Examining the impact of pollen diet composition on bee development and lifespan

by

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Abstract

Pollen is the sole protein source for most bees and the largest component of their larval diets. The high pollen requirements of bee larvae may select for a generalist foraging strategy that reduces dependency on individual pollen sources. Alternatively, specialization on low-quality pollens could be favoured by selection if it increases efficiency, reduces competition for floral resources, or lowers the risk of larval predation.

Some pollens, including those from the plant family Asteraceae, appear to be poor diets for generalist bees, possibly owing to nutrient deficiencies or the presence of secondary metabolites or digestive barriers that prevent nutrient assimilation. The high pollen and nectar yield of Asteraceae flowers combined with the long blooming period of these plants should otherwise make them an important pollen source for both specialist and generalist visitors. However, physiological adaptation—and an accompanying loss of ability to thrive on other diets—may be necessary to tolerate the unfavourable qualities of Asteraceae pollen. This could be the reason so many specialist bees are associated with this plant family while many generalist bees appear to avoid it.

In my thesis, I conducted feeding trials on larval and adult bees to examine how specialist and generalist foraging strategies affect performance on different pollen diets. First, I compared larval survival and development of both specialist and generalist *Osmia* (Megachilidae) bees on host and nonhost pollen diets. I then investigated whether the observed differences in survival and development on Aster provisions could be explained by nutritional deficiencies or low digestibility by analyzing the amino acid profiles of the different pollen diets and comparing the digestive efficiencies of bees reared on different pollens. I found that Aster specialists could develop on both host and nonhost provisions. However, Aster provisions were unsuitable larval

diets for bee species that do not collect Asteraceae pollen (i.e., *Osmia iridis* and *O. tristella*). These findings suggest that Aster specialists are physiologically adapted to the chemistry of their host pollen, but have not lost the ability to tolerate other pollens. The Aster provisions were not deficient in amino acids, and the Aster specialists could digest their host pollen as efficiently as the generalist species, *O. tristella*. Deficiencies in other essential nutrients or the presence of toxic secondary metabolites could be the reason *O. iridis* and *O. tristella* failed to develop on Aster provisions.

Mixing pollens might be another strategy for bees to exploit unfavourable pollens while still stabilising larval diet quality, if the different pollen sources help to complement nutrient deficiencies and alleviate the effects of toxic secondary metabolites. In my second study, I examined how the proportion of sunflower (*Helianthus annuus,* Asteraceae) pollen in the diet of captive-reared bumblebees (*Bombus impatiens*) affects the survival of queenless microcolonies. Bees fed sunflower pollen had significantly shorter lifespans than bees fed broad bean, rapeseed, or Cucurbitaceae pollen. However, mixed pollen diets containing 50% sunflower pollen did not reduce survival, which suggests that the other pollens were able to compensate for the low nutritive quality of the sunflower pollen. Due to agricultural intensification and a loss of wildflowers, farmland monocultures (e.g., sunflower crops) can be important floral resources for bumblebees. My study suggests that providing alternative floral resources of high nutritive quality could help mitigate the potential harmful effects of a monofloral sunflower diet on bumblebees.

In my thesis, I demonstrate that pollen specialization and pollen mixing can be alternative strategies to exploit low-quality pollen resources. Since the foraging decisions of mother bees ultimately determine the pollen composition and nutritional quality of offspring provisions,

feeding studies should be paired with host-selection experiments to assess the willingness of adult bees to exploit different plant species. Understanding both the host range and the nutritional requirements of different bee species is important for pollinator management since changes in the availability of host plants could lead to pollen shortages, potentially forcing bees to visit nonhost or less-preferred plants to feed their offspring.

Résumé

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Le pollen est la seule source de protéines pour la majorité des abeilles et constitue la plus grande partie de leurs alimentations larvaires. Les besoins importants de pollen des larves d'abeilles peuvent favoriser une stratégie d'alimentation généraliste qui réduit la dépendance à des sources uniques de pollen. Autrement, la spécialisation sur les pollens de faible qualité peut être favorisée si elle augmente l'efficacité, réduit la compétition pour les ressources florales ou diminue le risque de prédation larvaire.

Certains pollens, dont ceux de la famille Asteraceae, semblent être de mauvais régimes pour les abeilles généralistes, peut-être en raison de carences nutritives ou de présence de métabolites secondaires ou bien des barrières digestives qui empêchent l'assimilation de nutriments. Le rendement élevé en pollen et en nectar des fleurs d'Asteraceae, ainsi qu'une longue période de floraison, devrait en faire une source majeure de pollen pour les spécialistes et généralistes. Cependant, des adaptations physiologiques – et une perte de capacité à se développer sur d'autres régimes alimentaires – peuvent être nécessaires pour tolérer les aspects défavorables du pollen d'Asteraceae. Ceci pourrait être la raison pour laquelle tant d'abeilles spécialisées sont associées à cette famille de plantes.

Dans ma thèse, j'ai mené des essais d'alimentation sur des larves et des abeilles adultes pour examiner comment les stratégies d'alimentation des spécialistes et généralistes affectent leur développement sur différents régimes de pollen. Premièrement, j'ai comparé la survie larvaire et le développement des abeilles *Osmia* (Megachilidae) sur des régimes polliniques de plantes hôtes et non-hôtes afin de déterminer si la performance alimentaire des larves correspond aux réserves de pollen recueilli par leur mère. Par la suite, j'ai analysé les profils d'acides aminés des

différents régimes polliniques et comparé les efficacités digestives des abeilles élevées sur différents pollens. Ceci afin de déterminer si les différences observées sur les régimes de pollen d'Aster dans la survie et le développement pouvaient s'expliquer par des carences nutritionnelles ou une faible digestibilité. J'ai trouvé que les spécialistes d'Aster pouvaient se développer sur les pollens hôtes et non-hôtes. Cependant, les régimes de pollen d'Aster ne convenaient pas aux larves d'abeilles qui ne collectent pas ce type de pollen (c.-à-d. *Osmia iridis* et *O. tristella*). Ces résultats suggèrent que les spécialistes d'Aster sont adaptés à la chimie de leur pollen hôte, mais n'ont pas perdu la capacité à tolérer d'autres pollens. Les provisions d'Aster n'étaient pas déficientes en acides aminés, et les spécialistes d'Aster pouvaient digérer leur pollen d'hôte aussi efficacement que l'espèce généraliste *O. tristella*. Des carences en autres nutriments essentiels ou la présence de métabolites secondaires toxiques pourraient être la raison pour laquelle *O. iridis* et *O. tristella* n'ont pas réussi à se développer sur les provisions d'Aster.

Une autre stratégie pour exploiter les pollens défavorables tout en stabilisant la qualité du régime larvaire des abeilles pourrait être de les mélanger avec d'autres pollens qui préviennent les carences nutritives et atténuent les effets des métabolites secondaires toxiques. Dans une deuxième étude, j'ai examiné comment la proportion de pollen de tournesol (*Helianthus annuus*, Asteraceae) dans le régime alimentaire des bourdons (*Bombus impatiens*) affecte la survie des microcolonies sans reines. Les abeilles nourries au pollen de tournesol avaient une durée de vie significativement plus courte que les abeilles nourries au pollen de fève, de colza et de la famille Cucurbitaceae. Cependant, les régimes contenant 50% de pollen de tournesol ne réduisaient pas la survie, ce qui suggère que les autres pollens pouvaient compenser pour la faible qualité nutritive du pollen de tournesol. En raison de l'intensification de l'agriculture et la perte de fleurs indigènes, les monocultures de terres agricoles (telles les parcelles de tournesol) peuvent être en

elles-mêmes des ressources florales importantes pour les bourdons. Mon étude suggère que l’offre de ressources florales alternatives de haute qualité nutritive pourrait aider à atténuer les effets nocifs potentiels d'un régime monofloral de tournesol sur les bourdons.

