*, 12, e0183236. Available from: <https://doi.org/10.1371/journal.pone.0183236>
- Filipiak, Z.M. & Filipiak, M. (2020) The scarcity of specific nutrients in wild bee larval food negatively influences certain life history traits. *Biology*, 9, 462. Available from: <https://doi.org/10.3390/biology9120462>

- Forrest, J.R.K. & Thompson, J.D. (2011) An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. *Ecological Monographs*, 81, 469–491.
- Fox, J. & Weisberg, S. (2019) *Nonlinear regression, nonlinear least squares, and nonlinear mixed models in R*. *Population*, Vol. 150, p. 200.
- Gallinetti, J., Harputlugil, E. & Mitchell, J.R. (2013) Amino acid sensing in dietary-restriction-mediated longevity: roles of signal-transducing kinases GCN2 and TOR. *Biochemical Journal*, 449, 1–10. Available from: <https://doi.org/10.1042/BJ20121098>
- Ghosh, S., Wang, Y. & Jung, C. (2023) Editorial: nutritional ecology of wild and managed bees. *Frontiers in Ecology and Evolution*, 11. Available from: <https://doi.org/10.3389/fevo.2023.1223769>
- Ghranh, H.A. & Khan, K.A. (2023) Honey bees prefer pollen substitutes rich in protein content located at short distance from the apiary. *Animals*, 13, 885. Available from: <https://doi.org/10.3390/ani13050885>
- Glass, J.R. & Harrison, J.F. (2022) The thermal performance curve for aerobic metabolism of a flying endotherm. *Proceedings of the Royal Society B*, 289, 20220298. Available from: <https://doi.org/10.1098/rspb.2022.0298>
- Goulson, D., Nicholls, E., Botías, C. & Rotheray, E.L. (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, 347, 1255957. Available from: <https://doi.org/10.1126/science.1255957>
- Graham, K.K., Milbrath, M.O., Killewald, M., Soehnen, A., Zhang, Y. & Isaacs, R. (2023) Identity and diversity of pollens collected by two managed bee species while in blueberry fields for pollination. *Environmental Entomology*, 52, 907–917. Available from: <https://doi.org/10.1093/ee/nvad072>
- Greenleaf, S.S., Williams, N.M., Winfree, R. & Kremen, C. (2007) Bee foraging ranges and their relationship to body size. *Oecologia*, 153, 589–596. Available from: <https://doi.org/10.1007/s00442-007-0752-9>
- Harris, C., Balfour, N.J. & Ratnieks, F.L.W. (2024) Seasonal variation in the general availability of floral resources for pollinators in northwest Europe: a review of the data. *Biological Conservation*, 298, 110774. Available from: <https://doi.org/10.1016/j.biocon.2024.110774>
- Heinrich, B. (1979) “Majoring” and “minoring” by foraging bumblebees, *Bombus vagans*: an experimental analysis. *Ecology*, 60, 245–255. Available from: <https://doi.org/10.2307/1937652>
- Hendriksma, H.P., Oxman, K.L. & Shafir, S. (2014) Amino acid and carbohydrate trade-offs by honey bee nectar foragers and their implications for plant–pollinator interactions. *Journal of Insect Physiology*, 69, 56–64. Available from: <https://doi.org/10.1016/j.jinsphys.2014.05.025>
- Hendriksma, H.P., Pachow, C.D. & Nieh, J.C. (2019) Effects of essential amino acid supplementation to promote honey bee gland and muscle development in cages and colonies. *Journal of Insect Physiology*, 117, 103906. Available from: <https://doi.org/10.1016/j.jinsphys.2019.103906>
- Hendriksma, H.P. & Shafir, S. (2016) Honey bee foragers balance colony nutritional deficiencies. *Behavioral Ecology and Sociobiology*, 70, 509–517. Available from: <https://doi.org/10.1007/s00265-016-2067-5>
- Honda, Y., Fujita, Y., Maruyama, H., Araki, Y., Ichihara, K., Sato, A. et al. (2011) Lifespan-extending effects of royal jelly and its related substances on the nematode *Caenorhabditis elegans*. *PLoS One*, 6, e23527. Available from: <https://doi.org/10.1371/journal.pone.0023527>
- Janz, N. & Nylin, S. (2008) The oscillation hypothesis of host-plant range and speciation. In: Brakefield, J. (Ed.) *Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects*. Oakland, CA: University of California Press, pp. 203–215. Available from: <https://doi.org/10.1525/california/9780520251328.003.0015>
- Jeannerod, L., Carlier, A., Schatz, B., Daise, C., Richel, A., Agnan, Y. et al. (2022) Some bee-pollinated plants provide nutritionally incomplete pollen amino acid resources to their pollinators. *PLoS One*, 17, e0269992. Available from: <https://doi.org/10.1371/journal.pone.0269992>
