Nutritional Ecology of Wild Bees: How Pollen Macronutrient Composition Shapes Development and Survival

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Abstract

Bees rely on diverse floral resources to meet their nutritional needs, yet habitat loss, urbanization, and agricultural intensification are altering pollen availability and composition. Understanding the macronutrient profiles of pollen is central for pollinator health, as protein, lipids, and essential fatty acids influence bee development and survival. This thesis explores the nutritional composition of pollen from various plant species and its implications for bee health. Chapter I investigates the macronutrient content of 57 pollen species native to North America, revealing significant variation in non-esterified fatty acids (NEFAs), amino acids (AAs), and protein-to-lipid (P:L) ratios. Asteraceae pollen was particularly rich in essential amino acids, while mixed diets provided a balance of nutrients necessary for bee health. Chapter II examines how pollen macronutrient composition influences larval development in the small carpenter bee (*Ceratina calcarata*). Experimental rearing on black poplar (*Populus nigra*) and dandelion (*Taraxacum officinale*) pollen demonstrated that protein-rich arboreal pollen significantly enhanced bee growth, survival, and developmental rates. Bees on dandelion pollen alone exhibited delayed growth and reduced body size, while mixed diets mitigated nutritional stress. This research highlights the importance of nutritional diversity in pollen sources, emphasizing the role of tree pollen as an overlooked resource for pollinator conservation. As habitat loss and climate change continue to threaten pollinators, ensuring access to high-quality, nutritionally diverse floral resources may be key to sustaining bee populations and promoting ecosystem resilience.

Dedication

This thesis is dedicated to my parents, Carl and Cassandra, for their unwavering love, encouragement, and endless support throughout my academic journey. Your belief in me has been the foundation of my perseverance and success. I also dedicate this to everyone who has guided, challenged, and inspired me along the way. Your kindness, wisdom, and motivation have shaped every step that has brought me here.

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Chapter I: General Introduction

Pollinators, particularly bees, play an essential role in ecosystem stability, biodiversity, and global food production by ensuring the successful reproduction of flowering plants. Their pollination services contribute to both natural ecosystems and agricultural productivity, making them important for maintaining plant diversity and food security. Among the diverse group of native pollinators in North America, small carpenter bees (*Ceratina calcarata*) are particularly significant due to their extensive distribution and unique nesting and foraging behaviours (Nguyen & Rehan, 2022; Rehan, 2020). Like many other wild pollinators, these bees rely heavily on pollen as their primary source of proteins and lipids, which are essential for growth, development, and immune function (Vaudo et al., 2015; Engel et al., 2012).

However, pollinator populations are facing unprecedented declines due to increasing anthropogenic pressures such as habitat loss, agricultural intensification, urbanization, and pesticide exposure (Cameron & Sadd, 2020; Goulson et al., 2015). One of the most pressing challenges resulting from these environmental changes is nutritional stress, as the loss of floral diversity limits access to high-quality pollen sources. The decline in available nutritionally rich pollen may negatively impact bee development, reproduction, and resilience to environmental stressors (Vaudo et al., 2015; Engel et al., 2012; Chau & Rehan, 2024). Understanding which pollen sources provide optimal macronutrient profiles is crucial for developing conservation strategies that prioritize floral resources capable of supporting pollinator health.

The Role of Macronutrients in Pollinator Health

Foraging decisions in bees are largely driven by nutritional requirements, particularly their need for proteins, lipids, and essential fatty acids. Different plant species produce pollen with varied nutritional profiles, influencing the dietary choices of bees and their ability to sustain healthy colonies or populations. Among the 20,000+ bee species worldwide, foraging strategies differ based on species-specific nutritional demands, evolutionary history, and floral specialization (Winfree et al., 2011; Potts et al., 2016). Some bee species exhibit specialized pollen preferences, while others, like honey bees (*Apis mellifera*), forage broadly to meet their complex dietary needs (Dafni & Kevan, 1997; Vaudo et al., 2015).

Protein and lipid content in pollen play a particularly an important role in shaping bee physiology and foraging behaviour. Protein is essential for larval growth, tissue development, and immune function, while lipids contribute to energy storage, metabolic regulation, and hormone production (Vaudo et al., 2015). The balance of these macronutrients, often represented as the protein-to-lipid (P:L) ratio, influences which pollen species bees select. Essential amino acids (EAAs) and non-esterified fatty acids (NEFAs) are key determinants of pollen quality, with different plant families producing pollen that varies widely in these nutrients (Vaudo et al., 2015; Engel et al., 2012).

Environmental Changes and Nutritional Challenges

Climate change and human-driven alterations to landscapes are causing shifts in floral abundance, composition, and nutritional quality, making it more difficult for pollinators to maintain nutritionally balanced diets (Ruedenauer et al., 2020; Ziska et al., 2016). Urban expansion and monoculture farming reduce floral diversity, forcing bees to rely on suboptimal pollen sources that may not provide the complete range of nutrients they require. Honey bees, as social foragers, can adjust their foraging patterns to compensate for dietary imbalances, but solitary bees, such as *Ceratina calcarata*, lack this flexibility and may be more vulnerable to nutritional deficiencies (Barraud et al., 2022). The impact of these dietary constraints is particularly concerning for generalist species that rely on multiple floral resources to sustain their populations.

Understanding the nutritional composition of different pollen sources is essential for informing conservation efforts and ensuring that pollinators have access to the resources they need. While some widely available pollen sources, like dandelion (*Taraxacum officinale*), are abundant in degraded landscapes, their nutritional quality remains questionable, as their low protein content and poor amino acid composition may limit their suitability for larval development (Peng et al., 1985; Fan et al., 2018; Anderegg et al., 2021). In contrast, tree pollen, such as that from black poplar (*Populus nigra*), is often protein-rich and may provide superior nutritional benefits for developing bees.

Ceratina calcarata as a Model for Studying Bee Nutrition

Ceratina calcarata is a sub social bee species that provides extensive maternal care, making it an ideal model for investigating the relationship between pollen nutrition and bee health. Unlike eusocial bees, where colony-level dynamics can obscure individual dietary effects, the solitary nature of *C. calcarata* allows for precise assessments of how specific pollen sources impact development and survival (Rehan, 2020). Recent advancements in pollen metabarcoding, metagenomics, and molecular techniques have revealed key dietary patterns in *C. calcarata*, particularly its frequent use of Asteraceae pollen, a nutritionally diverse plant family (Lawson et al., 2016; Shell et al., 2022; Chau et al., 2023). The ability of

C. calcarata to persist in urban environments, such as the Greater Toronto Area, highlights its adaptability, but also raises questions about how nutritional availability affects its long-term survival (City of Toronto, 2016; Shell & Rehan, 2016). This study seeks to assess the impact of pollen nutrition on bee physiology, providing valuable data to guide conservation efforts and habitat restoration initiatives.

Thesis Objectives

This research investigates the relationship between pollen macronutrient composition and bee health, with a focus on *Ceratina calcarata*. Chapter 1 examines the protein and lipid content of 57 pollen species, analyzing NEFA, EAA, P:L ratios, and omega-6:3 fatty acid ratios. It also explores phylogenetic trends in pollen nutrition across plant families and genera. These findings will help inform habitat restoration strategies by identifying the most nutritionally valuable floral resources for pollinators. Based on previous studies (Chau & Rehan, 2024), I hypothesize that significant nutritional differences exist between native and introduced plant species.

Chapter 2 evaluates how pollen macronutrient composition affects the development and survival of *C. calcarata*. Using data from Chapter 1, I selected dandelion (*Taraxacum officinale*) and black poplar (*Populus nigra*) pollen, which have contrasting nutritional profiles and overlapping blooming periods in early spring. By rearing *C. calcarata* larvae on exclusive and mixed diets of these pollen types, I assess developmental rates, body sizes, and survival outcomes. Given the higher protein content and beneficial lipid profile of tree pollen, I hypothesize that bees fed black poplar pollen will exhibit improved physiological and developmental outcomes compared to those fed dandelion pollen. Additionally, bees on mixed diets will likely show intermediate results, reflecting nutritional complementarity observed in polyfloral diets (Eckhardt et al., 2014; Vaudo et al., 2016). Dandelion pollen remains controversial in pollinator nutrition because, despite being an abundant resource, its low protein content, poor amino acid composition, and spiny exine structure may limit its digestibility and nutritional value for developing larvae (Peng et al., 1985; Fan et al., 2018; Anderegg et al., 2021).

This research provides valuable insights into how pollen macronutrient composition influences bee health and development, highlighting the essential role of diverse floral resources in supporting pollinator populations. As habitat loss and environmental changes continue to alter the availability of nutritionally rich pollen, these findings emphasize the importance of conservation strategies that prioritize floral diversity to sustain resilient bee communities and ensure the stability of pollination networks.

Chapter II: Dietary Foundations for Pollinators: Nutritional Profiling of Plants for Bee Health

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Abstract

Human activities, including urban expansion, intensive farming practices, and the application of pesticides have significantly reshaped bee habitats. Understanding the nutritional content of pollen, the primary source of bees' proteins and lipids, is important for maintaining their diet and health. In this study, we set out to determine the nutritional composition of pollen from various plant families and genera. Our objectives were to analyze the levels of non-esterified fatty acids (NEFAs), amino acids (AAs), protein-to-lipid (P:L) ratios, and omega-6:3 ratios of 57 pollen species native to North America. These data suggest a potential trade-off between NEFA and AA content within pollen, suggesting that a diverse floral diet may benefit bees more than a single pollen source. The AA profiles showed considerable diversity, with all pollen species providing the essential amino acids (EAAs) required for bee health, except for methionine which was lacking in Rhus glabra pollen. The plant family Asteraceae was especially abundant in EAAs. P:L ratios varied widely further emphasizing bees' need for access to a diverse array of nutritional profiles. There were no overall nutritional differences between pollen from native and introduced plant species. This study highlights the significance of a diverse array of floral resources to meet the comprehensive nutritional needs of bees, contributing to the support of pollinator populations and the broader ecological system.

Keywords: Nutritional Ecology, Pollen Nutrition, Amino Acids, Fatty Acids, Pollinator Diet, Bee Health

Introduction

The mutualistic relationships between pollinators and flora have evolved through intricate ecological processes, with bees serving as a prime example of pollinators that directly rely on the flora they forage upon to fulfill their dietary needs. These plant-insect herbivore interactions have been observed in various insects, such as fruit flies, butterflies, and especially bees, which have been the focus of many studies in nutritional ecology (Filipiak, 2018). For instance, the diets of larval monarch butterflies (*Danaus plexippus*) depend on the species of milkweed consumed throughout their lifecycle, influencing the dispersal of traits and flight energetics in the adults (Pocius et al., 2022). Milkweed is also important for monarch butterfly diets, as it strengthens their resistance to a protozoan parasite (*Ophryocystis elektroscirrha*), highlighting the significance of these plant-insect interactions (Tan et al., 2018). The diversity of floral resources is instrumental in providing bees and other pollinators with the necessary nutrients, including proteins and lipids, which are imperative for their development, immune function, and overall health (Blüthgen and Klein, 2011; Vaudo et al., 2015). Pollinators often exhibit various preferences and adaptations for certain floral traits, such as flower scent, shape, color, nectar, and pollen production, which have co-evolved with the plants they pollinate (Janz and Nylin, 2008; Johnson, 2010). This interdependence has led to the formation of complex plant-pollinator networks, where certain bee species have evolved to preferentially forage on specific plant species, enhancing pollination efficiency and plant reproductive success (Mitchell et al., 2009; Gómez-Martínez et al., 2022). This interdependence between pollinators and the flora they forage upon highlights the intricate co-evolutionary processes that have shaped the dietary specializations observed in these species.

Human-induced changes, such as urbanization, agricultural intensification, and pesticide usage, have drastically altered bee habitats. These changes have led to a scarcity in the diversity and availability of pollen resources, essential for the health and proliferation of bee populations (Potts et al., 2010; Goulson et al., 2015). Declines in pollinator populations and alterations in their geographic spread disrupt pollination services, affecting plant communities and agricultural yield. Additionally, climatic alterations induced by human activity are now understood to affect the nutritional makeup of pollen. With the protein, lipid, and amino acid (AA) content of pollen influenced by rising temperatures and changing CO2 levels (Ziska et al., 2016; Ruedenauer et al., 2020), pollinators like bees face new nutritional challenges. Their ability to find the necessary nutrients for growth, development, and immunity is challenged, compounding the stressors imposed by habitat degradation.

Understanding the details of pollen nutrition is essential in this context. It encompasses more than measuring protein levels; it explores the broader nutritional ecology to address the decline of pollinator populations and their important roles in ecosystems (Filipiak, 2018). With over 20,000 described bee species worldwide providing pollination services for various flora and crops, they are important to global food security and ecosystem health (Winfree et al., 2011; Potts et al., 2016). Bees' foraging behaviuors are shaped by their nutritional needs, revealing preferences for certain macronutrients. For example, bumble bees (*Bombus* spp.) have been observed to select plants like *Solanum tridynamum*, *Solanum elaeagnifolium*, and *Exacum affine*, which offer pollen rewards concealed within poricidal anthers that are accessible only through buzz pollination demonstrating the bees' adaptation to meet their complex nutritional requirements (Russell et al., 2016; Ruedenauer et al., 2021). Additionally, generalist bee species such as the honey bee (*Apis mellifera*), often forage on a diverse array of plant species, including sturdy, irregular-shaped flowers like snapdragons or penstemon, to meet their varied nutritional needs (Dafni and Kevan, 1997; Vaudo et al., 2015). This understanding goes beyond simplistic botanical classifications, suggesting the importance of examining the relationships between bees and the diverse plant species that sustain them.