Dans ma thèse, je démontre que la spécialisation et l’incorporation de pollens de sources variées peuvent être des stratégies pour exploiter des ressources polliniques de faible qualité. Étant donné que les choix d’approvisionnement des abeilles déterminent la composition et la qualité nutritionnelle des provisions pour leur progéniture, les études sur l'alimentation devraient être jumelées à des expériences de sélection des plantes hôtes (pour évaluer la volonté des abeilles adultes à visiter les différentes espèces végétales). Comprendre à la fois la gamme d'hôtes et les besoins nutritionnels des différentes espèces d'abeilles est important pour la gestion des pollinisateurs, car les changements dans la disponibilité des plantes hôtes pourraient entrainer des pénuries de pollen, forçant potentiellement les abeilles à visiter des plantes non-hôtes ou des plantes moins propices pour nourrir leur progéniture.

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Chapter 1

General Introduction

Host Selection

The study of ecological specialization and generalization is a common theme in biology and spans research on many different living systems (Futuyma and Moreno, 1988; Simon and Toft, 1991; Eby, 1998). Among herbivorous insects, many species are specialists and feed on only a limited number of host plants (reviewed by Bernays and Graham, 1988; Jaenike, 1990). This trend towards specialization has led to several hypotheses about how natural selection shapes plant–insect interactions. For example, the 'oviposition-preference–offspring-performance' hypothesis (henceforth preference–performance hypothesis; also known as the 'mother-knows-best' hypothesis) predicts that selection should favour mother insects that oviposit on plants that optimize larval fitness (Jaenike, 1978; Mayhew, 2001). However, the fact that many herbivorous insects prefer plants that do not maximize larval performance (reviewed by Mayhew, 1997; Ballabeni *et al.*, 2001 and references therein) has led to additional hypotheses to explain observed patterns of host selection (Thompson, 1988; Scheirs *et al.*, 2000). For example, parent–offspring conflicts can arise when the host plants that maximize larval performance differ from the plants that increase adult longevity and egg production (Mayhew, 2001). In addition, search-time constraints as well as temporal and spatial separation of optimal adult and larval host plants could select against mothers ovipositing on the host plants that would be best for their larvae (Scheirs *et al.*, 2000). Mother insects that spend most of their time feeding on plants that increase their own longevity might also lay eggs more frequently on these plants, even if this behaviour reduces larval performance (the 'optimal-bad-mother' hypothesis; Mayhew, 2001). In addition, search-time constraints can also favour oviposition on common but nutritionally inferior plants

when high-quality host plants are rare (the 'patch-dynamic' hypothesis, Thompson, 1988). Natural enemies (predators, parasitoids, and competitors; Ballabeni *et al.*, 2001) as well as mutualists (e.g., Atsatt, 1981) could also exert selective pressures on host selection (reviewed by Thompson, 1988). For example, selection for enemy-free space can favour mothers that preferentially oviposit on a narrow range of host plants (Bernays and Graham, 1988) that either contain secondary compounds the larvae can sequester to use as chemical defense (e.g., Denno *et al.*, 1990; Ballabeni *et al.*, 2001) or possess morphological structures that provide some protection from natural enemies (e.g., Feder, 1995).

Another influential hypothesis on host specialization in phytophagous insects was developed from the assumption that plant chemistry drives herbivore specialization. The 'physiological-efficiency' hypothesis postulates that specialists have adapted to utilize their host plant and perform better on their preferred host than generalist species (reviewed by Kelly and Bowers, 2016). In addition, a by-product of this physiological adaptation is a loss of the ability to feed on other plants, which further narrows the dietary breadth of the species (Cornell and Hawkins, 2003). Some studies have found mixed support for both the preference–performance hypothesis and the physiological-efficiency hypothesis (e.g., Friberg *et al*., 2015; Kelly and Bowers, 2016), while others have failed to find support for the preference–performance hypothesis since they did not observe a significant relationship between oviposition preference and larval performance (Futuyma, 2008; Friberg and Wiklund, 2009 but see Gripenberg *et al.*, 2010).

Although most herbivores are quite specialized on one or a few host species, generalist species still exist. This has led to additional hypotheses to explain the persistence of generalist foraging strategies. One possible explanation is that specialization is a derived condition and an evolutionary dead end that may permanently restrict host breadth and increase a species'

likelihood of extinction (Futuyma and Moreno, 1988, Kelley and Farrell, 1998). However, this hypothesis is not well supported, since a number of studies on different phytophagous taxa have not found specialization to be an evolutionary dead end (reviewed by Kergoat *et al*., 2017).

Another possible explanation, known as the 'apparency' hypothesis, posits that plants that are defended by digestibility-reducing chemicals (referred to as 'apparent' plants) should favour more generalist foraging strategies, since it should be more difficult for herbivores to adapt to these defenses than to 'unapparent' plants that are defended by toxic chemicals (reviewed by Cornell and Hawkins, 2003). A generalist feeding strategy could be particularly apparent in 'grazing' insects that feed on multiple plants to complete development (Thompson, 1988). Selection on grazing insects to perform well on mixed plant diets could explain why MacFarlane and Thorsteinson (1980) observed greater survival, faster development, and higher adult weight of *Melanoplus bivittatus* grasshoppers fed mixed diets than on the corresponding single plant diets. For example, survival was 90% on a mixed diet containing flixweed (*Descurainia sophia*) and stinkweed (*Thlaspi arvense*), but flixweed alone only supported 40% survival and stinkweed could not support development beyond the fourth instar (MacFarlane and Thorsteinson, 1980). Mixed diets could be beneficial if they provide a better nutrient balance (i.e., nutrient complementation hypothesis: Pulliam, 1975; Rapport, 1980) or dilute harmful secondary metabolites (i.e., toxic dilution hypothesis: Freeland and Janzen, 1974; reviewed by Unsicker *et al.*, 2008).

Host selection is clearly a complex process, and ecological, neurological, and behavioural factors could all influence evolution of a species' host breadth. These factors could help explain why the dietary breadths of larvae can be larger than the host range of the adult insects (e.g., swallowtail butterflies [*Papilio*], reviewed by Thompson, 1988). Host selection is also an

important research area for bees, which are herbivorous insects that use pollen and nectar as their predominant food source. We might expect similarities in the host-selection processes observed in phytophagous insects and bees, since both must adapt to the defenses of their host plants.

'Mutual Exploitation' Between Plants and Bees

Unlike nectar, which is a replenishing floral reward to attract pollinators, pollen is a finite resource as well as the male fitness component of a plant (Westerkamp, 1997). Pollen is also the sole protein source for the majority of bees and the largest dietary component for their brood (Roubik, 1982). Consequently, a conflict of interest exists between plants and bee pollinators since both partners need pollen for their own reproductive success (Westerkamp, 1997).

The relationship between plants and their pollinators can probably best be thought of as a "balanced mutual exploitation" (Westerkamp 1996), and plants may defend their pollen to narrow the spectrum of floral visitors to only the most effective agents of pollen transfer. For example, certain floral morphologies such as complex keel blossoms and bilabiate flowers, found in diverse bee-pollinated taxa, most likely evolved to minimize pollen loss to bees (Westerkamp and Claβen-Bockhoff, 2007) or to exclude weaker, generalist bees. It has also been proposed that plants may chemically defend their pollen to reduce the spectrum of floral visitors (reviewed by Sedivy *et al.*, 2011; Haider *et al.*, 2014). It is well known that pollen composition is highly variable across different plant families (reviewed by Roulston and Cane, 2000). Numerous feeding experiments have also demonstrated that pollen can contain unfavourable elements that are detrimental to bees (Levin and Haydak, 1957; Williams, 2003; Sedivy *et al*., 2011, Haider *et al.*, 2013). There is also interspecific variation (Haider *et al.*, 2014) in the ability of bees to develop on diets with different pollen compositions, suggesting that bees are physiologically adapted to the pollen chemistry of their host plant (Praz *et al.*, 2008a). In the remaining sections I

discuss pollen specialization in bees, then describe how the nutritional quality of pollen for bees can vary across flowering plant species; lastly, I provide an outline of my thesis.

Floral Constancy and Pollen Specialization

The majority of the approximately 20 000 bee species worldwide are solitary (Michener, 2000). These bees, which have relatively short lifespans, are mostly pollen specialists, perhaps because their life span falls within the flowering period of a few plant species that can supply all of their foraging needs (Minckley *et al*., 1994; Minckley and Roulston, 2006). Most bee species rely solely on floral rewards to feed themselves and provision their brood (Michener, 2000). Nectar is an important carbohydrate source for bees, but most other macronutrients (i.e., lipids and protein) as well as micronutrients (i.e., vitamins and minerals) must be obtained from pollen (Roulston and Cane, 2000). Potentially due to the nutritional importance of pollen, bee species are more selective for pollen than nectar (Danforth, 1991), and restrict their pollen collection to a subset of the available floral resources (Linsley and MacSwain, 1958; Ginsberg, 1983).