- Johnson, S.D. (2010) The pollination niche and its role in the diversification and maintenance of the southern African flora. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, 365, 499–516. Available from: <https://doi.org/10.1098/rstb.2009.0243>
- Jones, J. & Rader, R. (2022) Pollinator nutrition and its role in merging the dual objectives of pollinator health and optimal crop production. *Philosophical Transactions of the Royal Society B*, 377, 20210170. Available from: <https://doi.org/10.1098/rstb.2021.0170>
- Lawson, S.P., Helmreich, S.L. & Rehan, S.M. (2017) Effects of nutritional deprivation on development and behavior in the subsocial bee *Ceratina calcarata* (Hymenoptera: Xylocopinae). *Journal of Experimental Biology*, 220, jeb.160531. Available from: <https://doi.org/10.1242/jeb.160531>
- Lawson, S.P., Kennedy, K.B. & Rehan, S.M. (2020) Pollen composition significantly impacts the development and survival of the native small carpenter bee, *Ceratina calcarata*. *Ecological Entomology*, 46, 232–239. Available from: <https://doi.org/10.1111/een.12955>
- Leonhardt, S.D., Blüthgen, N. & Schmitt, T. (2011) Chemical profiles of body surfaces and nests from six Bornean stingless bee species. *Journal of Chemical Ecology*, 37, 98–104. Available from: <https://doi.org/10.1007/s10886-010-9900-5>
- Leonhardt, S.D., Peters, B. & Keller, A. (2022) Do amino and fatty acid profiles of pollen provisions correlate with bacterial microbiomes in the mason bee *Osmia bicornis*? *Philosophical Transactions of the Royal Society B*, 377, 20210171. Available from: <https://doi.org/10.1098/rstb.2021.0171>
- Ma, L., Wang, Y., Zhang, W., Wang, H., Liu, Z. & Xu, B. (2016) Alterations in protein and amino acid metabolism in honeybees (*Apis mellifera*) fed different l-leucine diets during the larval stage. *Journal of Asia-Pacific Entomology*, 19, 769–774. Available from: <https://doi.org/10.1016/j.aspen.2016.07.005>
- Manning, R. (2001) Fatty acids in pollen: a review of their importance for honey bees. *Bee World*, 82, 60–75. Available from: <https://doi.org/10.1080/0005772X.2001.11099504>
- Naug, D. (2009) Nutritional stress due to habitat loss may explain recent honeybee colony collapses. *Biological Conservation*, 142, 2369–2372. Available from: <https://doi.org/10.1016/j.biocon.2009.04.007>
- Obata, F. & Miura, M. (2024) Regulatory mechanisms of aging through the nutritional and metabolic control of amino acid signaling in model organisms. *Annual Review of Genetics*, 58, 19–41. Available from: <https://doi.org/10.1146/annurev-genet-111523-102042>
- Ollerton, J. (2021) *Pollinators and pollination: nature and society*, 1st edition. Exeter: Pelagic Publishing.
- Ogle, D.H., Doll, J.C., Wheeler, P. & Dinno, A. (2021) FSA: fisheries stock analysis. R package version 0.9. 1. R Package Vignette.
- Ostaf, D.P., Mosseler, A., Johns, R.C., Javorek, S., Klymko, J. & Ascher, J.S. (2015) Willows (*Salix* spp.) as pollen and nectar sources for sustaining fruit and berry pollinating insects. *Canadian Journal of Plant Science*, 95, 505–516. Available from: <https://doi.org/10.4141/cjps-2014-339>
- Peng, Y., Nasr, M.E., Marston, J.M. & Fang, Y. (1985) The digestion of dandelion pollen by adult worker honeybees. *Physiological Entomology*, 10, 75–82. Available from: <https://doi.org/10.1111/j.1365-3032.1985.tb00021.x>
- R Core Team. (2022) *R: language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.
- Rehan, S.M. & Richards, M.H. (2010) Nesting biology and subsociality in *Ceratina calcarata* (Hymenoptera: Apidae). *The Canadian Entomologist*, 142, 65–74. Available from: <https://doi.org/10.4039/n09-056>
- Roulston, T.H. & Cane, J.H. (2000) Pollen nutritional content and digestibility for animals. *Plant Systematics and Evolution*, 222, 187–209. Available from: <https://doi.org/10.1007/BF00984102>
- Schmid-Hempel, P. (2005) Evolutionary ecology of insect immune defenses. *Annual Review of Entomology*, 50, 529–551. Available from: <https://doi.org/10.1146/annurev.ento.50.071803.130420>