Maintaining a diverse array of pollen sources is important, as it allows generalist bee species to forage on multiple plant species to fulfill their nutritional requirements. Most bees are generalists and they may visit a variety of plant species including ancient bee-pollinated flowers like magnolias and water lilies (Bernhardt and Thien, 1987; Vaudo et al., 2024). For instance, Kleijn et al. (2015) highlighted key North American bees such as *Bombus impatiens*, *Anthophora urbana*, and *Andrena vicina,* noting their frequent visits to specific crops like Alfalfa, Apple, and Carrot. This diversity in foraging options demonstrates the importance of these species in both natural and cultivated ecosystems. This supports healthy bee populations and facilitates successful plant restoration efforts by ensuring adequate pollination services (Winfree et al., 2011; Potts et al., 2016). Nectar provides bees with energy-rich carbohydrates, while pollen is the primary source of proteins, lipids, free AAs, and other micronutrients essential for their cognitive functions, development, and immunity (Nicolson, 2011). Nutritional profiles vary widely among plant species and even within the same family or genus, which has implications for both specialist and generalist bees (Williams, 2003). Bee nutrition also differs among species, influencing foraging selectivity to fulfill specific dietary needs (Leonhardt et al., 2011). Honey bees, for instance, adjust their foraging preferences based on their colony's nutritional demands, seeking out plants with suitable lipid and protein pollen profiles (Barraud et al., 2022). Honey bees also have been observed to prefer plants with lower lipid content to avoid impairing their sensory functions (Bennett et al., 2022). In contrast, the solitary mason bee, *Osmia cornuta*, favours plant species with higher pollen protein and lower lipid content (Sedivy et al., 2011). The generalist bumblebee *Bombus terrestris* is known for its broad foraging range, covering over 400 plant genera from more than 80 families (Goulson et al., 2008). While these findings are significant, there is a notable gap in bee nutrition research, particularly regarding solitary bees, which represent about 85% of identified wild bee species pollinating a substantial portion of crops across North America and worldwide (Reilly et al., 2020; Khalifa et al., 2021). This underlines the need for further study of the nutritional profiles across diverse plant genera and families to enrich our knowledge of wild bee nutrition and support their conservation (Crone et al., 2022; Ghosh et al., 2023).

Fatty acids are pivotal for the energy production, cellular integrity, and physiological regulation in bees (Kaplan et al., 2016). Bees must obtain essential non-esterified fatty acids (NEFAs), like omega-3 (linolenic acid) and omega-6 (linoleic acid), through their diet since they cannot synthesize them (Jeannerod et al., 2022). These fatty acids are essential for bee growth, overall health, development, and provide an energy reserve during low nectar and pollen periods (Arien et al., 2018). Additionally, they are key for reproductive and immune functions (Arien et al., 2020). The ideal omega-6:3 ratio for honey bees is around 1 or lower; higher omega-6 levels are linked to impaired learning and physiological issues (Arien et al., 2015; Bennett et al., 2022). Deficiencies in these fatty acids negatively impact bee development, immune response, and lifespan, and can reduce their resistance to environmental stressors including diseases (Vaudo et al., 2016a). Bees can synthesize some fatty acids from pollen and nectar components (Hsu et al., 2021). The availability and quality of these dietary sources are important, as they affect fatty acid synthesis and utilization which in turn influences bee health and physiological functions (Arien et al., 2020). However, the understanding of fatty acid metabolism in bees is not as comprehensive as it is in mammals, pointing to an area for further study to improve our knowledge of how dietary fats impact bee metabolism and health.

The protein-lipid (P:L) ratio in pollen is a metric used to evaluate the nutritional quality of pollen and understand foraging preferences across bee species (Crone and Grozinger, 2021). Bees are known to modulate their pollen consumption to attain an optimal P:L ratio for their dietary needs (Vaudo et al., 2020). Pollen species fall on a diverse P:L spectrum, typically ranging from 0.5 to 6.0, with the majority of bee-pollinated flora featuring a P:L ratio of 1–4 (Vaudo et al., 2020; Barraud et al., 2022). Honey bees show a preference for pollen with a P:L ratio of about 2–3, which offers a balanced mix of proteins and lipids (Vaudo et al., 2020). In contrast, bumble bees, such as *Bombus terrestris* and *Bombus impatiens*, actively regulate their pollen intake to achieve higher P:L ratios—14:1 for *Bombus terrestris* and 12:1 for *Bombus impatiens* (Vaudo et al., 2016a,b). Environmental stressors, including insecticide exposure, can be more harmful to bees when coupled with higher dietary P:L ratios, suggesting that the P:L ratio also influences bees' resilience to such stressors (Crone and Grozinger, 2021).

The nutritional value of pollen protein is often characterized by its essential amino acid (EAA) content (Taha et al., 2019). De Groot (1953) identified 10 EAAs necessary for honey bees—arginine, histidine, lysine, phenylalanine, tryptophan, methionine, threonine, leucine, isoleucine, and valine—a list corroborated by recent studies quantifying these EAAs' requirements by evaluating growth and protein content in bees on diets with varying AA levels (Jeannerod et al., 2022). In light of previous research showing that the nutritional value of pollen is not solely dependent on its protein content, but also on the presence of essential fatty acids and a balanced profile of AA, it is important to incorporate these metrics into nutritional evaluations (Arien et al., 2015, 2018). Deficiencies in EAAs impair protein synthesis and bee fitness (Mariotti, 2017). Bees show a preference for pollen high in EAAs, especially isoleucine, leucine, and valine, mandatory for their health (Cook et al., 2003). Weeks et al. (2018) further highlighted methionine's significance, finding that bees cannot sustain brood on methionine-deficient pollen and typically avoid pollen or nectar from such plants. This avoidance behavior emphasizes methionine's essential role in bee nutrition. Additionally, lysine is used for producing nitric oxide, a neurotransmitter that enhances memory in bees, while leucine influences protein regulation and impacts gene expression, indicating the roles EAAs play in bee health and development (Gage et al., 2020). Thus, a balanced EAA composition in pollen is essential for bee development and brood health.

This study has three aims to describe the nutritional profile of several pollen species from different families and genera: (1) document the content of NEFAs, AA, P:L, and omega-6:3 ratios in 57 North American pollen species; (2) identify phylogenetic signals of plant family and genus with NEFA and AA content; and (3) assess the overall nutritional value of the pollen species as important food sources for wild bees. This study sets the stage for future experimentation on how diverse floral resources meet the nutritional needs of bees. By examining these nutritional components, we aim to inform floral plantings and future strategies for ecological conservation and sustainable agriculture, with the goal of supporting healthy bee populations and robust pollinator networks.

Materials and methods

We analyzed a total of 57 pollen samples, which include 20 former samples from Chau and Rehan (2024) and 37 newly acquired species, collectively spanning 47 genera and 27 families. Included in the former samples were *Taraxacum officinale* and *Plantago lanceolata*, which were specifically reanalyzed due to the acquisition of new pollen samples. The samples were sourced from wild plants in the Greater Toronto Area (Canada), with additional samples commercially obtained from the United States, Australia, and China (Supplementary Table S1). The targeted plant species were chosen for their ecological significance to northeastern wild bees and their prevalence. The collection method was tailored to each pollen species to optimize pollen extraction based on their distinct floral structures and pollen characteristics. The pollen was hand-collected in spring and summer 2022 and 2023 either from fresh blooms and then transported to the laboratory in sealed plastic bags, or from collected flowers that had been allowed to dry in the laboratory. The extraction process involved either brushing the pollen from flowers or tapping them over clean white paper to collect the pollen, which was then sieved to remove impurities (45µm, Hogentogler & Co, Columbia), weighed in grams, and kept frozen at −80°C until further analysis.

Pollen analyses

Pollen samples were sent to the Mayo Clinic in Rochester, Minnesota to determine the content of NEFA and AA. The preparatory process for each pollen sample involved amalgamating the pollen with a phosphate-buffered saline solution, followed by homogenization through sonication and mixing via vortex to ready the samples for NEFA and AA measurements. The quantification of 12 NEFAs was executed employing a Thermo Quantum Ultra triple quadrupole interfaced with Waters' Acquity liquid chromatography system, measured against the established protocol outlined by Persson et al. (2010). Subsequently, a 10 mg aliquot of pollen was treated with a blend of isotopic internal standards prior to the extraction process. Post-extraction, the samples were dried and reconstituted with a running buffer in preparation for the liquid chromatography-mass spectrometry (LC/MS) analysis using negative electrospray ionization.

For each pollen species analyzed, we quantified the total free fatty acids present, evaluated the composition of NEFAs, and determined the ratios of imperative NEFAs, specifically omega-3 (linolenic acid) and omega-6 (linoleic acid), measured in nmols/mg of pollen. The quantification of AA was carried out via LC/MS, adhering to the method delineated by Lanza et al. (2010). Briefly, to each 2 mg of pollen solution, isotopic internal standards were introduced prior to the removal of proteins using chilled methanol. Subsequently, the clear liquid was treated with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate as specified by the Waters' MassTrakTM protocol. Following the addition of internal standards, a calibration curve of 10 points was prepared using the same derivatization method. These derivatized standards and pollen samples underwent analysis on a Thermo Quantum Ultra triple quadrupole mass spectrometer, paired with a Waters Acquity liquid chromatography system where a selective ion monitoring (SRM) technique facilitated data collection. The calculated concentrations of 42 analytes per sample were compared against their calibration standards with results expressed in nmols/mg of pollen.

Additionally, we estimated the total AA content, identified as EAAs for bees (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine; de Groot, 1953), and outlined the AA profile for each pollen species. The percentage of each EAA, in comparison to the requirements for a honey bee diet (de Groot, 1953), was also determined and depicted through a heatmap for the top 10 pollen species that most closely match the honey bee EAA requirements. The nutritional evaluation included calculating the P:L ratio for each pollen species, which was calculated by dividing the total content of AA and metabolites in nmols/mg of pollen by the total NEFA content for each pollen species. We also analyzed the omega-6:3 fatty acid ratios using the total linoleic acid content divided by the total linolenic acid content for each pollen species, as the omega-6:3 ratio obtained from diet has been found to impact bee cognitive function (Arien et al., 2015, 2018; Bennett et al., 2022).

Statistical analyses

Variations in the quantities of total NEFA, AA, EAA, and non-essential amino acids (NEAA) across various pollen species were visualized with non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarity indices (Chau and Rehan, 2024). Data were transformed using the "total" option in the *decostand* function from the vegan package in R (Oksanen et al., 2017), standardizing the data to relative total amounts for each pollen species. Further investigation determined whether the nutritional content (total AA, EAA, NEAA, NEFA, P:L ratio, and omega-6:3 ratio) exhibited significant variation among families with two or more species (Asteraceae, Brassicaceae, Theaceae, Cornaceae, Rosaceae, Oleaceae, Fabaceae, Pinaceae, Salicaceae, Anacardiaceae, and Adoxaceae). For this, we applied Bartlett's test and the Shapiro-Wilks test, utilizing the *bartlett.test* and *shapiro. test* functions in base R to examine the homogeneity of variances and normality of distribution, respectively. Although variances were homogeneous across families, the data did not follow a normal distribution. Consequently, the Kruskal-Wallis test was employed, using the *kruskal.test* function in base R, to evaluate whether nutritional content differed by family. All statistical analyses were conducted in R version 4.3.1 (R Core Team, 2022).

To determine the evolutionary relationship between total NEFA and various AA profiles, we conducted phylogenetic generalized least squares (PGLS) analyses using the *nlme* package in R (Pinheiro and Bates, 2000; Pinheiro et al., 2023). This analysis considered the relationships of (1) total NEFA vs. total AA, (2) total NEFA vs. total EAA, (3) total NEFA vs. total NEAA, and (4) total omega vs. total EAA, all calculated per pollen species. The phylogenetic tree (Figure 1) used for this analysis was constructed using ribulose-1,5-bisphosphate carboxylase large subunit (rbcL) sequences from chloroplasts sourced from NCBI Genbank for each species (NCBI accession numbers in Supplementary Table S1). For three plant species that were not taxonomically identified to the species level (*Trifolium* sp., *Prunus* sp., and *Rosa* sp.), we substituted with rbcL sequences from *Trifolium repens* (HQ644078.1), *Prunus serotina* (NC_036133.1), and *Rosa virginiana* (MG247335.1) respectively, which are distributed in North America to construct the phylogenetic tree. A phylogenetic tree was constructed by employing the ultrafast IQ-TREE software (version 1.6.12) which used 1,000 bootstrap replicates to develop a maximum likelihood tree (Nguyen et al., 2015; Hoang et al., 2018). To align the tree with established angiosperm relationships, we used Mesquite version 3.70 (Maddison and Maddison, 2021) to revise branches to ensure adherence to the angiosperm phylogeny outlined by Li et al. (2021).

Next, each pollen species was classified as “native” or “introduced” to North America, to determine if the nutritional content shows a significant difference based on endemicity. Plant distribution data were sourced from the USDA PLANTS database (USDA and NRCS, 2024). Endemicity for three plants from Chau and Rehan (2024) were sourced from literature: *Taraxacum officinale* (Stewart-Wade et al., 2002), *Viburnum opulus* (Česonienė et al., 2010), and *Rubus idaeus* (Fernald, 1919). When pollen was identified only to the genus level, we utilized species-specific data corresponding to the same taxa as those used for rbcL sequence retrieval, including *Trifolium repens*, *Rosa virginiana*, and *Prunus serotina*. Given that the Shapiro-Wilks test indicated the data followed a non-normal distribution for total EAA, NEAA, AA, NEFA, P:L ratio, and omega-6:3 ratio with respect to endemicity, we applied the Mann–Whitney-Wilcoxon test using the *wilcox.test* function in base R to evaluate if there was a statistically significant difference in nutritional content between native and introduced pollen species.