Two distinct forms of floral specialization have been described for bees, one at the level of the individual and the other at the level of the species. The term "floral constancy" usually refers to individual-level specialization and has been used to characterize the foraging behaviour of an individual bee that will gather pollen from one plant species on a foraging bout (Linsley and MacSwain, 1958). However, in this case, the species as a whole may be generalized, using multiple pollen hosts.

The second, species-level form of floral specialization was first clearly defined by Robertson (1925). He recognized that floral specialization occurs with respect to pollen (not nectar), and he introduced terms such as oligolectic (pollen specialist for flowers of a single genus, subfamily, or family) and polylectic (pollen generalist that will exploit pollen from multiple plant families) to describe the range of species-level floral specialization in bees. Many studies have confirmed the

presence of pollen-specialist bees, but the degree of specialization can vary from part of a plant genus to an entire plant family (Linsley, 1958; Linsley and MacSwain, 1958; Michener, 1979; Thorp, 1969). For example, Müller (1996) described host-plant specialization in different anthidiine bee species (Megachilidae: Anthidiini) at the plant tribe, subfamily, and family level. However, monolecty, in which bees restrict their pollen foraging to a single plant species, is rarely observed (e.g., González-Varo *et al.*, 2016) and in some cases may reflect the isolation of a plant species from its close relatives rather than a species-level preference by the bees (Linsley, 1978; Thorp, 1969).

Pollen Nutritional Quality

There is evidence that the pollens of different plant species differ significantly in their nutritional content, including the proportion of protein (Roulston *et al.*, 2000; Roulston and Cane, 2000). Previous studies have demonstrated that pollen can be a difficult resource for bees to exploit, and bees may have to adapt physiologically to tolerate unfavourable properties of pollen (see references cited above).

Pollen is a vital source of protein as well as lipids and other nutrients for bees (Dobson and Peng, 1997), and protein is considered to be the most important nutritional component of pollen for larval development (Roulston and Cane, 2000). It is also well established that pollen diets deficient in protein can negatively affect larval bee development (Levin and Haydak, 1957; Regali and Rasmont, 1995; Roulston and Cane, 2002) and shorten adult lifespan (in honeybees; Schmidt *et al.*, 1987). Pollen can also be of low quality for bees due to a lack of essential nutrients (e.g., amino acids, sterols), inhibition of digestion or nutrient assimilation, or the presence of secondary metabolites (Roulston and Cane, 2000; Roulston *et al.*, 2000).

Amino Acid Composition

De Groot (1953) identified 10 essential amino acids and determined the ideal composition to maximize honeybee development (arginine 11%, histidine 5%, isoleucine 14%, leucine 16%, lysine 11%, methionine 5%, phenylalanine 9%, threonine 11%, tryptophan 4%, and valine 14%). He also demonstrated that development was impossible for honeybees fed solely a protein source devoid of one of these essential amino acids (De Groot 1953). However, not all pollens collected by bees contain all the essential amino acids, and tryptophan and phenylalanine are sometimes absent (reviewed by Roulston and Cane, 2000), as is the case for dandelion pollen, *Taraxacum officinale* (Asteraceae: Cichorioideae; Auclair and Jamieson, 1948). Dandelion pollen is also deficient in the essential amino acid arginine (Herbert *et al.*, 1970; Loper and Cohen 1987), and the addition of all three amino acids (tryptophan, phenylalanine, and arginine) to dandelion pollen was needed for honeybees to complete development (Herbert, 1992).

Structure and Digestion

Pollen can also be a poor-quality diet for bees if nutrients are difficult to extract from within the pollen grains. The majority of the nutrients in pollen are contained within the inner protoplasm, which is protected by a durable protein wall capable of resisting both decay and digestion (Roulston and Cane, 2000). The protein wall consists of two sections: the inner intine layer composed of cellulose and pectin, and an outer exine wall made up of sporopollenin (Roulston and Cane, 2000). A coating of neutral lipids, hydrocarbons, and terpenoids on the surface of the pollen is known as the pollenkitt (Dobson, 1988). Bees consume pollen grains whole and digestion primarily occurs in the midgut where the cytoplasm is gradually extracted through perforations in the pollen wall known as germinal pores (honeybees (adults): Peng *et al.*, 1985; *Osmia* (larvae): Suárez-Cervera *et al.*, 1994; *Chelostoma florisomne* (larvae): Dobson and Peng, 1997). Digestive efficiency could vary among different types of pollen owing to structural

differences in pollen grain morphology (mentioned by Dobson and Peng, 1997). For example, the thickness of the intine covering germinal pores (Suárez-Cervera *et al.*, 1994), the complexity of the exine layers (Human *et al.*, 2007), and the amount of pollenkitt surrounding the pollen grains (Williams, 2003; Human *et al.*, 2007) could all affect digestion. Peng *et al.* (1985) observed incomplete digestion of *Taraxacum* pollen in the honeybee digestive tract and proposed that poor digestion could also contribute to the poor performance of honeybees fed dandelion pollen (Herbert, 1992).

Secondary Metabolites

Pollen can contain a wide variety of secondary compounds including some that are highly toxic to animals (Detzel and Wink, 1993; Kempf *et al.*, 2010; Gosselin *et al*., 2013). Although the major compounds found in pollen tend to also be found in other chemically-defended structures including the leaves, flowers, and roots, the ratio of these compounds in the pollen can be distinct from that in other plant tissues, making it unlikely that their presence in the pollen is simply a pleiotropic consequence of herbivore defense (Detzel and Wink, 1993; Gosselin *et al*., 2013). Chemically-protected pollen could function to reduce pollen losses by narrowing the spectrum of floral visitors (Hargreaves *et al.*, 2009). For example, natural concentrations of pyrrolizidine alkaloids in *Echium vulgare* pollen were found to be toxic to honeybee larvae (Reinhard, 2011 as cited by Haider *et al.*, 2014) and natural concentrations of amygdalin in almond pollen, *Amygdalus communis* L., had toxic effects on adult honeybees (Kevan and Ebert, 2005). However, the concentration of ranunculin in *Ranunculus acris* pollen did not significantly impact the development of two bee species, *Heriades truncorum* and *Chelostoma rapunculi* (Sedivy *et al.*, 2012). Nevertheless, both species failed to develop on diets of pure *R. acris* pollen (Praz *et al.*, 2008a), suggesting that another mechanism, such as an unknown secondary

metabolite or a lack of an essential nutrient, might be responsible for the detrimental effects on larval development (Sedivy *et al.*, 2012).

Since the nutritional quality of pollen for bees is highly variable, floral specialization by mothers may be a means of maintaining consistent food quality for larvae (Velthuis, 1992). In addition, physiological adaptations may be required to overcome pollen protective qualities, and pollen specialization may thus be favoured to protect larval development (Williams, 2003; Praz *et al*., 2008a). Conversely, pollen mixing could also stabilize food quality for larvae by reducing nutrient deficiencies or reducing the harmful effects of toxic secondary metabolites (Eckhardt *et al*., 2014).

Thesis Outline

In my thesis, I conducted feeding trials on larval and adult bees to examine how specialist and generalist foraging strategies affect performance on different pollen diets. My study species included solitary mason bees (*Osmia* spp., Megachilidae; Chapter 2) as well as the common eastern bumblebee (*Bombus impatiens*, Apidae; Chapter 3). I was particularly interested in Asteraceae pollen since at least some taxa in this plant family are deficient in essential amino acids (Auclair and Jamieson, 1948; Herbert *et al.*, 1970), and the pollen structure or chemistry may interfere with digestion (Peng *et al.*, 1985; Williams, 2003). Consequently, physiological adaptation may be necessary to tolerate the unfavourable qualities of Asteraceae pollen. This could be the reason so many specialist bees are associated with this plant family (*Helianthus* spp.: Hurd *et al.*, 1980, Asteroideae: Müller and Kuhlmann, 2008), while many generalist bees appear to avoid it (Müller, 1996).