- Shell, W.A. & Rehan, S.M. (2018) The price of insurance: costs and benefits of worker production in a facultatively social bee. *Behavioral Ecology*, 29, 204–211.
- Smart, M.D., Otto, C.R.V. & Lundgren, J.G. (2019) Nutritional status of honey bee (*Apis mellifera* L.) workers across an agricultural land-use gradient. *Scientific Reports*, 9(1), 16252. Available from: <https://doi.org/10.1038/s41598-019-52485-y>
- Splitt, A., Skórka, P., Strachecka, A., Borański, M. & Teper, D. (2021) Keep trees for bees: pollen collection by *Osmia bicornis* along the urbanization gradient. *Urban Forestry & Urban Greening*, 64, 127250. Available from: <https://doi.org/10.1016/j.ufug.2021.127250>
- Stephen, K.W., Chau, K.D. & Rehan, S.M. (2024) Dietary foundations for pollinators: nutritional profiling of plants for bee health. *Frontiers in Sustainable Food Systems*, 8, 1411410. Available from: <https://doi.org/10.3389/fsufs.2024.1411410>
- Therneau, T.M. & Grambsch, P.M. (2001) *Modeling survival data: extending the Cox model*. Statistics for biology and health. New York: Springer.
- Timberlake, T.P., Vaughan, I.P. & Memmott, J. (2019) Phenology of farmland floral resources reveals seasonal gaps in nectar availability for bumblebees. *Journal of Applied Ecology*, 56, 1585–1596. Available from: <https://doi.org/10.1111/1365-2664.13403>
- Tomberlin, J.K., Miranda, C., Flint, C., Harris, E. & Wu, G. (2023) Nutrients limit production of insects for food and feed: an emphasis on nutritionally essential amino acids. *Animal Frontiers*, 13, 64–71. Available from: <https://doi.org/10.1093/af/vfad032>
- Topal, E., Mărgăoan, R., Bay, V., Takma, Ç., Yücel, B., Oskay, D. et al. (2022) The effect of supplementary feeding with different pollens in autumn on colony development under natural environment and in vitro lifespan of honey bees. *Insects*, 13, 588. Available from: <https://doi.org/10.3390/insects13070588>
- Toth, A.L. & Robinson, G.E. (2005) Worker nutrition and division of labour in honeybees. *Animal Behaviour*, 69, 427–435. Available from: <https://doi.org/10.1016/j.anbehav.2004.03.017>
- Tucker, E.M. & Rehan, S.M. (2016) Wild bee pollination networks in northern New England. *Journal of Insect Conservation*, 20, 325–337. Available from: <https://doi.org/10.1007/s10841-016-9870-1>
- van der Kooij, C.J., Vallejo-Marín, M. & Leonhardt, S.D. (2021) Mutualisms and (a)symmetry in plant–pollinator interactions. *Current Biology*, 31, R91–R99. Available from: <https://doi.org/10.1016/j.cub.2020.11.020>
- Vanderplanck, M., Gilles, H., Nonclercq, D., Duez, P. & Gerbaux, P. (2020) Asteraceae paradox: chemical and mechanical protection of *Taraxacum* pollen. *Insects*, 11, 304. Available from: <https://doi.org/10.3390/insects11050304>
- Vanderplanck, M., Martinet, B., Carvalho, L.G., Rasmont, P., Barraud, A., Renaudeau, C. et al. (2019) Ensuring access to high-quality resources reduces the impacts of heat stress on bees. *Scientific Reports*, 9, 12596. Available from: <https://doi.org/10.1038/s41598-019-49025-z>
- Vaudo, A.D., Dyer, L.A. & Leonard, A.S. (2024) Pollen nutrition structures bee and plant community interactions. *Proceedings of the National Academy of Sciences of the United States of America*, 121, e2317228120. Available from: <https://doi.org/10.1073/pnas.2317228120>