We expanded our analysis on P:L ratios across pollen families by incorporating P:L ratios obtained from Vaudo et al. (2024) supplementary data which expanded our dataset from 57 to 167 species. To match the P:L ratio units from the study of Vaudo et al. (2024) which is in µg/mg, we converted our total protein and total lipid values from nmols/mg to µg/mg by multiplying our protein and lipid values by the molecular weight of each respective protein and lipid molecule, and then converting the units to µg/mg. The molecular weight was obtained in g/mol for each NEFA, AA, and metabolite by searching each molecule on PubChem (Kim et al., 2023). We then analyzed the expanded P:L ratios only from families with two or more species by first testing for normal distribution among P:L ratio means using a Shapiro–Wilk test. The data were not normally distributed. Hence, we used the Kruskal-Wallis test to test for differences in P:L ratio means among families. The Welch two sample *t*-test (the *t.test* function in base R) was used to determine the difference in mean total protein, omega, P:L and total lipid in native and introduced species.

Finally, to identify which pollen species had ideal EAA compositions compared to honey bee EAA requirements (de Groot, 1953), Euclidean distance was used as a measure of nutritional alignment with the bees' optimal dietary profile. Utilizing base R functions, we calculated the average Euclidean distances for each pollen family to determine the top 10 most suitable pollen species for wild bee diet based on honey bee EAA requirements. Specifically, we used the *apply* function to compute the square root of the sum of squared differences between the AA ratios of each species and the optimal profile, interpreting lower scores as indicative of a more suitable match to the honey bees' nutritional preferences.

Results

NEFA and AA content across pollen species

Among the 57 pollen species studied, all contained a range of 7–10 NEFA (Supplementary Table S2). Notably, *Rhus glabra* pollen presented the lowest NEFA concentration at 1.55nmols/mg, while *Impatiens capensis* had the highest at 49.67 nmols/mg. Some fatty acids were only detected in small quantities and among few species studied. For instance, docosahexaenoic acid was found exclusively in *Cichorium intybus* and *Impatiens capensis*. Arachidonic acid was identified in *Coreopsis lanceolata*, *Cornus sericea*, *Daucus carota*, *Leucanthemum vulgare*, *Pinus resinosa, Plantago lanceolata, Ranunculus acris*, *Rudbeckia hirta*, and *Viburnum prunifolium.* Elaidic acid was detected only in *Rubus idaeus.* Furthermore, eicosapentaenoic acid was only detected in six pollen species: *Ambrosia acanthicarpa, Leucanthemum vulgare, Plantago lanceolata, Ranunculus acris, Rosa rugosa*, and *Viburnum prunifolium*. Five NEFAs were universally found across all examined species, including essential linoleic and linolenic fatty acids, along with oleic, palmitic, and palmitoleic acids. Of these, linolenic, palmitic, and linoleic acids were identified as the three most prevalent NEFA across a majority of the pollen species. The concentration of NEFA linoleic acid was observed to vary, with a range between 0.19 nmols/mg in *Actinidia arguta* to 17.26 nmols/mg in *Quercus rubra*. Similarly, for linolenic acid, the range extended from 0.23 nmols/mg in *Rhus glabra* to 34.72 nmols/mg in *Brassica napus*.

The composition of pollen from each species included 26–34 AA and metabolites, with total protein concentrations ranging from 8.63 nmols/mg in *Acer rubrum* to 416.08 nmols/mg in *Populus nigra* (Supplementary Table S3). Almost all pollen genera contained all 10 EAAs. The levels of EAAs varied considerably, with the lowest levels detected in *Prunus salicina* (1.01 nmols/mg) and the highest levels in *Quercus rubra* (59.08 nmols/mg). Histidine was the most abundant EAA comprising 34.98% of the total AA content across all pollen species, followed by arginine at 21.74%, and the least prevalent being methionine at 0.978%. Among 17 species that recorded a total EAA concentration of 20% or higher, seven species were classified under the Asteraceae family (*Coreopsis lanceolata*, *Leucanthemum vulgare*, *Rudbeckia hirta*, *Ambrosia acanthicarpa*, *Chrysanthemum morifolium*, *Helianthus divaricatus*, and *Cichorium intybus*).

The NEAA content in pollen ranged with the least amount detected in *Acer rubrum* with 6.70 nmols/mg and the highest in *Populus nigra* with 358.39 nmols/mg (Supplementary Table S3). The pollen species with total NEAA concentrations exceeding 200 nmols/mg included one from the Asteraceae family (*Vernonia noveboracensis*), one from the Salicaceae family (*Populus nigra*), and one from the Fabaceae family (*Lotus corniculatus*). None of the pollen species analyzed contained detectable levels of the NEAAs anserine, carnosine, cystathionine, or hydroxylysines. The most prevalent NEAA was proline, accounting for 56.23% of the total, followed by asparagine at 15.26%, with the least common NEAAs being cysteine at 0.00015% and hydroxylysine 1 at 0.00016%.

The P:L ratios varied drastically, reaching levels as low as 0.11 in *Acer rubrum* and as high as 32.85 in *Prunus* sp., resulting in an average P:L ratio of 6.71 among the various pollen species (df = 56, *p* value = 0.47; Supplementary Table S4). Expanded analysis incorporating data from Vaudo et al. (2024) corroborates high variability in P:L ratios among pollen species (Supplementary Table S4; Figure 2). In the combined dataset with an additional 110 species, P:L ratios exhibited a considerable range, with the lowest value being 0.44 in *Prunus salicina* (Vaudo et al., 2024) and the highest was 32.85 in *Prunus* sp. (this study) resulting in a slightly lower average P:L ratio of 6.20 (df = 144, *p* value = 0.48; Supplementary Figure S2).

We observed a wide spectrum of total omega content among the pollen species analyzed. Total omega content ranged from 0.42 nmols/mg in *Rhus glabra* to 37.67 nmols/mg in *Brassica napus*, averaging 9.9 nmols/mg. Seven of the species analyzed exhibited omega-6:3 ratios exceeding 20% including: *Acer rubrum* 27.35 nmols/mg, *Brassica napus* 37.67 nmols/mg, *Impatiens capensis* 22.25 nmols/mg, *Plantago lanceolata* 24.16 nmols/mg, *Quercus rubra* 34.05 nmols/mg, *Taraxacum officinale* 29.75 nmols/ mg, and *Trifolium* sp. 25.00 nmols/mg.

Nutrition content by plant family

Examination of total NEFA content revealed that pollen species were not clearly segregated into distinct groups based on family (Figure 3A). Clustering of total AA content was only evident for Rosaceae (Figure 3B). However, clustering of total EAA content revealed that Asteraceae, Brassicaceae, and Fabaceae families formed distinct clusters (Figure 3C). A notable clustering was observed within the Brassicaceae family when analyzing total NEAA content (Figure 3D). PGLS revealed a significant negative correlation between total NEFA and total AA (t-value = −9.18, df = 57, $p < 0.0001$; Figure 4A) suggesting that total AA decreases as total NEFA increases.

Moreover, EAA exhibited a notable inverse correlation with NEFA (t-value = −4.25, df = 57, $p < 0.0001$), which suggests a concurrent increase in EAA content with a decrease in NEFA content (Figure 4B). The relationship between NEFA and NEAA was also negatively correlated (t-value = −7.52, df = 57, $p < 0.0001$), highlighting that an increase in NEAA corresponds with a reduction in NEFA (Figure 4C). Further analysis revealed a significant negative correlation between total omega fatty acids and EAA (t-value = −2.92, df = 57, p value = 0.0051), showing that an elevation in EAA is potentially linked with a decline in omega fatty acids within the species studied (Figure 4D).

Top pollen species ideal for bee nutrition

The majority of pollen species exhibited a predominance of one of two AA: histidine or arginine (Figure 5; Supplementary Table S5). Our results indicated that Ranunculus acris presented the most similar alignment with honey bee dietary requirements, achieving the lowest Euclidean average similarity score of 12.66. This was closely followed by *Actinidia arguta (*13.36), *Lotus corniculatus* (13.56), Cornus sericea (14.54), *Rosa multiflora* (14.78), *Rubus idaeus (*15.12), Rosa virginiana (15.50), *Hibiscus syriacus* (17.04), *Rhus typhina* (18.29), *and Viburnum opulus* (18.63) comprising the top 10 plant species most aligned with the honey bees' nutritional requirements.

Nutritional content by plant endemicity

Across the focal 57 plant species, 30 (53%) are introduced and 27 (47%) are native to eastern North America (Supplementary Table S6). Average mean lipid content was not significantly different between introduced (16.02 µg/mg) and native (12.02 µg/mg) pollen species ($t = 1.10$, df = 25.5, p value = 0.28). For protein content, native species show a non-significantly higher average of 134.05 µg/mg compared to the 116.17 µg/mg in introduced species ($t = -0.82$, df = 30.15, p value = 0.42). Average P:L ratio was not significantly different between native and introduced pollen species, with a mean P:L ratio of 0.57 in native compared to 0.56 in introduced species ($t = -0.30$, df = 34.1, p value = 0.76). Additionally, native species have a mean total omega content of 6.81 nmols/mg, which is lower than the introduced species (8.79 nmols/mg), but non-significantly different ($t = 1.03$, df = 31.2, p value = 0.31). Overall, nutritional content did not statistically differ in pollen species based on endemicity.

Discussion

Here we profiled the nutritional content of 57 pollen species to examine their value for bee health and long term conservation restoration initiatives. There was a consistent presence of five specific NEFAs across pollen species: linoleic (omega-6), linolenic (omega-3), oleic, palmitic, and palmitoleic acids. Notably, linolenic and linoleic acids, known for their roles in bee immune function and developmental support (Manning, 2001), were present and highly abundant across the majority of pollen species, with palmitic acid also commonly abundant across species. Further analysis revealed significant variability in the AA content among these species with all pollen samples containing EAA. However, the presence of arginine and histidine was notably predominant. Our findings also highlight extensive diversity in the P:L ratios, indicative of the vast range of P:L content among the different pollen species, a trait observed even within the same genus. This underscores the complexity of the nutritional landscape bees navigate and the diversity of plants required to meet pollinator dietary needs. We also note that there were no significant nutritional differences between the pollen nutritional profiles of native and introduced plant species. This suggests that wild bees, particularly generalist foragers, have the capacity to obtain their nutritional needs from an array of plant sources, regardless of the plant's origin. Our findings indicates that several plant genera and families provide pollen with nutritional profiles that match the dietary requirements of honey bees, and potentially wild bees, though experimental research is much needed. This demonstrates the importance of diverse floral landscapes play in fulfilling the dietary needs of wild bees, which is imperative for sustaining ecological balance and supporting conservation efforts.

Role of fatty acids on bee nutrition

Non-esterified fatty acids support the complex dietary needs of pollinators. Pollen species typically comprised of 7–10 different NEFAs. *Rhus glabra* exhibited the lowest NEFA concentration, contrasting with the highest levels found in *Impatiens capensis*. Interestingly, specific fatty acids such as docosahexaenoic acid, eicosapentaenoic acid, and arachidonic acid were exclusive to a few species. Docosahexaenoic acid and eicosapentaenoic acid are omega-3 fatty acids that are important components in the diet of many animals, including bees (Arien et al., 2015). Although these fatty acids are rare in pollen, their presence significantly enhances its nutritional profile and can lead to improved cognitive abilities and overall health in pollinator species, marking them as key components in bee nutrition (Arien et al., 2015). The occasional presence of these omega-3 fatty acids in certain pollen types introduces diversity in nutritional content available to fulfill the dietary requirements of various pollinating species. Linolenic, palmitic, and linoleic acids were predominant across the majority of species analyzed. Omega-3 (linolenic acid) strengthens the immune response and supports the development of the nervous system in bees, both essential for effective foraging and navigation (Manning, 2001). Omega-6 fatty acids, particularly linoleic acid, have been directly linked to influencing key physiological processes in bees, including growth, reproduction, and larval development, thereby contributing to the colony’s health and resilience (Arien et al., 2020). An increase in dietary lipid levels was found to positively correlate with brood development, while imbalances in the omega-6:3 ratios are linked to decreased adult survival and impaired brood rearing (Arien et al., 2020). NEFA content did not show a uniform distribution among pollen species by plant family, except for some grouping within the Rosaceae based on total AA content. When assessing EAA content, both our results and those of Chau and Rehan (2024) indicate that pollen species within the Asteraceae are uniquely grouped, distinguishing them from other families. Similarly, distinct clusters were also observed in the Brassicaceae, and Fabaceae families. We found significant negative correlations between total NEFA and AA levels, suggesting that an increase in NEFA corresponds with a decrease in AA concentrations, a trend consistent for both EAA and NEAA contents. Furthermore, a significant inverse relationship was detected between total omega fatty acids and EAA, indicating that a rise in EAA may be associated with a decrease in omega fatty acid content within these pollen species. The contribution of various fatty acids to bee immune defense is well-documented, with stearic acid found in beeswax and pollen known to strengthen bee immune function (Alaux et al., 2010), and omega-3 fatty acids such as linolenic acid integral for the immune response and nervous system development in bees (Manning, 2001). Myristic acid is activates enzymes for immune responses, though its direct impact on immunity requires further investigation (Brodschneider and Crailsheim, 2010). Additionally, oleic acid is recognized for its anti-inflammatory and antimicrobial properties and may support bee health while serving as a natural hive preservative (Vaudo et al., 2016a).

Role of amino acids on bee nutrition

All 10 EAAs were present across the examined pollen species, with the sole exception of smooth sumac (*Rhus glabra*) which lacked methionine. Methionine plays an essential role in the development of honey bee larvae, particularly during pupation and eclosion, and is an important l-methyl donor that influences caste differentiation in bee colonies, affecting the development of female larvae into worker bees (de Groot, 1953; Chen et al., 2021). Regardless, methionine was generally the least abundant EAA detected across all pollen species. Red oak (*Quercus rubra*) displayed the most diverse and abundant EAA profile.