In Chapter 2, my research objective was to test whether the diet breadths of larval bees match the pollens collected by their mothers by comparing larval survival of *Osmia* (Megachilidae) on host and nonhost pollen diets. Furthermore, I investigated whether observed differences in

performance on Asteraceae provisions could be explained by nutritional deficiencies or low digestibility by analyzing the amino acid profiles of the different pollen diets and quantifying the digestive efficiencies of bees reared on different pollens.

In Chapter 3, I examined how mixed and monofloral pollen diets affect adult bee lifespan. Specifically, I investigated how the proportion of sunflower pollen in the diet of bumblebees (*B. impatiens*) affects the survival of worker bees. Previous studies have demonstrated that sunflower pollen has deleterious effects on both honeybees (*Apis mellifera*: Schmidt *et al.*, 1995) as well as European bumblebees (*B. terrestris*: Tasei and Aupinel, 2008a), and I was interested in whether mixing sunflower pollen with other pollen species could improve the quality of the pollen diet through nutrient complementation or dilution of toxic substances.

In Chapter 4, I discuss how my findings fit into the existing literature on host selection in herbivorous insects, and identify some areas for future research.

Chapter 2

Do bees specialize on pollens because of pollen nutritional quality for bee larvae?

Introduction

Pollen is a vital source of protein, lipids, and other nutrients for bees (Dobson and Peng, 1997). Pollen-feeding in honeybees and stingless bees is accomplished by the adult bees, and the pollen consumption then stimulates the production of glandular secretions from their hypopharyngeal and mandibular glands to feed the larvae (Haydak, 1970). However, for over 90% of bee species (including all the solitary bees), pollen is consumed primarily by the larvae and represents an essential component of their larval diet (Dobson and Peng, 1997). The adults harvest pollen which they bind with nectar to create a provision mass for each larva (Roubik, 1982). Since the pollen preferences of the larva do not affect the provision mass they are provided, host selection is made by the adult bees and reflects either their innate preferences or learned floral associations (i.e., floral conditioning, Rojas and Wyatt, 1999).

Bees differ greatly in host breadth (reviewed by Wcislo and Cane, 1996; Cane and Sipes, 2006). Specialist bees, termed oligoleges, restrict their pollen foraging to within a plant genus, subfamily, or family, whereas generalists, also known as polyleges, will forage from multiple plant families for pollen provisions (Robertson, 1925; Cane and Sipes, 2006; Müller and Kuhlmann, 2008). Specialization may be favoured if it improves foraging efficiency (Laverty and Plowright, 1988) or stabilizes larval diet (Velthuis, 1992), since pollen chemistry is highly variable (Roulston *et al.*, 2000). For example, specialist bees may be more efficient than generalists at foraging on their preferred plant species (Strickler, 1979; Cane and Payne, 1988; but see Pesenko and Radchenko, 1993). In addition, specialists can be highly adapted to the

pollen composition of their host plant, and in some cases, can develop more rapidly when fed host pollen (*Evylaeus galpinsiae*: Bohart and Youssef, 1976; but see Williams, 2003).

However, specialization could also arise due to selective pressures to escape natural enemies which could favour physiological adaptation to tolerate pollens with "unfavourable" or protective properties (e.g., kleptoparasites: Spear *et al.*, 2016). However, this adaptation could come at the cost of a reduced capability to exploit other pollen sources. This trade-off in performance on different resources is known as the physiological-efficiency hypothesis, and was initially applied to specialized herbivorous insects (Cornell and Hawkins, 2003). It is still unknown whether this trade-off applies to most specialist bee species since different larval feeding studies comparing survival and development on host and nonhost pollens have reported mixed results. Some studies have found support for this trade-off when specialist bees have either failed to develop on nonhost pollens (Praz *et al.*, 2008a), or did not develop as well on nonhost pollens as broadly generalist species (Haider *et al.*, 2014). However, the performance of specialist bees can vary across nonhost pollens (Haider *et al.*, 2014) and between specialist species due to differences in their ability to tolerate nonhost pollen (Praz *et al.*, 2008a). In some cases, specialists can be even more tolerant of nonhost pollens than generalist species (e.g., Williams, 2003). Furthermore, the nutritional or digestive mechanisms to explain these differences in performance on nonhost pollens remain largely unknown (e.g., Sedivy *et al.*, 2012).

When Müller and Kuhlmann (2008) examined the scopal pollen loads of bees in the genus *Colletes,* they found that Asteroideae (Asteraceae) was an important pollen source for the specialist bees but was not equally represented in the pollen diet of the polylectic bees. For example, 14 of the 26 bees they classified as oligoleges were specialized on Asteroideae pollen

whereas only 7 of the 34 polylectic bee species collected marginal amounts of Asteroideae pollen, which overall represented only 2.7% of the pollen loads of the generalist bees (Müller and Kuhlmann, 2008). The high pollen and nectar yield within these compound flowers combined with the long blooming period of these plants should make them an important pollen source for both specialist and generalist visitors (Minckley and Roulston, 2006). However, Asteraceae pollen seems to be a difficult-to-utilize resource and is considered a poor diet for honeybees (*Apis mellifera*; Rayner and Langridge, 1985) as well as bumblebees (*Bombus terrestris*; mentioned by Regali and Rasmont, 1995; Tasei and Aupinel, 2008a). In addition, several broadly polylectic solitary bees, *Megachile rotundata* (Guirguis and Brindley, 1974), *O. bicornis* (Sedivy *et al.*, 2011), *O. cornuta* (Sedivy *et al.*, 2011), and *O. lignaria* (Levin and Haydak, 1957), also failed to develop on diets of pure Asteraceae pollen. In general, Asteraceae pollen is low in protein (Roulston *et al.*, 2000); pollen of at least some constituent taxa is deficient in essential amino acids (Auclair and Jamieson, 1948; Herbert *et al.*, 1970); and the structure or chemistry of Asteraceae pollen may interfere with digestion (Peng *et al.*, 1985; Williams, 2003). Consequently, physiological adaptation—and an accompanying loss of ability to thrive on other diets—may be necessary to tolerate the unfavourable qualities of Asteraceae pollen. This could be the reason that Asteraceae taxa are associated with so many specialist bees (*Helianthus* spp.: Hurd *et al.*, 1980, Asteroideae: Müller and Kuhlmann, 2008), yet collected in only marginal amounts by many generalists (Müller, 1996).

Here, I tested whether the dietary breadths of larval bees match the pollen diets provided by their mothers by comparing larval survival and development on host and nonhost pollen diets. I then investigated whether the observed differences in survival and development on Asteraceae pollen provisions could be explained by nutritional deficiencies or low digestibility. To do this, I

analyzed the amino acid profiles of the different pollen diets and quantified the digestive efficiencies of bees reared on different pollens.

Methods

Study Species

I studied the solitary mason bee species *Osmia coloradensis, O. iridis*, *O. montana*, *O. subaustralis, O. tersula,* and *O. tristella* (Megachilidae). Female mason bees collect pollen and nectar to provision their brood (Michener, 2000). These species normally build their nests in tunnels they find in dead trees, but they will also nest in artificial "trapnests" that consist of holes drilled into blocks of wood (Cane *et al*., 2007). Along the length of the tunnel, eggs are laid on individual pollen provisions that are separated by walls the mother bees build from mud and/or leaf material (Cane *et al*., 2007). These species engage in mass provisioning rather than interval feeding, since the mother bee provisions enough food to suffice for all larval growth of each egg, and she seals each cell soon after laying the egg (Michener, 2000). She constructs her brood cells sequentially such that she completes the wall of the previous brood cell before she begins gathering pollen and nectar for the following offspring (Michener, 2000).

Four of the study species (*O. coloradensis, O. iridis, O. montana,* and *O. subaustralis*), are oligolectic, whereas *O. tristella* and *O. tersula* are polylectic. *Osmia iridis* is a specialist on Fabaceae pollen from the plant tribe Fabeae (specifically, *Lathyrus lanszwertii* and potentially *Vicia americana* at the study site); the other specialists restrict their foraging to plants from tribes in the Asteraceae family including Lactuceae, Helenieae, Heliantheae, and Senecioneae. In our study area, *Osmia tristella* collects pollen from multiple plant families including Boraginaceae, Fabaceae, Lamiaceae, and Plantaginaceae. *Osmia tersula* collects pollen from the plant families Boraginaceae, Fabaceae, Lamiaceae, Plantaginaceae, Rosaceae, and Violaceae.