- Vaudo, A.D., Patch, H.M., Mortensen, D.A., Tooker, J.F. & Grozinger, C.M. (2016) Macronutrient ratios in pollen shape bumble bee (*Bombus impatiens*) foraging strategies and floral preferences. *Proceedings of the National Academy of Sciences of the United States of America*, 113, E4035–E4042. Available from: <https://doi.org/10.1073/pnas.1606101113>
- Vaudo, A.D., Tooker, J.F., Grozinger, C.M. & Patch, H.M. (2015) Bee nutrition and floral resource restoration. *Current Opinion in Insect Science*, 10, 133–141. Available from: <https://doi.org/10.1016/j.cois.2015.05.008>
- Vaudo, A.D., Tooker, J.F., Patch, H.M., Biddinger, D.J., Coccia, M., Crone, M.K. et al. (2020) Pollen protein:lipid macronutrient ratios may guide broad patterns of bee species floral preferences. *Insects*, 11, 132. Available from: <https://doi.org/10.3390/insects11020132>
- Verrier, E., Bretagnolle, V., Aupinel, P., Decourtye, A., Henry, M., Rebaudo, F. et al. (2024) Semi-natural habitats mitigate the impact of food shortage on honey bees in farmlands. *Science of the Total Environment*, 950, 175309. Available from: <https://doi.org/10.1016/j.scitotenv.2024.175309>
- Walters, J., Fisher, R., Sharkey, T.D., Isaacs, R. & Santiago, J.P. (2025) Extreme heat affects blueberry pollen nutrition, bee health, and plant reproduction. *Scientific Reports*, 15, 6249. Available from: <https://doi.org/10.1038/s41598-025-90676-y>
- Wang, Y., Kaftanoglu, O., Brent, C.S., Page, R.E. & Amdam, G.V. (2016) Starvation stress during larval development facilitates an adaptive response in adult worker honey bees (*Apis mellifera* L.). *Journal of Experimental Biology*, 219(7), 949–959. Available from: <https://doi.org/10.1242/jeb.130435>
- Weeks, E.N.I., Schmehl, D.R., Baniszewski, J., Tomé, H.V.V., Cuda, J.P., Ellis, J.D. et al. (2018) Safety of methionine, a novel biopesticide, to adult and larval honey bees (*Apis mellifera* L.). *Ecotoxicology and Environmental Safety*, 149, 211–216. Available from: <https://doi.org/10.1016/j.ecoenv.2017.11.026>
- Westreich, L.R., Westreich, S.T. & Tobin, P.C. (2023) Native solitary bee reproductive success depends on early season precipitation and host plant richness. *Oecologia*, 201, 965–978. Available from: <https://doi.org/10.1007/s00442-023-05354-5>
- Wright, G.A., Nicolson, S.W. & Shafir, S. (2018) Nutritional physiology and ecology of honey bees. *Annual Review of Entomology*, 63, 327–344. Available from: <https://doi.org/10.1146/annurev-ento-020117-043423>
- Wu, P., Dai, P., Wang, M., Feng, S., Olhnuud, A., Xu, H. et al. (2021) Improving habitat quality at the local and landscape scales increases wild bee assemblages and associated pollination services in apple orchards in China. *Frontiers in Ecology and Evolution*, 9, 621469. Available from: <https://doi.org/10.3389/fevo.2021.621469>

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Head width measurements. Head width (mm) of adult bees across different treatments. Measurements are taken at the widest part of the head capsule post-eclosion.

Table S2. Development time. Total days from egg to adult emergence for individual bees under different treatments. Development duration reflects treatment impact on life cycle progression.

Table S3. Body mass. Final dry mass (mg) of adult bees following treatment conditions. Values inform analysis of nutritional and environmental effects on growth outcomes.

Table S4. Lipid values. Total lipid content (µg) measured per individual bee across treatments. Lipid data were derived from chemical extraction and quantification methods.

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