Among the subset of pollen species with an EAA concentration exceeding 20% of the total AA profile, those from the Asteraceae family stood out. Specifically, seven species within this family, including lanceleaf tickseed (*Coreopsis lanceolata*) and chicory (*Cichorium intybus*), demonstrated the highest EAA levels, exceeding 20% of the total AA content. This is consistent with previous research highlighting the Asteraceae family as a particularly rich source of EAAs for bees (Vaudo et al., 2015). The diverse array of Asteraceae species, many of which are bee-pollinated, likely contributes to this family's prominence as a valuable nutritional resource for wild bee communities. Histidine was especially prevalent, constituting nearly 35% of the total AA profile in all pollen samples, followed by arginine, indicating the prominence of these AAs in bee nutrition. Nonetheless, methionine was consistently found in lower quantities relative to the other EAAs examined.

While our study found that *Ranunculus acris* pollen most closely matches the EAA needs of honey bees, no single pollen species was found to perfectly match honey bee nutritional EAA requirements. Rather, several pollen species portrayed EAA amounts conducive to honey bee EAA needs. This suggests that rather than seeking an optimal pollen source, a diversified approach incorporating a variety of plants is more beneficial for bee health. Future research should broadly profile the nutritional content of various plants, considering their availability across seasons and bees' foraging preferences in different environments. It is also important to understand how diet variations affect bee resilience to challenges such as diseases and climate change, which will be pivotal in developing support strategies for bee populations, essential for both natural ecosystems and agriculture globally.

The NEAA proline emerged as the most prevalent metabolite across pollen species, often constituting over half of the total AA content. Proline serves as an alternative energy source for bees, especially during foraging activities (Jeannerod et al., 2022). Its presence in floral nectars enhances the attractiveness of these plants to various pollinators and may improve nectar’s nutritional quality (Carter et al., 2006). Diets high in proline have been linked to faster development rates in bee broods, suggesting that proline could reduce the time required for bees to reach maturity (Stec et al., 2021). Additionally, proline acts as a potent feeding stimulant, influencing honey bees’ dietary choices and contributing to key reproductive functions, such as egg-laying in queens (Bouchebti et al., 2022). The prevalence of proline in both pollen and nectar highlights its integral role in bee health and the broader pollination ecology.

Interactions between lipids and proteins

This study reveals a complex balance between NEFAs and AAs within pollen nutrition, highlighting a trend where pollen types rich in NEFAs typically have lower levels of AAs. In contrast, pollen with a higher content of EAAs tends to have lower omega fatty acid concentrations. Hendriksma et al. (2019) support this finding, noting that while a diet high in lipids can boost brood production, an elevated omega-6:3 ratio may increase mortality and reduce brood rearing. This suggests that pollen with high NEFA levels, especially those with a skewed omega-6:3 ratio, may naturally adjust EAA levels to maintain an optimal nutritional profile for bee health. This balancing act is reflected in bee foraging behavior, where bees seem to preferentially select pollen with an optimized lipid composition, likely to support reproductive processes (Ruedenauer et al., 2020). Additionally, Arien et al. (2018) highlighted the importance of the omega-6:3 fatty acid ratio in bee diets, demonstrating that an imbalance in these essential lipids can affect not just bee health, but also their cognitive functions such as learning and memory. All studies highlight the diverse nutritional profiles across pollen species which emphasizes the need for varied floral diets. Such diversity is ideal for enhancing bee health and mitigating the effects of lipid overabundance, which is known to impair sensory acuity and alter foraging patterns detrimentally (Bennett et al., 2022).

We found a wide range of P:L and omega-6:3 ratios across the various pollen species. Incorporating data from Vaudo et al. (2024) revealed large P:L ratio differences even within the same genus. For instance, the highest P:L was in *Prunus salicina* (Vaudo et al., 2024) and the lowest was in another *Prunus* sp. (this study). The study by Vaudo et al. (2020) emphasizes that pollen P:L ratios can significantly guide the floral preferences of bees. Specifically, they found that bumble bees (*Bombus impatiens*) preferentially foraged on pollen with higher P:L ratios, as these macronutrient profiles better matched their nutritional requirements for growth, development, and colony fitness (Vaudo et al., 2016a). This suggests that bees may selectively forage on pollen that optimizes their nutritional intake. The wide variation in P:L and omega-6:3 ratios across pollen species, even within the same plant genus, illustrates the complex nutritional landscape that bees navigate. This diversity of pollen nutritional profiles likely enables bees, especially specialist species, to selectively forage on the resources that best meet their unique dietary requirements.

Ideal pollen species

The top 10 pollen species from this study demonstrated that EAA ratios aligned with honey bee dietary needs vary considerably in their total lipid content, indicating that focusing exclusively on EAAs overlooks other important nutritional factors. This broader perspective is important for appreciating the complex dietary landscape bees encounter. Reinforcing these observations, Di Pasquale et al. (2013) found that while high EAA diets enhance certain developmental factors in bees, a diet balanced with NEAAs is essential for overall colony health and longevity, particularly as EAA dominance in pollen may elevate susceptibility to pathogens like the *Nosema ceranae* parasite. Together, these insights emphasize the complexity of bee nutrition, suggesting that multiple dietary components contribute to their health and colony sustainability.

While EAAs are essential for supporting larval growth and sustaining adult bees (Vaudo et al., 2016a,b), we should also consider the overall lipid profile. Our findings suggest that an optimal pollen diet for bees balances EAAs with a mix of lipids, maintaining an ideal omega-6:3 ratio close to 4:1 (Arien et al., 2020), and favorable P:L ratios, which range from 1:1 to 2:1 for honey bees (Vaudo et al., 2020). In this study, pollen species like *Ranunculus acris*, *Actinidia arguta*, *Rosa* sp. and *Rubus idaeus* all exhibited P:L ratios around 2.24, and are identified as particularly well-suited for wild bee nutrition. Similarly, Chau and Rehan (2024) found that pollen from *Rosa* sp., *Trifolium* sp., and *Rubus idaeus* were considered ideal for bumble bees and honey bees, with P:L ratios greater than 2:1 and EAA compositions similar to honey bee EAA requirements.

Nutritional content between native and introduced plants

Our findings reveal that the nutritional content of pollen from both native and introduced plant species shows no significant differences, with marginally higher average omega fatty acid levels in introduced species. Average P:L ratios were also found to be consistent regardless of the plants' endemicity status. Further analysis indicates that both native and introduced flora provide key lipids essential for bee development, with little difference in average lipid or protein content between them. This continuity in pollen nutrition regardless of endemicity demonstrates the remarkable potential for adaptability of pollinators to new and ever changing resources in their ecosystems. For instance, studies have shown that moderate levels of omega-3 polyunsaturated fatty acids (PUFAs), such as alpha-linolenic acid, can enhance immune function and longevity in honey bees (Arien et al., 2015). Additionally, omega-6 PUFAs like linoleic acid are essential for proper development and growth in bees (Vaudo et al., 2016b). Therefore, the slightly elevated omega fatty acid profiles observed in introduced plant species may actually expand the nutritional options available to native pollinators, provided the overall lipid composition remains balanced. The ability of bee species like the small carpenter bee (*Ceratina calcarata*) to adjust to varied dietary resources, such as foraging on clover (*Trifolium* sp.) despite its non-native origin, further highlights the resilience and flexibility of native pollinators given alternative food sources (Kooyers and Olsen, 2012; Lawson et al., 2020). However, this may be due to the polylectic nature of many native bees, whereas oligolectic bees are more limited in foraging resources. This continuity in pollen nutrition regardless of endemicity demonstrates the need to characterize foraging preferences and dietary requirements of wild bees more broadly to understand the adaptability of pollinators to new and native resources in their ecosystems.

Conservation implications and future directions

The global decline of bee populations is an alarming issue, compounded by a multitude of stressors such as disease, climate change, parasites, poor nutrition, habitat destruction, and pesticide use (Klein et al., 2017; Cameron and Sadd, 2020). Endangered species, such as *Bombus affinis* and *Bombus franklin* as well as various solitary bees that have specialized plant dependencies, suggests a need for conservation measures (Strange and Tripodi, 2019; Graves et al., 2020; Kline and Joshi, 2020). A significant gap in our understanding lies in the inadequately studied dietary requirements of most bees species and nutritional profiles of many plant species for bee health (Kriesell et al., 2017). Effective conservation strategies must prioritize habitat restoration and the cultivation of diverse plant species, catering to the complex dietary requirements of bees (Brown and Paxton, 2009). Specifically, we recommend pollen species from roses (*Rosa* sp.), clovers (*Trifolium* sp.), red raspberry (*Rubus idaeus*), tall buttercup (*Ranunculus acris*), and Tara vine (*Actinidia arguta*) to be emphasized in wild flower restoration projects based on their ideal P:L ratios for wild bee nutrition. Our study reinforces this need, highlighting the role that varied pollen sources and diverse floral landscapes play in ensuring the nutritional welfare of pollinators. This diversity is essential for sustaining ecological balance and supporting robust plant-pollinator interactions, which are fundamental to the well-being of ecosystems.

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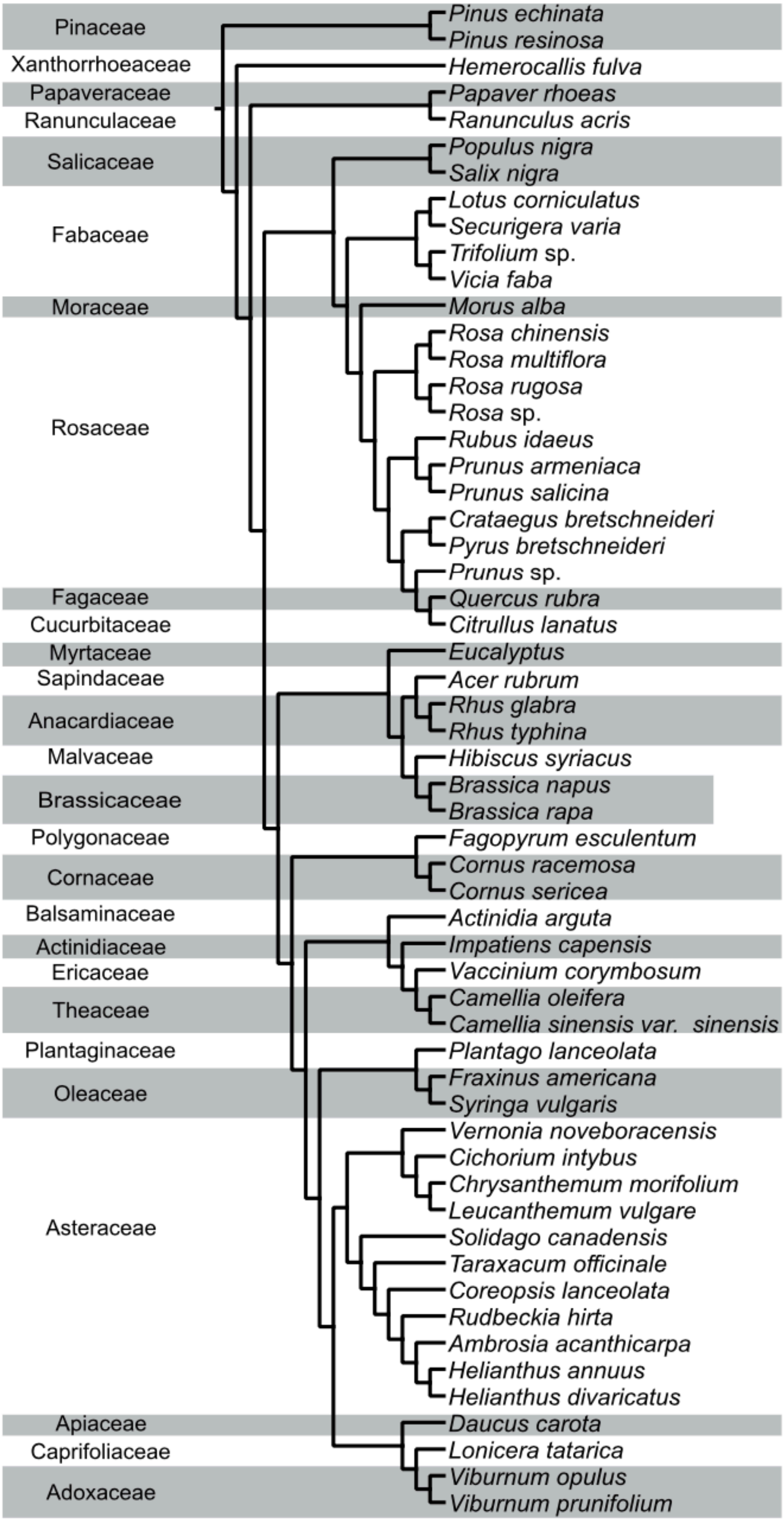
Figures for Chapter 1.


Pinaceae
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Oleaceae
Asteraceae
Apiaceae
Caprifoliaceae
Adoxaceae
Pinus echinata
Pinus resinosa
Hemerocallis fulva
Papaver rhoeas
Ranunculus acris
Populus nigra
Salix nigra
Lotus corniculatus
Securigera varia
Trifolium sp.
Vicia faba
Morus alba
Rosa chinensis
Rosa multiflora
Rosa rugosa
Rosa sp.
Rubus idaeus
Prunus armeniaca
Prunus salicina
Crataegus bretschneideri
Pyrus bretschneideri
Prunus sp.
Quercus rubra
Citrullus lanatus
Eucalyptus
Acer rubrum
Rhus glabra
Rhus typhina
Hibiscus syriacus
Brassica napus
Brassica rapa
Fagopyrum esculentum
Cornus racemosa
Cornus sericea
Actinidia arguta
Impatiens capensis
Vaccinium corymbosum
Camellia oleifera
Camellia sinensis var. sinensis
Plantago lanceolata
Fraxinus americana
Syringa vulgaris
Vernonia noveboracensis
Cichorium intybus
Chrysanthemum morifolium
Leucanthemum vulgare
Solidago canadensis
Taraxacum officinale
Coreopsis lanceolata
Rudbeckia hirta
Ambrosia acanthicarpa
Helianthus annuus
Helianthus divaricatus
Daucus carota
Lonicera tatarica
Viburnum opulus
Viburnum prunifolium

Figure 1. Phylogenetic tree of pollen species grouped by their plant families from this study and from Chau and Rehan (2024). In total, 57 pollen species from 45 genera and 27 families are analyzed for lipid and protein content in this study.