Egg-Transfer Experiment

The egg-transfer experiment was conducted in a 5 × 3 factorial design with five different bee species (*O. coloradensis*, *O. iridis*, *O. montana*, *O. subaustralis*, and *O. tristella*) and three different pollen provision types, since the provisions of the three Asteraceae specialists were considered interchangeable (Table 2.1.). The experiment was performed over two consecutive years from late June to August (2016: 26 June to 27 August; 2017: 21 June to 15 August). *Osmia tersula* was not included in the experiment in either year due to the low numbers of collected nests for this species. The three provision types included 'Aster' (pollen provisions of the Asteraceae specialists *O. coloradensis*, *O. montana*, and *O. subaustralis*), 'Fabeae' (pollen provisions of the Fabeae specialist *O. iridis*) and 'Various' (the majority were provisions of the generalist *O. tristella*, and two were likely *O. tersula* but the mother bee could not be identified). Bee eggs were assigned sequentially (as much as possible given the availability of different types of provisions at the time of transfer) to different treatments (control or novel provision type [Aster, Fabeae or Various]), with bees from neighbouring cells receiving opposite treatments. A systematic assignment process was used to prevent a sex bias in treatment assignment, since female eggs are more often laid in the inner cells of a nest and male eggs tend to be found in the outer cells.

Bee nests were collected from established trapnests near the Rocky Mountain Biological Laboratory in Colorado, USA, in 2016 (38 nests, 5 sites) and 2017 (26 nests, 4 sites). To obtain bee nests from the field, the holes of the trapnests were lined with paper straws that could be easily extracted with hemostat pliers. Individual eggs (or larvae) were transferred within 8 days of when their nest was brought back to the lab, and nests prior to transfer were stored in a growth chamber on a 10°C to 25°C ramping diurnal cycle (dark). Eggs were used when possible, but

sometimes early-stage larvae within 15 days of their estimated date laid, were included. In total, 34 of the 107 bees included in the experiment were transferred as larvae.

The experimental protocol for transferring eggs or young larvae (henceforth "eggs") was based on the methods employed in previous egg-transfer experiments (Williams, 2003; Praz *et al.*, 2008a; Haider *et al*., 2014; Appendix A). A scalpel and microscissors were used to cut the straw and separate individual nest cells. Once the cells were separated, microscissors were used to remove the top half of the straw and expose the egg and pollen provision. A metal spatula and a pair of forceps were then used to pick up and transfer the egg to a new pollen provision, from the same or a different nest. Each egg was positioned on the novel provision in a similar orientation to its position on its original provision. Trace amounts of host pollen (never more than the volume of the egg itself) were frequently (unavoidably) transferred along with the egg. Each pollen provision, together with the lower half of the straw containing it, was stored in an individual well of a wood block. Each well consisted of an approximately 2 cm long and 1 cm wide cavity drilled into the side of a wood block (30.8 cm by 3.7 cm) with 15 wells per block. Glass coverslips were taped across the top of the unit, and the front edge of the unit was also covered with either a coverslip, a piece of paper, or a strip of wood to protect the eggs and larvae and minimize disturbance to the bees during observations.

The bees were kept in the growth chamber and assessed every other day to monitor bee status (alive/dead) and stage of development. Developmental stages included "pre-defecating larva" (a stage that includes second-, third- and fourth-instar larvae, since the first fecal particles are only deposited once larvae reach the fifth instar; Torchio, 1989), "defecating larva" (recognized by the presence of at least one fecal pellet), "spinning larva" (recognized by the presence of silk

threads), and cocoon (defined here as the stage at which the spinning larva could no longer be seen through the silk). The developmental stages are illustrated in Appendix B.

Digestive-Efficiency Experiment

Frass samples were taken in August 2016 from bees that had reached the defecating larval stage, and were stored in a freezer until microscope slides could be prepared. Samples could not be taken from three *O. montana* bees because the frass pellets were embedded within residual pollen. Samples were subsequently thawed and soaked overnight in Alexander stain (Alexander, 1969, 1980), which stained the pollen wall (exine and any remnant intine) green and the inner protoplasm of the pollen grains red. Since bees digest the protoplasm and excrete mainly empty, exine shells (Peng *et al.*, 1985; Suárez-Cervera et al., 1994; Dobson and Peng, 1997), the digestion of the pollen grains can be compared based on the extent to which the grains retained their internal contents (Roulston and Cane, 2000). For each bee, approximately 300 pollen grains were examined at 400x magnification and scored as "empty" (<25% protoplasm), "partially empty" (25–50% protoplasm), "partially full" (50–80% protoplasm) and "full" (>80% protoplasm; Appendix C).

Bee Provision Amino Acid Analysis

The amino acid profiles of pollen provisions from *O. coloradensis* (n = 1 sample), *O. iridis* (3), *O. montana* (3), *O. subaustralis* (3), *O. tersula* (1), and *O. tristella* (1) were analyzed at the SPARC BioCentre of the Sick Kids Hospital (Toronto, Ontario, CA) (Table 2.2., pollen provision description). Each sample underwent three analyses: a standard amino acid analysis (AA analysis) and a free amino acid analysis (FAA analysis), both of which excluded cysteine and tryptophan, and lastly a tryptophan analysis, which included both bound and free tryptophan. Cysteine was not quantified since this is not an essential amino acid for bees (De Groot, 1953). The Waters Pico-Tag System was used to analyze the amino acids, and the detection limit for all

three analyses was 25 pmol (Heinrikson and Meredith, 1984; White *et al.*, 1986). Prior to analysis, samples from *O. coloradensis*, *O. montana*, and *O. subaustralis*, which were solid and dry, were pulverized with a mortar and pestle; samples from *O. iridis*, *O. tersula*, and *O. tristella*, which were more viscous, were thoroughly mixed.

Statistical Analysis

All statistical analyses were performed in R (version 3.3.3; R Core Team, 2017).

Egg-Transfer Experiment

The age of bees at transfer, which was calculated as the difference between the estimated lay date and transfer date, was compared across provision types and bee species using an analysis of variance (type 3) with the 'Anova' function from the 'car' package (Fox and Weisberg, 2011). Pairwise comparisons were then conducted using Tukey's HSD test ('TukeyHSD' function).

Cox proportional hazard (henceforth Cox PH) models were used to compare bee survival and development on the different pollen provision types (i.e., Aster, Fabeae, and Various) using the 'coxph' function from the 'survival' package (Therneau, 2015). Hazard ratios were calculated for each bee species, and represent the proportional risk of either dying (survival) or reaching a developmental stage (defecating, spinning, or cocoon stage) for bees reared on different provision types. Cluster terms were included in the model for nest ID. These terms are used to compute a robust variance for the model by accounting for non-independence and correlation among observations. Year (2016 or 2017) was included as an additional fixed factor for *O. iridis* and *O. montana* since these species were sampled in both experimental years. The assumption of proportional hazards was met for all species except *O. coloradensis* (Schoenfeld residuals; *O. coloradensis*: $\chi^2 = 12.23$, $P = 0.0022$, $n = 21$; *O. iridis*: $\chi^2 = 0.645$, $P = 0.886$, $n = 43$; *O. montana*: $\chi^2 < 0.001$, $P = 1$, $n = 11$; *O. subaustralis*: $\chi^2 = 0.094$, $P = 0.954$, $n = 21$; *O. tristella*: χ^2

< 0.001, $P = 1$, $n = 11$). Despite the violation of the model assumption, outliers were not removed because of the fairly low sample size for this bee species.

Digestive Efficiency

I conducted binomial generalized linear models with pollen type as an explanatory variable, using the 'glm' function from the 'stats' package, to compare the proportion of pollen grains in frass that were less than 25% full (i.e., containing <25% protoplasm) or less than 50% full (i.e., containing <50% protoplasm) between the four bee species included in the 2016 experiment (i.e., *O. iridis*, *O. montana*, *O. subaustralis*, and *O. tristella*). Two additional binomial generalized linear models were run on the bees fed Fabeae pollen provisions to compare the proportions of pollen grains < 25% and < 50% full between these four species. There was some deviation in the distribution of the residuals for all four models, but the analyses could not be run without outliers due to low sample size, so I report the results of models including all data, with the caveat that these should be interpreted with caution.