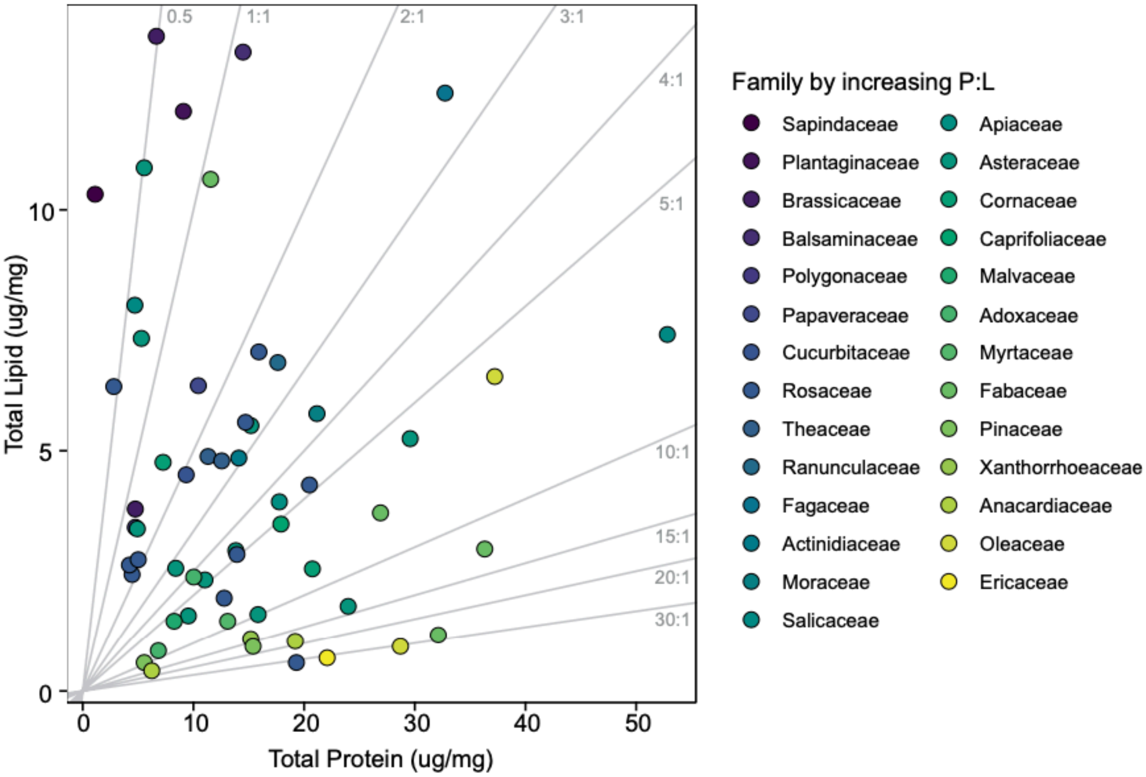

Figure 2. Scatterplot of pollen protein and lipid concentrations (μg/mg). Each point corresponds to a specific pollen species, with the color of the marker varying according to the plant family. Gray lines indicate P:L ratios and range from low to high moving left to right.

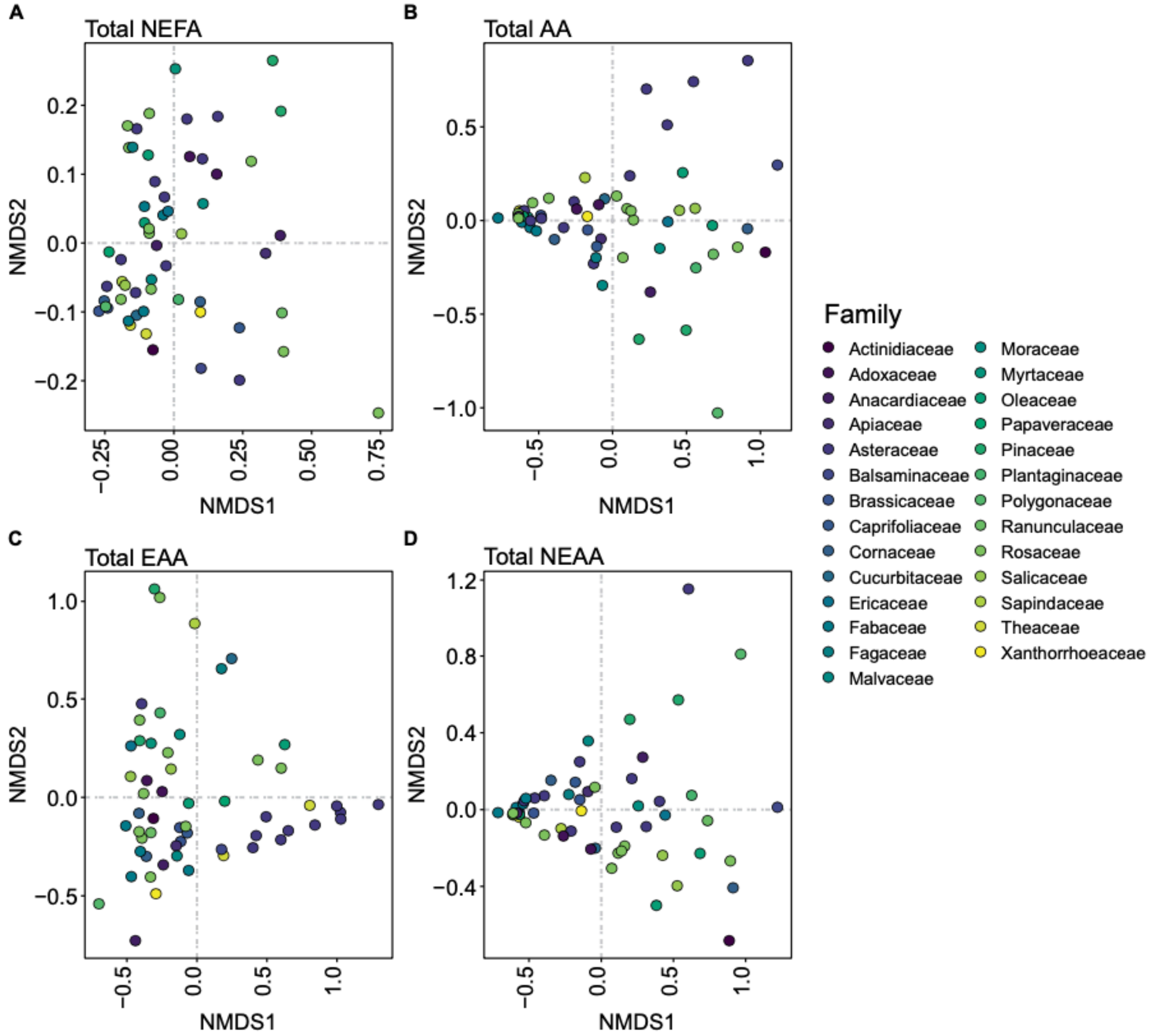

Figure 3. Non-metric dimensional scale (NMDS) plots representing the distribution of each pollen species per family. (A) Total non-esterified fatty acid (NEFA) content, (B) total amino acid (AA) content (essential and non-essential amino acids), (C) total essential amino acid (EAA) content, and (D) total non- essential amino acid (NEAA) content.

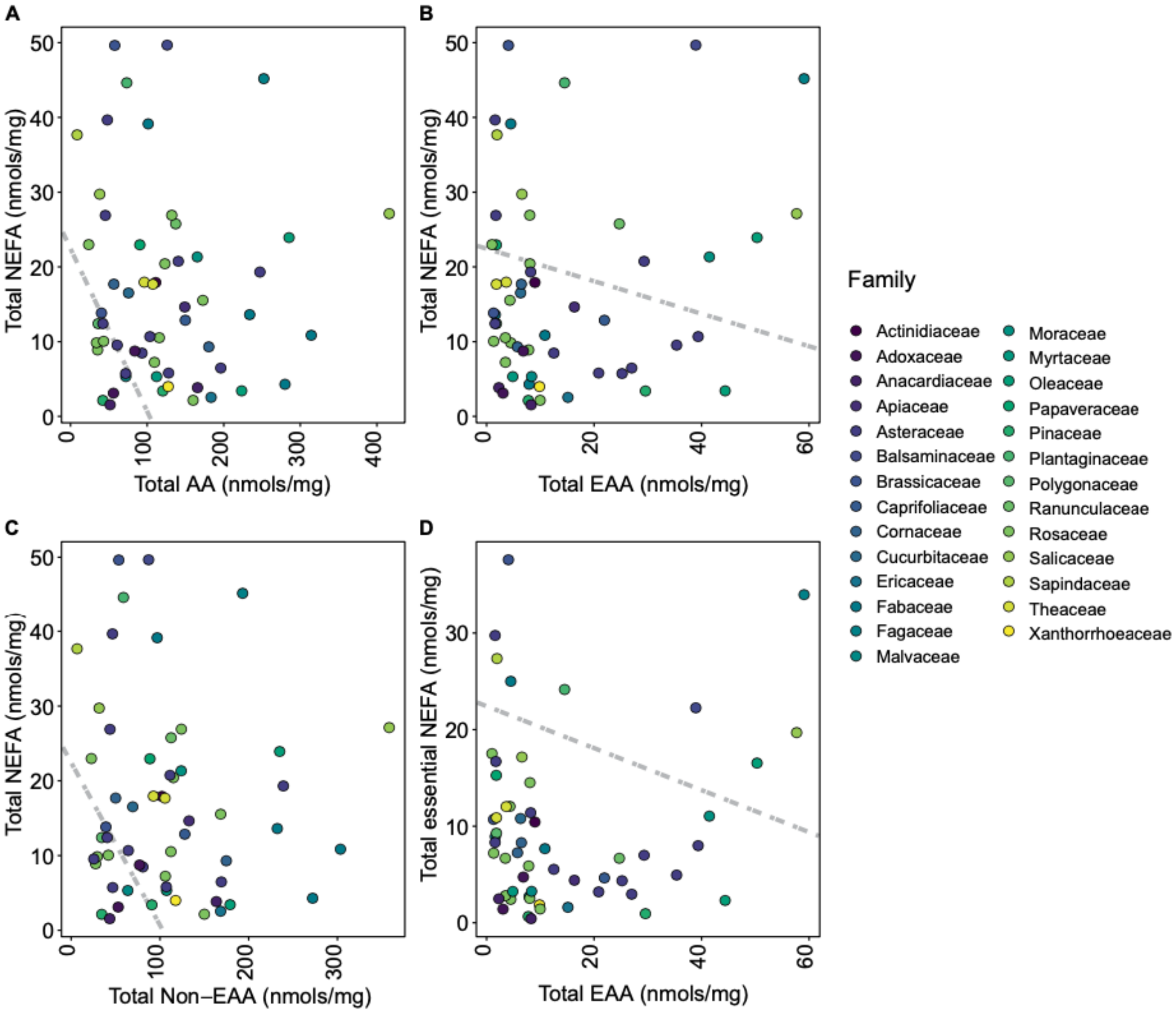

Figure 4. Phylogenetic generalized least square (PGLS) plots representing distribution of each pollen species per family. (A) Total non-esterified fatty acids (NEFA) against total amino acids (AA), (B) total NEFA against total essential amino acids (EAA), (C) total NEFA against total non-essential amino acids (NEAA), and (D) total essential NEFA (linoleic acid and linolenic acid) against total EAA.

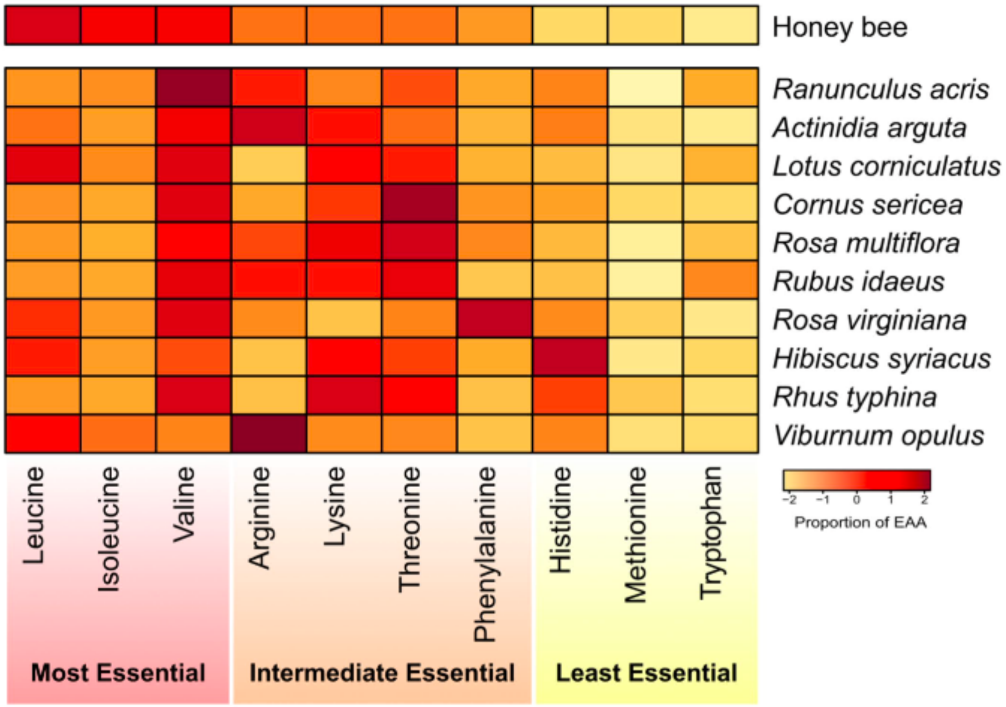

Figure 5. Heatmap representing the percentage composition of each essential amino acid (EAA) within different pollen species. Each color-coded square indicates the proportion of each AA relative to the total EAA present. The heatmap displays the percentage requirements of each EAA for honey bees as determined by de Groot (1953). The EAAs are arranged in descending order of their importance to honey bee nutrition, and the pollen species are ranked from the most beneficial to the least beneficial for honey bees based on Euclidean distances.