Amino Acid Analysis

The amino acid profiles (bound plus free amino acids) for the pollen provisions of the six bee species (*O. coloradensis*, *O. iridis*, *O. montana*, *O. subaustralis*, *O. tersula*, and *O. tristella*) were compared using non-metric multidimensional scaling (NMDS) with the 'metaMDS' function in the 'vegan' package (Oksanen *et al.*, 2017). Two NMDS ordinations were performed, both using Manhattan dissimilarity matrices, two

dimensions, and 20 runs. These parameters were sufficient to reach a stress level less than 0.07 and model convergence. The first NMDS was based on absolute amounts of each amino acid (i.e., µg of each amino acid per mg of dry sample); the second used proportions (i.e., each amino acid expressed as a percent [by mass] of total amino acid content). Permutational multivariate analyses of variance (perMANOVA; 1000 permutations) using the 'adonis' function

were then performed to compare the Aster specialists (i.e., *O. coloradensis*, *O. montana*, and *O. subaustralis*) to the 'other bee species' (i.e., *O. iridis*, *O. tersula*, and *O. tristella*). Each pollen provision type (i.e., Aster, Fabeae, and Various) could not be compared to all others due to the small samples sizes. Prior to these analyses, I verified homogeneity of group covariances using permutational multivariate homogeneity of group dispersions tests (PERMISP; 1000 permutations) with the 'betadisper' function. To determine if certain amino acids were particularly representative of either group (i.e., Asteraceae specialist or other bee species), indicator values for each amino acid (based on proportions of total amino acid content) were calculated using the 'indval' function of the labdsv package (Roberts, 2016). Lastly, the proportions of essential amino acids (EAA) in the pollen provisions of each group were compared to the EAA requirements for honeybees (*Apis mellifera*) outlined by De Groot (1953) (arginine (11%), histidine (5%), isoleucine (14%), leucine (16%), lysine (11%), methionine (5%), phenylalanine (9%), threonine (11%), tryptophan (4%), and valine (14%)) by running an additional NMDS on a Manhattan dissimilarity matrix (2 dimensions and 20 runs) with these requirements included as an additional "sample". A perMANOVA and pairwise comparisons ('pairwise.perm.manova' function, 'RVAideMemoire' package; Hervé, 2017; Bonferroni-corrected) were then performed to compare each group with the EAA requirements for honeybees.

Results

Egg-Transfer Experiment

The age of the bees at transfer did not differ between the three provision treatments but did differ among bee species (ANOVA, provision treatment: $F_{2,100} = 1.06$, $P = 0.35$; bee species: $F_{4,100} = 4.38$, $P = 0.0027$). However, only *Osmia iridis* (8.05 ± 0.43 days) and *O. tristella* (11.18 ± 1.13 days) differed significantly in age at transfer (Tukey's HSD, $P = 0.022$), and mean ages at

transfer differed by no more than 3.1 days among species (*O. coloradensis*: 8.33 ± 0.44 days, *O. montana*: 10.36 ± 1.11, *O. subaustralis*: 10.14 ± 0.75).

O. coloradensis

In total, 21 bees (of 35) consumed pollen after they were transferred. None of the bees reared on Aster or generalist provisions survived (10 and 1 bees, respectively), whereas 6 out of the 10 bees provided Fabeae provisions survived until the end of the experiment (Figure 2.1.; Table 2.3.). The six bees that survived on Fabeae provisions were also the only bees that reached defecating and spinning developmental stages, and no bees completed cocoons before the end of the experiment (Figures 2.2.–2.4.).

O. iridis

Forty-three bees fed after they were transferred (of 75 bees transferred in total) and were included in the analysis. The 24 bees transferred to Aster provisions all died, but none of the bees provided Fabeae or generalist provisions died during the experiment (18 and 1 bees, respectively, Figure 2.1.). In addition, only the bees provided Fabeae and generalist provisions reached the defecating and spinning stages (Figures 2.2., 2.3.). The 16 bees that finished their cocoons before the end of the experiment were all provided Fabeae pollen provisions (Figure 2.4.). Survival and development did not differ significantly between years (Cox PH (reference year: 2016), survival: hazard ratio (HR) = 0.571, $P = 0.22$; defecating: HR = 0.411, $P = 0.16$; spinning: HR = 0.691, $P = 0.55$, cocoon: HR = 0.543, $P = 0.27$).

O. montana

Eleven of the 30 bees that were transferred fed on the novel provision and were included in the analysis. All seven bees provided Aster provisions survived until the end of the experiment (Figure 2.1.). One of the three bees given Fabeae provisions died and the only bee that fed on a generalist provision died. Only five bees provided Aster provisions and two bees given Fabeae provisions reached the defecating larval stage (Figure 2.2.). None of the bees reached the

spinning or cocoon developmental stages (Figures 2.3., 2.4.). Bee survival was greater in 2016 than in 2017, but there was no difference between years in development to the defecating stage (Cox PH (reference year: 2016), survival: HR < 0.01, $P < 0.01$; defecating: HR = 1.93, $P = 0.44$). Despite the difference in survival between years, the data could not be subdivided by year due to the low sample size for this species.

O. subaustralis

Twenty-one bees out the 36 that were transferred fed on the novel provision and were included in the analysis. Survival was comparable on Aster and Fabeae provisions, with 7 bees (of 12) and 4 bees (of 7) respectively surviving until the end of the experiment (Figure 2.1.). However, survival was significantly higher on generalist provisions compared to the other treatments, and the two bees that fed on generalist provisions both survived until the end of the experiment. Both bees provided generalist provisions also reached the defecating larval stage, as did eight and three bees given Aster and Fabeae provisions, respectively (Figure 2.2.). In addition, both bees provided generalist provisions and one bee that fed on Fabeae pollen reached the spinning stage, but none of the bees that fed on Aster provisions reached this stage (Figure 2.3.). The only bee that finished its cocoon before the end of the experiment was provided Fabeae pollen (Figure 2.4.).

O. tristella

During the experiment, 14 bees were transferred and 11 bees fed on their novel pollen provision. All of the bees provided Fabeae and generalist provisions survived until the end of the experiment (three and four bees respectively, Figure 2.1.). All four bees provided Aster provisions died before reaching the defecating stage (Figure 2.2.). The bees fed Fabeae provisions all reached the defecating and spinning stages but only one bee completed its cocoon before the end of the experiment (Figures 2.3., 2.4.). All bees provided generalist provisions

reached the defecating stage and three bees (of four) reached the spinning stage and completed their cocoons before the end of the experiment.

Digestive Efficiency

Bees fed host pollen provisions

Pollen grains in *O. iridis* frass were significantly emptier (i.e., were more often <25% or <50% full) than those in frass from the Aster specialists or the generalist *O. tristella* (Figure 2.5., Table 2.4.). Frass from *O. montana* and *O. subaustralis* also contained significantly more "empty" pollen grains (<25% full) than frass from *O. tristella.* However, *O. tristella* samples contained significantly more partially empty pollen grains (<50% full) than those from the Aster specialists.

Bees fed Fabeae (Fabaceae) pollen provisions

In a comparison of bee species reared on Fabeae (Fabaceae) pollen provisions, the frass samples of *O. iridis* (the only species specialized on Fabeae pollen) contained significantly more empty and partially empty pollen grains than those of other bee species (Figure 2.6., Table 2.4.). *Osmia tristella* samples contained significantly more empty and partially empty pollen grains than those of the Aster specialists. There was only a marginal difference in the proportion of empty pollen grains between the two Aster specialists, and no difference was detected in the proportion of partially empty pollen grains.