Chapter III: Optimizing Bee Nutrition: How Macronutrient Composition in Pollen Affects Development and Survival in *Ceratina calcarata*

Khara W. Stephen and Sandra M. Rehan

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*) from artificial nest substrates and reared them on diets formulated with black poplar (*Populus nigra*) and dandelion (*Taraxacum officinale*) 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 lacks the protein and essential fatty acids necessary for optimal larval growth. In contrast, black poplar pollen, despite being primarily wind-dispersed, emerged as a superior protein source, significantly enhancing bee body size, developmental rates, and survival. Bees reared on black poplar pollen exhibited faster maturation, larger head widths, and greater body mass, highlighting the 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 an overlooked nutritional resource and accentuate the need 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: Nutritional ecology, Pollen nutrition, Amino acids, Fatty acids, Pollinator diet, Bee health, Dietary diversity, Larval development, Wind-pollinated trees, Body size and fitness

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 (Vaudo et al., 2015; Filipiak & Filipiak, 2020).

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, fueling adult metabolic processes, flight, and thermoregulation (Filipiak & Filipiak, 2020). Additionally, a minority of bee species utilize specialized floral oils, further diversifying their nutritional resources (van der Kooi et al., 2021; Manning, 2001).

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 ten 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 (Hendriksma et al.,2019; Jeannerod et al., 2022; Filipiak & Filipiak, 2020). Consequently, pollen sources rich in essential amino acids significantly influence bee foraging behavior and dietary preferences, ultimately shaping pollinator health and community dynamics across ecosystems.

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). Understanding these lipid dynamics, therefore, is essential for accurately assessing bee nutritional requirements and predicting population resilience under changing environmental conditions.

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 pollen processing helps regulate lipid content and optimize fatty acid ratios, particularly omega-6 to omega-3 fatty acid proportions, to ensure balanced larval nutrition and promote optimal developmental outcomes (Leonhardt et al., 2022). Although adult nurse bees themselves require relatively lower lipid reserves, the careful modulation of lipid content and fatty acid balance during processing significantly influences larval health, growth rates, and immune development (Wright et al., 2018; Leonhardt et al., 2022). In contrast, solitary bees such as *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 (Shell & Rehan, 2018; Lawson et al., 2020).

Recent studies have highlighted the nutritional significance of blueberry (*Vaccinium corymbosum*) pollen for bee health, primarily due to its unique amino acid and fatty acid composition. However, blueberry pollen is generally considered to have a poor nutritional profile for bees, with a crude protein content of approximately 13.9%, which is lower than that of other floral sources (Graham et al., 2023). The broader implications of pollen nutritional quality extend beyond immediate developmental outcomes to influence bee foraging behaviour and reproductive success. For example, studies have shown that pollen nutritional content directly affects bee larval growth and subsequent adult health, reinforcing that nutrition not only shapes individual fitness outcomes but also drives broader ecological patterns of pollinator behaviour and community structure (Jones and Rader., 2022). Consequently, integrating nutritionally beneficial plants into agroecosystems and restoration landscapes could significantly strengthen pollinator populations and improve ecosystem resilience.

In the context of blueberry cultivation, research has revealed that different cultivars produce varying quantities and qualities of pollen. For instance, the 'Elliott' cultivar produces nearly twice the amount of pollen per flower by weight compared to the 'Duke' cultivar (Chakrabartiet al., 2025). Both cultivars exhibit relatively low pollen protein content and comparable pollen lipid levels (Chakrabarti et al., 2025). These differences in pollen production and nutritional quality can influence bee foraging preferences and, subsequently, pollination efficiency. Understanding these cultivar-specific variations is helpful for optimizing pollination strategies and supporting healthy bee populations. Moreover, environmental factors such as extreme heat events can alter the nutrient composition of blueberry pollen, making it less suitable for bee nutrition (Walters et al., 2025). Hence, it is important to consider both plant selection and environmental conditions in agroecosystem management to ensure the availability of high-quality pollen resources for pollinators.

Seasonality also significantly shapes nutritional availability and quality for pollinators (Harris et al., 2024). While early-season arboreal pollen sources like willow (*Salix spp.*), maple (*Acer spp.*), and poplar (*Populus spp.*) provide resources for spring-emerging bees, different nutritional challenges arise later in the season when floral diversity typically declines (Timberlake et al., 2019; Sponsler et al., 2020). Late-summer and fall bees depend heavily on late-blooming taxa such as asters (*Asteraceae*) and goldenrods (*Solidago spp.*) to sustain nutritional demands before overwintering (Smart et al., 2019). Thus, understanding seasonal variation in floral resource availability and nutritional quality is essential for effective conservation and restoration strategies aimed at maintaining pollinator populations year-round (Timberlake et al., 2019; Splitt et al., 2021).

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). Contrary to this oversight, recent studies have revealed that bees frequently utilize these pollen sources, suggesting that such pollen plays an important nutritional role even if the trees are primarily wind-pollinated (Westreich et al., 2023; Ghosh et al., 2020). Furthermore, flowering trees such as apple, pear, redbud, and serviceberry represent high-quality nutritional resources routinely visited by bees for pollination services, showcasing the need for more comprehensive assessments of arboreal pollen's nutritional contributions to bee populations (Wu et al., 2021; Ostaff et al., 2015).

The small carpenter bee *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 (Shell & Rehan, 2018; Lawson et al., 2017). This species emerges in the spring but maintains prolonged activity throughout the summer and into early fall, encountering a wide range of floral resources and thus reflecting nutritional demands representative of 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*. This research explicitly addresses these gaps by evaluating the effects of pollen macronutrient composition especially proteins, lipids, and fatty acid ratios on 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 the higher protein content and beneficial lipid profiles typically found in tree pollen, 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. Additionally, acknowledging the nutritional complementarity commonly observed in polyfloral diets (Eckhardt et al., 2014; Vaudo et al., 2016), we predict intermediate physiological and developmental outcomes for bees reared on mixed pollen diets (particularly 50:50 blends of poplar and dandelion). Dandelion pollen is especially controversial in pollinator nutrition because, despite being a widely accessible resource 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 (Peng et al., 1985; Fan et al., 2018; Anderegg et al., 2021). Ultimately, this study aims to contribute essential insights toward understanding nutritional ecology in wild solitary bees, informing targeted conservation strategies that prioritize diverse floral resources throughout seasonal periods, and supporting resilient pollinator communities amid ongoing environmental challenges.

Materials and Methods

Nest Collection, Dissection, and Brood Extraction

Raspberry canes (*Rubus idaeus*) were prepared as artificial nesting substrates and placed at several sampling sites within Toronto, Ontario, 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, eggs and small larvae were randomly assigned to experimental diet treatments to ensure unbiased distribution across dietary groups.

Pollen Diet Preparation and Larval Rearing

Dandelion (*Taraxacum officinale*) and black poplar (*Populus 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, 75:25, 50:50, 25:75, and 0:100. 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 *Ceratina calcarata* females when provisioning larvae (Lawson et al., 2016, 2020). Standardized 4-gram 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., 2016). 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 mg ±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 two days, categorizing 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 micrometer, 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 analyzed to quantify protein, carbohydrate (sugar), amino acids, and fatty acids. To standardize dry weight, pollen samples were air-dried for four days prior to analyses. Sugar content was assessed by suspending pollen samples in 200 µl distilled water, centrifuging at 2500 rpm for five minutes, 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 analyzer), 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 NEFA analysis, a 10 mg aliquot was spiked with isotopic internal standards, extracted, dried, and reconstituted before analysis. Quantification of 12 NEFAs was performed on a Thermo Quantum Ultra triple quadrupole mass spectrometer interfaced with a Waters Acquity liquid chromatography system, as per Persson et al. (2010), using negative electrospray ionization. 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 analyzed 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 three 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 hours at room temperature. Extracts were centrifuged at 14,000 rpm for 10 minutes (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 sulfuric 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 analyzed using one-way ANOVAs, followed by Tukey’s post-hoc tests. Non-parametric data, including development duration and sugar concentration, were analyzed with Kruskal-Wallis tests followed by Dunn’s multiple comparison tests (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 and Grambsch 2001). Macronutrient differences between pollen types (protein, amino acids, fatty acids, sugar) were analyzed using independent-sample t-tests or Wilcoxon rank-sum tests, based on the normality of data distribution.

Results

Macronutrient Composition

The macronutrient profiles of black poplar and dandelion pollen revealed significant differences in protein, sugar, amino acid, and fatty acid content (Fig. 1, Table 1). 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 nmols/mg 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/mg), in black poplar pollen compared to dandelion pollen (23.5 nmols/mg 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, $p = 0.024$; Fig. 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 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, $p < 0.001$; Fig. 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, $p = 0.002$; Fig. 3). Bees fed exclusively dandelion pollen exhibited greater survival and prolonged developmental durations during larval stages, indicative of delayed maturation. In contrast, 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 ($F = 4.9$, $df = 5,80$, $p < 0.001$; Fig. 4). Mean body mass was 13.3 mg for males and 12.3 mg for females. Bees fed the 25:75 black poplar: dandelion diet were significantly heavier than bees fed 100% dandelion diets (mean difference = 6.3 mg; 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).

Lipid content also varied significantly across dietary treatments ($F = 9.2$, $df = 10,94$, $p < 0.001$; Fig. 5). Bees fed the 75:25 black poplar: dandelion diet had the highest lipid levels (mean = 1.33 mg), significantly higher compared to those consuming 100% dandelion (0.07 mg; $p < 0.001$), 50:50 diets (0.29 mg; $p < 0.001$), and 25:75 diets (0.32 mg; $p < 0.001$). Bees fed exclusively black poplar pollen also had significantly higher lipid levels than those fed exclusively dandelion (mean difference = 1.13 mg; $p < 0.001$), 50:50 diets (mean difference = 0.91 mg; $p = 0.001$), and 25:75 diets (mean difference = 0.88 mg; $p = 0.003$). Control bees exhibited intermediate lipid content (0.88 mg), significantly higher than bees consuming 100% dandelion pollen (mean difference = 0.81 mg; p = 0.003) but lower than bees consuming the 75:25 diet (mean difference = 0.46 mg; 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 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 clearly demonstrated 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. 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 limit 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 highlight the complexity of the nutritional landscape bees navigate in nature and highlight the importance of incorporating diverse and high-quality pollen sources, particularly those from wind-pollinated trees such as black poplar, into conservation and restoration strategies aimed at sustaining healthy pollinator populations.

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 (Vaudo et al., 2015; Di Pasquale et al., 2013; Filipiak & Filipiak, 2020). Such differences likely underpin the variations we noted in developmental rates, body mass, and lipid reserves.

Protein-rich pollen sources can significantly enhance larval growth, survival, and subsequent adult fitness in bees (Vaudo et al., 2015; Hendriksma et al., 2019). Honey bees (*Apis mellifera*) that consume protein-deficient diets experience delayed brood development, reduced worker performance, and elevated mortality (Di Pasquale et al., 2013; Brodschneider & Crailsheim, 2010). 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, especially when coupled with sufficient sugars, serves as a key determinant for optimal larval nutrition and overall bee health.

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 (Obata and Miura, 2024; Delescluse et al., 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 (Ma et al., 2016; Honda et al., 2011). 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 and 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 (Ma et al., 2016; Honda et al., 2011). 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. 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 (Leonhardt et al., 2011; Hendriksma et al., 2014).

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) 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 (Vaudo et al., 2015; Hendriksma & Shafir, 2016). Larger bees typically exhibit enhanced flight performance, more efficient foraging, and improved thermoregulatory capacity (Glass and 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 and 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 and thermoregulation (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, ultimately constraining colony growth and productivity.

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 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 (Naug, 2009; Di Pasquale et al., 2013; 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 (Blanckenhorn, 2000; Schmid-Hempel, 2005; Alaux et al., 2010). 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; Hendriksma et al., 2014; Filipiak, 2018). Polyfloral pollen diets have consistently been associated with enhanced survival, stronger pathogen resistance, and elevated colony productivity (Topal et al., 2022; Filipiak et al., 2017). 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 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 centered 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 emphasize the ecological role of arboreal pollen, particularly in early spring, when herbaceous plants have not yet begun flowering (Ostaff et al., 2015; Splitt et al., 2021; Ghosh et al., 2020). Our results highlight the importance of rethinking conservation priorities to include wind-pollinated species such as black poplar. Doing so could bolster pollinator health by buffering seasonal pollen deficits and mitigating the adverse impacts of phenological mismatches driven by climate change (Splitt et al., 2021; Eckhardt et al., 2014). In line with these studies, our findings highlight that wind-pollinated trees such as *Populus* and *Salix* can be indispensable contributors to the nutritional landscape for 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 quantifying long-term fitness consequences of diets rich in wind-pollinated pollen, assessing reproductive outcomes, immune competence, and stress resistance across multiple generations. 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 behavior, and environmental stressors such as pesticides and pathogens (Ghosh et al., 2020; Arien et al., 2018). 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. By incorporating diverse pollen sources, including those from wind-pollinated trees into habitat restoration and conservation initiatives, land managers can help mitigate nutritional deficits, foster ecological resilience, and support the long-term viability of both managed and wild bee communities.

Chapter 3 Tables and Figures.

Table 1. Macronutrient composition of Populus nigra (Black Poplar) and Taraxacum officinale (dandelion) pollen. "fold_diff" represents the ratio of Black Poplar to Dandelion values for each macronutrient, while "log2_fold_diff" is the base-2 logarithm of that ratio. Groups include essential amino acids (nmols/mg), essential fatty acids (nmols/mg), non-essential fatty acids (NEFAs) (nmols/mg), protein, and carbohydrate (mg).

Group	Macronutrient	*T. officinale*	*P. nigra*	fold_diff	log2_fold_diff
Carbohydrate (mg)	Sugar (Sucrose)	4.30	9.55	2.22	1.15
Essential amino acid mols/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	31.1	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
Essential fatty acid nmols/mg	Linoleic	6.23	3.11	0.50	-1.00
	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

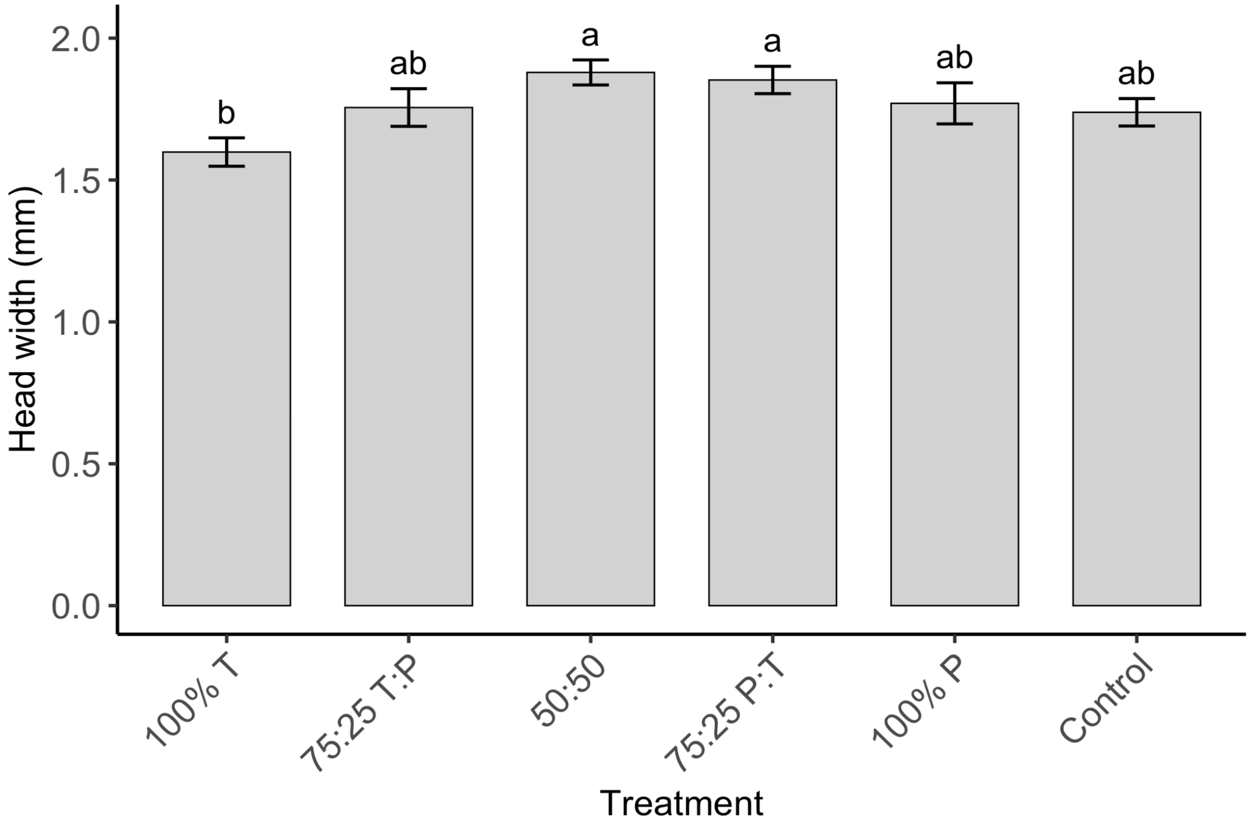

Figure 1. Mean head width (mm) of *Ceratina calcarata* reared on different pollen diet treatments. Treatments are abbreviated as follows: "100% T" (representing 100% *T. officinale*), "75:25 T:P" (75% *T. officinale*, 25% *P. nigra*), "50:50," "75:25 P:T" (75% *P. nigra*, 25% *T. officinale*), "100% 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.

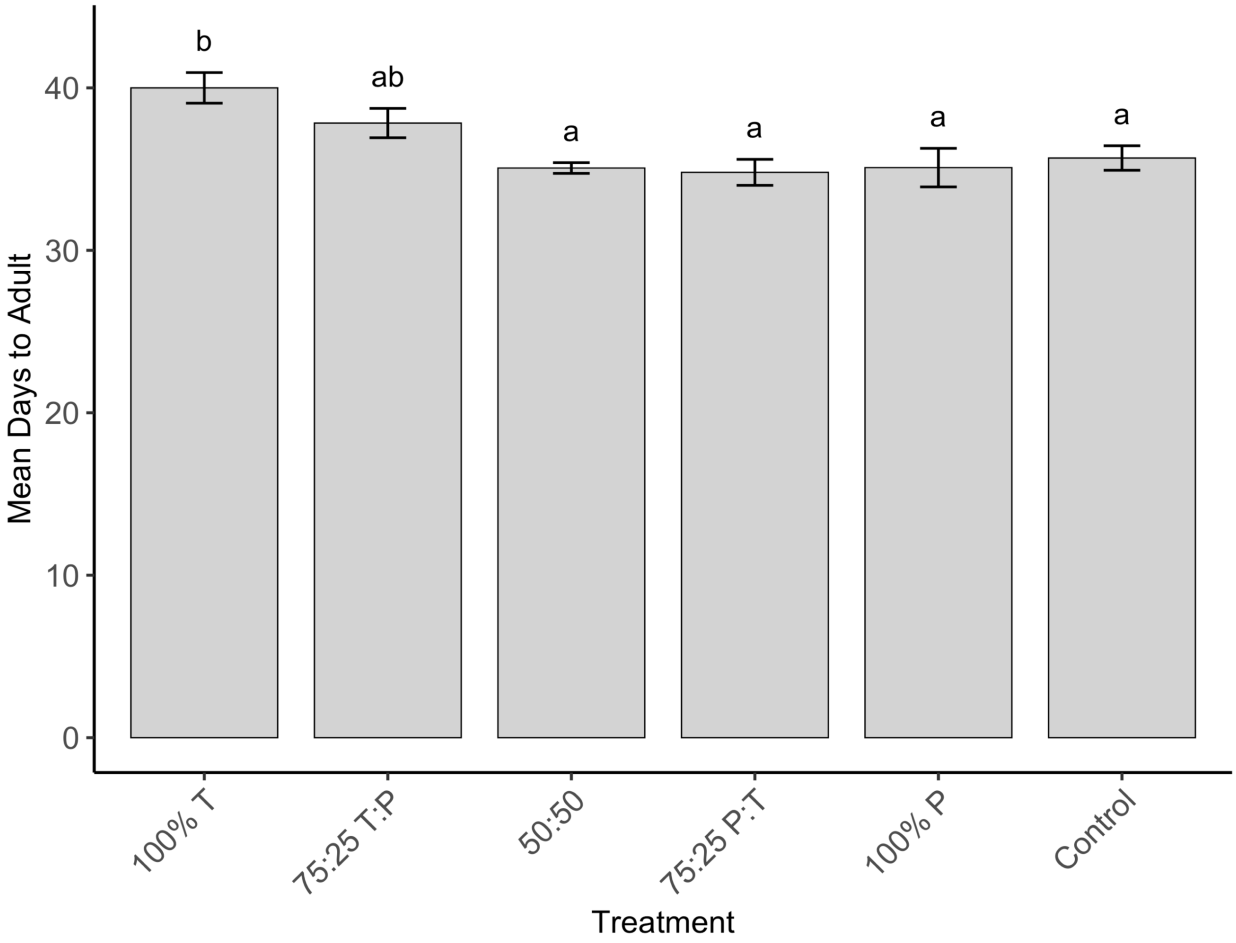

Figure 2. Mean days to adult for *Ceratina calcarata* reared on different pollen diet treatments. Treatments are abbreviated as follows: “100% T” (representing 100% *T. officinale*), “75:25 T:P” (75% *T. officinale*, 25% *P. nigra*), “50:50,” “75:25 P:T” (75% *P. nigra*, 25% *T. officinale*), “100% P”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

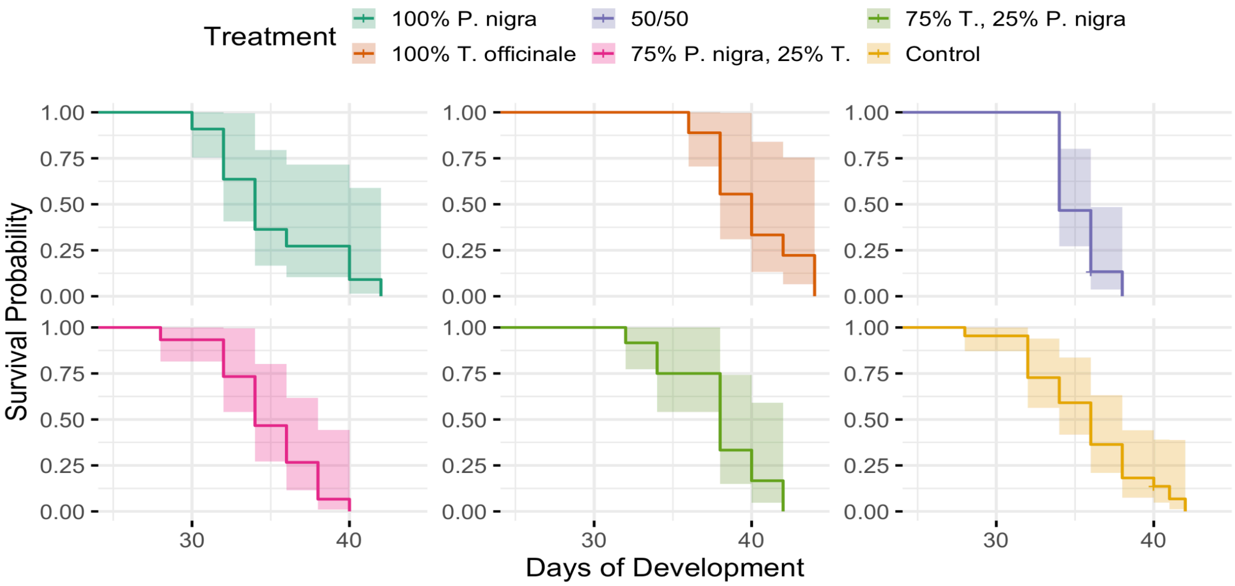

Figure 3. Kaplan–Meier survival curves showing the probability of reaching adulthood over days of development for *Ceratina calcarata* reared on six different pollen diet treatments. Each panel corresponds to one of the following treatments: “100% T” (representing 100% *T. officinale*), “75:25 T:P” (75% *T. officinale*, 25% *P. 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.

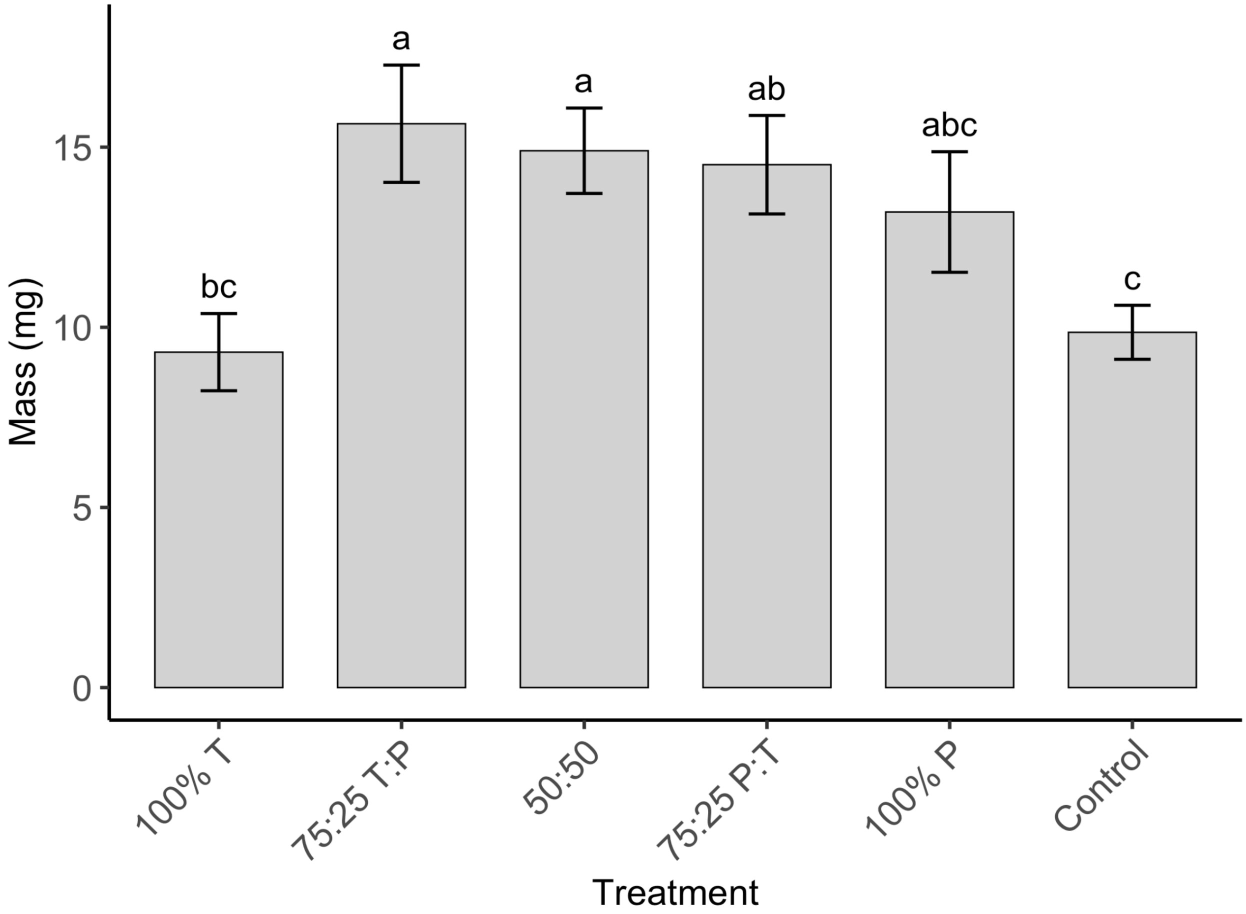

Figure 4. Mean body mass (mg) of *Ceratina calcarata* reared on different pollen diet treatments. Treatments are abbreviated as follows: “100% T” (100% *T. officinale*), “75:25 T:P” (75% *T. officinale*, 25% *P. nigra*), “50:50,” “75:25 P:T” (75% *P. nigra*, 25% *T. officinale*), “100% P” (100% *P. nigra*), 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.