Pollen Provision Amino Acids

The pollen provision samples from every bee species contained all the essential and nonessential amino acids that were assessed. Free amino acids accounted for 6.04 to 12.19% of the total amino acid content per sample (Appendix D). There were no differences between the Aster specialist provisions and the 'other bee species' in terms of absolute amounts of amino acids (i.e., µg/mg of dry sample; perMANOVA, $R^2 = 0.10$, $F_{1,10} = 1.14$, $P = 0.33$; Figures 2.7., 2.8.) or the proportions of the amino acids (perMANOVA, $R^2 = 0.13$, $F_{1,10} = 1.55$, $P = 0.19$;

Figure 2.9.). In addition, none of the amino acids was indicative of either group (Table 2.5.). There were marginal differences in the relative proportions of essential amino acids (perMANOVA, $R^2 = 0.43$, $F_{2,10} = 3.79$, $P = 0.047$; Figure 2.10.), but only between the Aster specialist and other bee species provisions (pairwise comparisons, $P = 0.042$); neither provision type significantly differed from the essential amino acid requirements for honeybees (pairwise comparisons: honeybees and Aster specialist provisions ($P = 0.69$); honeybees and other bee species provisions ($P = 1.00$). These marginal differences between the Aster specialists and the other bee species were probably driven by histidine and tryptophan, as the two groups did not differ from one another or from the requirements for honeybees when these amino acids were excluded from the analysis and the proportions of the remaining essential amino acids were adjusted accordingly (perMANOVA, $R^2 = 0.28$, $F_{2,10} = 1.97$, $P = 0.10$).

Discussion

Aster provisions were inadequate larval diets for bee species that do not normally use this pollen type. However, the Aster specialists (i.e., *Osmia coloradensis*, *O. montana*, and *O. subaustralis*) could survive and develop on nonhost pollens. I did not find that the Aster provisions were amino acid-deficient, and the Aster specialists did not have more difficulty digesting their host provisions than did the generalist, *O. tristella*. However, it is still possible that *O. iridis* and *O. tristella* could not survive on Aster provisions due to difficulties with digestion and nutrient assimilation. Unfortunately, their digestive efficiency on Aster provisions could not be examined since both species died before reaching the defecating larval stage.

Larval Performance on Host and Nonhost Provisions

All three Aster specialists were able to survive and develop on nonhost pollen provisions. These results suggest that the pollen dietary breadth of the larvae could be larger than the host range of the adults, which is consistent with previous studies. For example, larval survival of the

Asteroideae specialist, *Heriades truncorum,* was comparable between diets of host pollen and three nonhost pollens including *Sinapis* (Brassicaceae), *Campanula* (Campanulaceae), and *Echium* (Boraginaceae)—although bee development was significantly longer on the *Sinapis* pollen diet than the other three pollen diets (Praz *et al.*, 2008a). However, mother bees refused to collect either *Echium* or *Campanula* pollen even in the absence of host plants, suggesting that host selection could be neurologically constrained in this species (Praz *et al.*, 2008b). Similarly, the Heliantheae (Asteraceae) specialist, *O. californica*, grew larger when fed a diet containing 20% and 50% nonhost pollen (*Phacelia tanacetifolia,* Boraginaceae) and developed equally quickly on pollen diets with up to 80% *P. tanacetifolia* (Williams, 2003)—yet adult *O. californica* ceased nesting rather than collect nonhost pollen when host plants were unavailable (Williams, 2003). Together, these results suggest that the larval diet breadth of Asteraceae specialists often exceeds the host range of the adult bees.

In my study, I found that the Aster specialists seemed to develop more slowly on their host pollen compared to the non-Aster specialist bees that were also reared on host provisions. For example, by the end of the experiment, both *O. subaustralis* and *O. montana* bees that were provided host pollen had only reached the defecating stage. Conversely, the majority of *O. iridis* and *O. tristella* bees fed host pollen had completed their cocoons. The incomplete development of *O. montana* and *O. subaustralis* could be a function of relatively late egg-laying dates, since the median estimated lay dates for both species (*O. montana*: 14 July 2016; 7 July 2017; *O. subaustralis*: 11 July 2016) were more than 1.5 weeks later than those of the Fabeae specialist *O. iridis* (29 June 2016; 18 June 2017) and the other Aster specialist *O. coloradensis* (18 June 2017). However, the generalist species *O. tristella* had a median estimated lay date (15 July

2016) that was comparable to both *O. subaustralis* and *O. montana*, suggesting that the lay date is not the only factor influencing the slower development of these two species.

For an unknown reason, *O. coloradensis* did not develop on host Aster provisions, and the average lifespan of bees fed Aster provisions was less than 16 days. It is unclear why *O. coloradensis* bees failed to develop on their host pollen during the experiment since they can evidently develop successfully in the field. Interestingly, unmanipulated nest data collected in 2017, suggest that *O. coloradensis* bees may also develop more slowly than *O. iridis*. For example, *O. coloradensis* bees (33 of 35 bees [94%]) from five nests initiated within a 2-week period in June 2017, had only reached the defecating stage by the end of the experiment. However, 47 of 49 *O. iridis* bees (95%) from 19 unmanipulated nests, which were also initiated during the same 2-week window, had reached at least the defecating larval stage, and eight (16%) and 11 (22%) of those bees, respectively, were at the spinning and cocoon stages. Slower development of an Asteraceae specialist was also reported by Praz *et al.* (2008a), who noted longer development times for *Her. truncorum*, an Asteroideae specialist, than for three other specialist bee species (*Chelostoma florisomne*, *C. rapunculi*, and *Hoplitis adunca*), despite it being the smallest of the species examined.

Interestingly, some of the Aster specialists I studied seemed to develop more quickly on nonhost pollen than on their host provisions. For example, *O. subaustralis* bees reached the spinning stage on both Fabeae and generalist provisions, and one bee fed Fabeae pollen completed its cocoon before the end of the experiment. In addition, *O. coloradensis* bees that were transferred to Fabeae pollen also reached the spinning larval stage prior to the end of the experiment. However, *O. montana* bees fed Fabeae provisions did not develop more quickly and only reached the defecating stage. These findings differ somewhat with past feeding studies on

Aster specialists that have reported either comparable (Williams, 2003; Praz *et al.*, 2008a) or slower (Praz *et al.*, 2008a) larval development times on diets containing nonhost pollen.

Bees that were not Aster specialists (*O. iridis* and *O. tristella*) failed to develop on Aster provisions, but both species can potentially develop on the other two provision types (i.e., Fabeae and Various). These findings are consistent with previous studies that have found Asteroideae pollen to be a difficult resource to utilize for bee species that do not normally collect this pollen (see references cited above). Williams (2003) reported that increasing concentrations of Heliantheae (Asteraceae) pollen in the larval provisions of the blue orchard bee, *O. lignaria*, resulted in longer development time and reduced larval mass (Williams, 2003). Heliantheae pollen was also rejected by nesting *O. lignaria*, and these bees ceased nest construction when no other floral resources were available (Williams, 2003). After nectar-feeding from Heliantheae flowers, the bees would also groom themselves to remove the pollen before continuing to fly around the cage (Williams, 2003). Consequently, physiological adaptation may be necessary to tolerate the unfavourable qualities of Asteraceae pollen.

Digestibility and Amino Acid Composition of Pollen Provisions

Digestive Efficiency

Overall, my results suggest that Aster provisions are not difficult to digest for the Aster specialists (*O. montana* and *O. subaustralis*). Although *O. montana* and *O. subaustralis* did not digest their host Aster provisions as completely as *O. iridis* digested its host Fabeae provisions, the digestive efficiency of the Aster specialists was comparable to *O. tristella* bees fed generalist provisions. However, I could not compare digestion of *O. iridis* or *O. tristella* fed Aster provisions to that of Aster specialists because neither of the former species lived long enough on this provision type to reach the defecating larval stage. It is therefore possible these species could not survive on Aster provisions due to difficulties with digestion and nutrient assimilation.

Furthermore, the Aster specialists may have adapted physiologically to digest and utilize Asteraceae pollen. The Fabeae specialist, *O. iridis*, may have also adapted to its host pollen, since this species digested its host provisions more completely than either the Aster specialists or the generalist species (*O. tristella*) that were also fed Fabeae provisions.

Asteraceae pollen could be difficult to digest due to the morphology of the pollen grains. For example, Human *et al.* (2007) proposed that the spiny (echinate) structure and thick pollenkitt of sunflower (*Hel. annuus,* Asteroideae) pollen could impede digestion. In addition, dandelion pollen (*T. officinale*, Cichorioideae) is not completely digested by honeybees (*Apis mellifera*, Peng *et al.*, 1985), which could further reduce its nutritional value and contribute to the poor performance of honeybees fed dandelion pollen (Herbert, 1992).