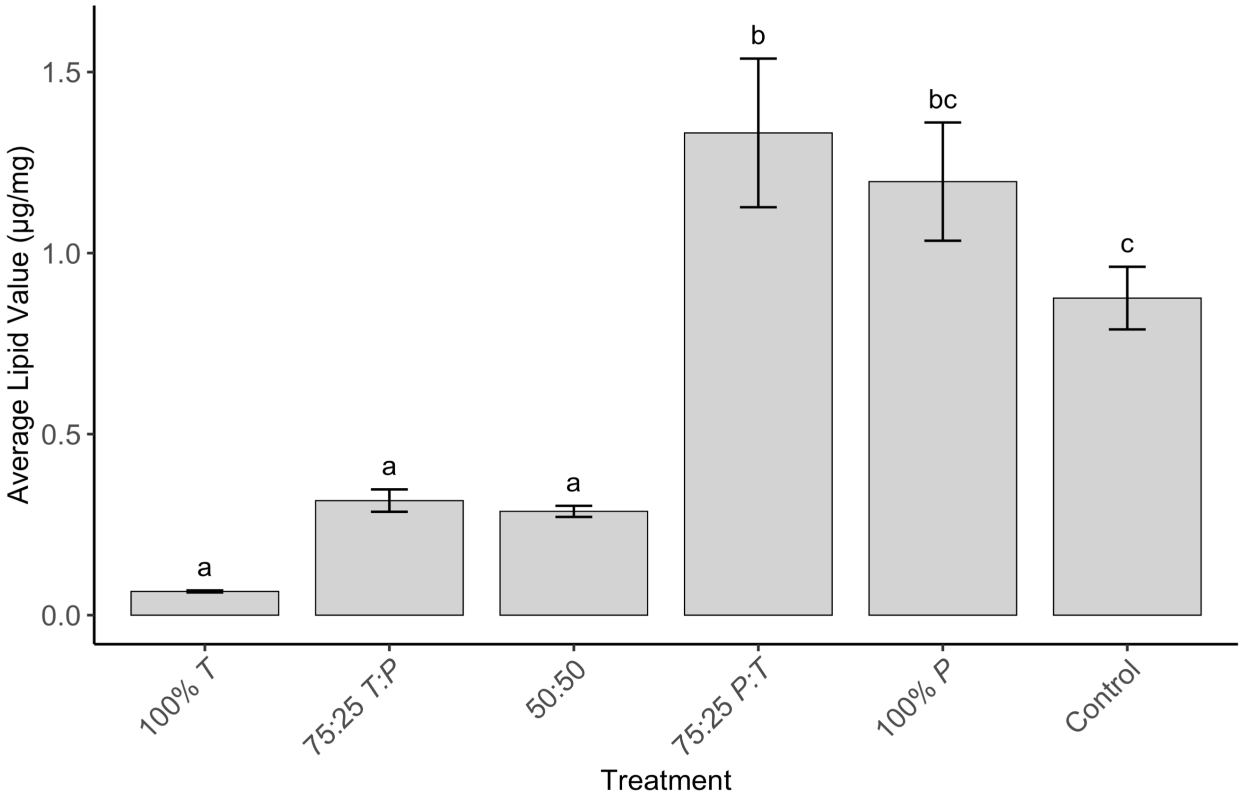

Figure 5. Mean lipid content (µg/mg) of *Ceratina calcarata* reared on different pollen diet treatments. Treatments are abbreviated as follows: “100% T” (100% *T. officinale*), “75:25 T:P” (75% *T. officinale*, 25% *P. nigra*), “50:50,” “75:25 P:T” (75% *P. nigra*, 25% *T. officinale*), “100% P” (100%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

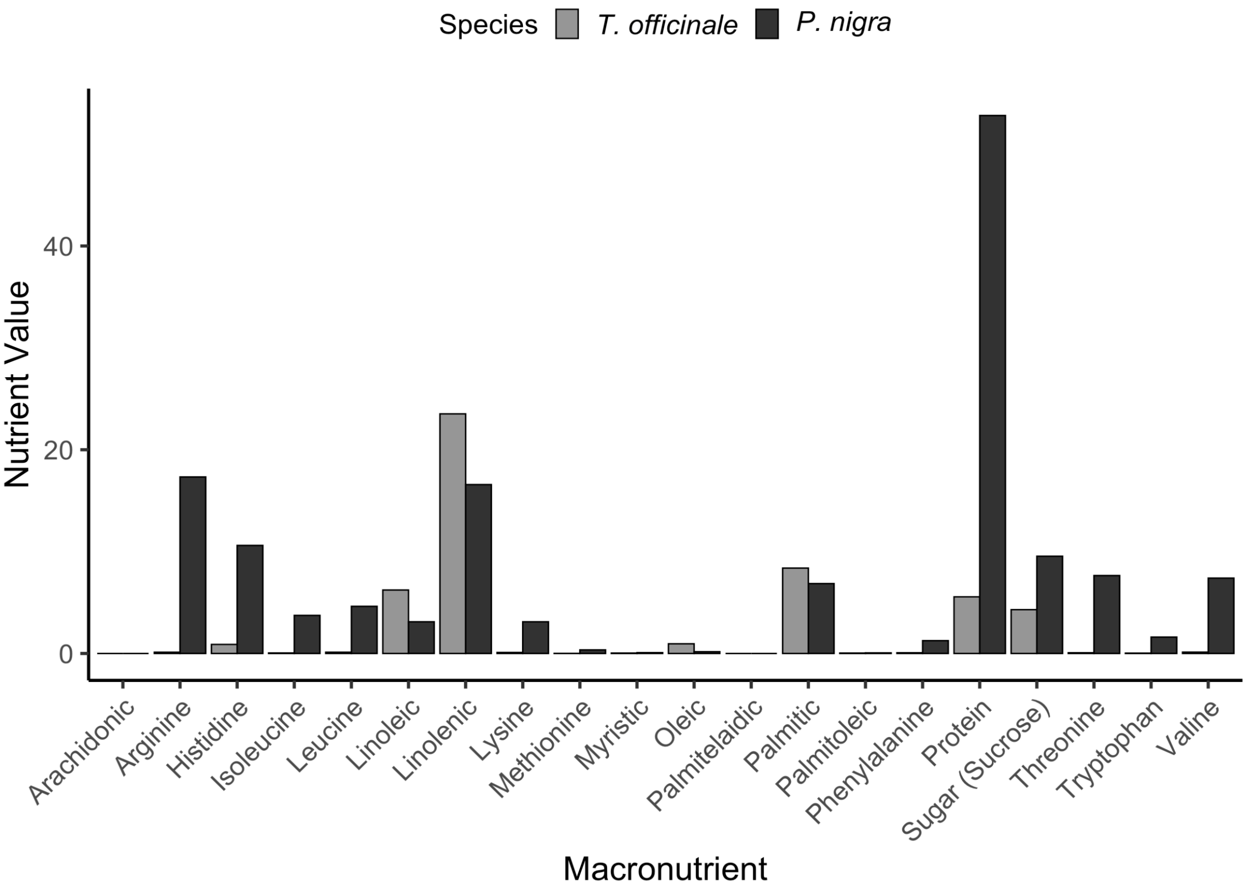

Figure 6. Macronutrient composition of *T. officinale* and *P. nigra* pollen. *P. nigra* pollen contained higher protein and sugar content.

Chapter IV: General Discussion

Bees rely on diverse floral resources to meet their nutritional requirements, yet habitat loss, agricultural intensification, and climate change are altering pollen availability and composition, potentially compromising pollinator health (Vaudo et al., 2015; Goulson et al., 2015; Zattara & Aizen, 2021). As pollen serves as the primary source of proteins, lipids, and essential fatty acids, understanding the macronutrient profiles of pollen species is central for evaluating their role in bee development, survival, and overall fitness (Vaudo et al., 2016; Filipiak et al., 2017). This thesis investigates the nutritional composition of pollen from diverse plant species and examines how macronutrient composition affects larval development in the small carpenter bee, *Ceratina calcarata*. By bridging nutritional ecology and pollinator health, this research provides insights into how diet composition influences growth and survival, emphasizing the importance of diverse, high-quality floral resources in conservation efforts (Di Pasquale et al., 2013).

In Chapter I, I characterized the macronutrient composition of 57 pollen species native to North America, analyzing their protein, amino acid (AA), non-esterified fatty acid (NEFA), and omega-6:3 fatty acid ratios. My findings revealed substantial variation in pollen nutritional content, with certain plant families, particularly Asteraceae, offering high levels of essential amino acids (EAAs), while others exhibited nutrient deficiencies (Somerville & Nicol, 2006; Weiner et al., 2010). Notably, all pollen species contained the essential amino acids required for bee health, except *Rhus glabra* pollen, which lacked methionine. My results suggest that no single pollen source provides a complete and balanced macronutrient profile, reinforcing the need for a diverse floral diet to ensure optimal nutrition for bees (Filipiak et al., 2017). Additionally, I found no significant nutritional differences between native and introduced plant species, suggesting that introduced plants can still serve as valuable pollen resources for pollinators (Harmon-Threatt & Kremen, 2015). These findings stress the importance of floral diversity in supporting bee health, as reliance on a single pollen source may not fulfill all macronutrient needs (Vaudo et al., 2016).

In Chapter II, I examined how differences in pollen macronutrient composition affect the development and survival of *Ceratina calcarata*. Using an experimental rearing approach, I reared larvae on black poplar (*Populus nigra*) and dandelion (*Taraxacum officinale*) pollen diets, two nutritionally contrasting pollen sources. Dandelion pollen, while abundant and carbohydrate-rich, lacked sufficient protein and essential fatty acids, leading to delayed development, reduced body size, and lower survival rates (Roulston & Cane, 2000; Nicolson, 2011). In contrast, black poplar pollen, despite being primarily wind-dispersed, provided significantly higher protein content, resulting in faster larval maturation, increased body mass, and improved survival (Requier et al., 2017; Di Pasquale et al., 2013). The mixed 50:50 diet group exhibited intermediate developmental outcomes, suggesting that dietary diversity helps mitigate nutritional stress and optimize larval growth. These findings emphasize the role of protein-rich pollen sources in bee development and highlight arboreal pollen as an overlooked yet nutritionally valuable resource (Filipiak & Filipiak, 2020). Given the increasing pressures of land-use change and floral resource depletion, ensuring access to a variety of high-quality pollen sources may be a key strategy in pollinator conservation (Goulson et al., 2015).

Having established the link between pollen macronutrient composition and bee development, this thesis highlights the nutritional trade-offs associated with different pollen sources and reinforces the importance of diverse floral landscapes for sustaining healthy pollinator populations (Vaudo et al., 2015; Woodard & Jha, 2017). Bees are highly sensitive to changes in pollen quality, and with the ongoing declines in floral diversity due to habitat loss and agricultural expansion, understanding which plant species provide optimal nutrition can inform land management and conservation planning (Winfree et al., 2011). My research suggests that promoting both herbaceous and arboreal pollen sources may enhance pollinator diet quality, development, and survival outcomes.

While this thesis advances our understanding of how pollen nutrition affects bee health, several key questions remain. Future research should examine how macronutrient intake influences immune function, reproductive success, and cognitive ability in bees (Alaux et al., 2010; Di Pasquale et al., 2013). Additionally, seasonal and regional differences in pollen macronutrient availability should be explored to determine how shifts in floral composition across time and space impact bee fitness (Requier et al., 2018). Incorporating other wild bee species with varying foraging strategies into nutritional studies will further clarify how pollen diet diversity affects broader pollinator communities (Woodard & Jha, 2017).

From a conservation perspective, my findings underline the importance of nutritionally diverse floral landscapes in mitigating the effects of habitat loss (Goulson et al., 2015; Zattara & Aizen, 2021). Current restoration efforts often focus on increasing floral abundance, but nutritional quality should also be prioritized to ensure that bees receive macronutrient-rich resources (Vaudo et al., 2016). As urbanization and climate change continue to reshape pollinator habitats, conservation strategies should incorporate tree pollen sources and nutritionally diverse floral plantings to support pollinator resilience (Winfree et al., 2011).

This thesis demonstrates that pollen macronutrient composition plays a fundamental role in bee development and survival. My results highlight arboreal pollen as an important but often overlooked nutritional resource and emphasize the need for dietary diversity in supporting pollinator health. Given the ongoing threats to floral diversity, ensuring access to high-quality, nutritionally rich pollen sources may be an important component of pollinator conservation efforts. By integrating nutritional ecology into habitat restoration and land management practices, we can develop more effective strategies for sustaining pollinator populations, preserving biodiversity, and promoting ecosystem stability.

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