Another factor that can influence pollen grain digestion by bees is the thickness of the intine, the inner pollen wall that covers the germinal pores. Suárez-Cervera *et al.* (1994) found that pollen digestion by *Osmia* bees was most complete for pollen grains with thinner intines, in which protoplasm visibly protruded through the apertures of the pollen wall in unconsumed pollen grains from the food provision. For thinner-walled pollen grains, there may be a degree of intine disruption at the germinal pores prior to consumption by the larvae. In addition, differences in the amount of nectar added to pollen provisions might influence the degree of protoplasmic extrusion and, consequently, subsequent larval digestion (mentioned by Dobson and Peng, 1997). A supporting finding was reported for honey possums, *Tarsipes rostratus*, in which the addition of a 36% (w/w) sucrose solution to *Banksia ericifolia* (Proteaceae) pollen during feeding trials reduced the time needed for the majority of excreted pollen grains to be empty (Turner, 1984). There could be differences in the volume of nectar added to pollen provisions across the bee species, since the provisions of the Aster specialists tend to be dry and

crumbly and the provisions of *O. iridis* and *O. tristella*, are wetter and stickier. In the future, it would be interesting to examine samples from the provisions of the different bee species (prior to consumption by larvae) for signs of intine disruption and protoplasmic extrusion.

Amino Acid Composition

None of the pollen provisions lacked any of the essential or nonessential amino acids that were examined, and amino acid profiles were comparable between the Aster specialists and the other bee species (i.e., *O. iridis*, *O. tersula*, and *O. tristella*). There were marginal differences in the proportions of essential amino acids between the Asteraceae specialists and the other bee species (apparently driven by differences in histidine and tryptophan), but neither group significantly differed from the amino acid requirements outlined for honeybees (De Groot, 1953).

Previous studies have reported amino acid deficiencies in the pollen of a number of Asteraceae taxa (Auclair and Jamieson, 1948; Herbert *et al.*, 1970; Wille *et al.*, 1985 as cited by Müller and Kuhlmann, 2008; Nicolson and Human, 2013) and I also found sunflower (*Hel. annuus*) pollen to have a lower content of total and essential amino acids compared to three other pollens (see Chapter 3). However, in this study, the concentration of total amino acids and essential amino acids were comparable between the Aster specialists and the other bee species (Appendix D). I suspect that this apparent contrast may be because the Aster bees add less nectar to their provisions than do the other bee species. Thus, the pollen as well as the protein content in the provisions of the non-Aster specialist bees may be more diluted—resulting in similar amino acid levels in provisions across all six species. Regardless, given the similar amino acid contents, it is unlikely that the reason *O. iridis* and *O. tristella* could not survive and develop on Aster provisions was due to deficiencies in essential amino acids.

Conclusion and Implications

My results show that specializing on Asteraceae pollen has not reduced the ability of the Aster specialist bees to tolerate novel pollen, which is inconsistent with the physiological-efficiency hypothesis. My results also suggest that the dietary breadth of Aster specialist larvae may be larger than the host range of adults. However, since the pollen preferences of the larva do not affect the provision they are provided, host selection by mother bees most likely reflects their own larval conditioning or innate preference (Rojas and Wyatt, 1999). The imprinting theory, proposed by Linsley (1958, 1961), suggests that specialized bees develop a preference for their host plants from exposure to pollen odors while in the nest as either larvae or pre-emerged adults. However, Praz *et al.* (2008b) found that specialization on Asteraceae pollen was hereditary for the bee species *Heriades truncorum* and was unaffected by rearing diet. Since the foraging decisions of mother bees ultimately determine the pollen composition and nutritional quality of offspring provisions, larval feeding studies should be paired with host-selection experiments to assess the willingness of adult bees to exploit novel pollen sources in the absence of their preferred host plants (e.g., Williams, 2003; Praz *et al.*, 2008b).

Although generalist foraging could be advantageous in reducing dependency on individual pollen sources (Eickwort and Ginsberg, 1980), neurological constraints, such as sensory limitations (suggested for phytophagous insects, Bernays, 2001) could restrict the host range of specialist bees (Praz *et al.*, 2008b). Alternatively, selection could favour specialization on Asteraceae pollen for a variety of reasons including the accessibility and availability of floral resources (i.e., 'predictable plethora', Wcislo and Cane, 1996), or to escape natural enemies (e.g., interspecific competitors, Thorp 1969). Although a complete escape from competitors is unlikely since generalists tend to also visit plants associated with specialist bees (Minckley and Roulston, 2006), competition could still be reduced by specializing on pollens that contain toxic secondary

metabolites or lack essential nutrients (suggested by Weiner *et al.*, 2010). In addition, Spear *et al.* (2016) found that the Aster specialists (same species as present study) were not attacked as frequently by kleptoparasitic wasps (*Sapyga*) as other bee species (*O. iridis*, *O. tersula*, and *O. tristella*), and that the wasps could not develop on Aster provisions.

In my study, I found that the Aster provisions were not deficient in amino acids, however, deficiencies in other essential nutrients (e.g., sterols: Vanderplanck *et al.*, 2014) or the presence of harmful secondary metabolites (e.g., alkaloids and glucosides: Detzel and Wink, 1993), could also affect the nutritional quality of Asteraceae pollen and should be the subject of future comparative studies. In addition, future research is needed to improve our understanding of the different nutritional requirements of bee species (Vaudo *et al.*, 2015).

Although the Aster specialists in my study were able to survive at least the experiment on nonhost provisions, it remains to be determined whether they can successfully overwinter on novel provisions. It is also still unclear whether cocoon completion is a good indicator of overwintering survival for the bee species I studied, in particular for the Aster specialists. Most bees that successfully overwintered after the 2016 experiment had finished their cocoons by 27 August 2016, when I left the field station (7 out of 10 bees: 4 *O. iridis* and 3 *O. tristella*). However, five bees that had completed their cocoons before the end of the experiment did not survive (one *O. iridis*, two *O. subaustralis*, and two *O. tristella*), suggesting that reaching the cocoon stage before the end of the experiment is not a perfect predictor of overwintering survival. Interestingly, the two *O. subaustralis* bees that survived had only reached the spinning larval stage and the sole *O. montana* that survived was at the defecating stage and had not started spinning by the end of the experiment. In addition, the three Aster specialist bees that survived

overwintering were all fed Aster provisions, so it remains to be determined if Aster specialists can complete development on novel host pollens.

Specialized bee species with narrow host ranges could be at a greater risk of local extinction during host shortages (Gathman and Tscharntke, 2002), notably those species that diapause and cease nesting in the absence of their host plant (e.g., Bohart and Youssef, 1976; Minckley *et al*., 1994). In addition, reproductive success can also be negatively affected by pollen shortages when foraging is associated with an increased risk of predation and parasitism (Goodell, 2003). Understanding the potential host range of bee species can help us not only reconstruct the evolutionary history of host shifts (e.g., Müller, 1996; Sipes and Tepedino, 2005; Sedivy *et al.*, 2008), but also potentially predict future host shifts in the face of changing host-plant availability. The latter is particularly important, as habitat destruction as well as agricultural intensification continue to reduce both the diversity and abundance of floral species worldwide (Müller *et al*., 2006).

Tables

Table 2.1. Sample size of bees in each of the three pollen treatments across both the 2016 and 2017 experiments. The bees are separated based on whether they were transferred as eggs or early-stage larvae. The three pollen treatments included 'Aster', which comprised pollen provisions from the Asteraceae-specialist bees *Osmia coloradensis*, *O. montana* and *O. subaustralis*; 'Fabeae', which were pollen provisions from the legume specialist *O. iridis*; and lastly 'Various', to represent mixed pollen provisions. Due to limitations in the availability of bee nests, *O. subaustralis* bees were only included in the 2016 experiment. In addition, only a single nest from both *O. coloradensis* and *O. tristella* was available in one of the two experiments and the bees that were transferred in that experiment either died before feeding on the provision or failed to hatch. As a result, the samples for these two species came from a single experiment.

Stage	**Year**	***O. coloradensis***			***O. iridis***			***O. montana***			***O. subaustralis***			***O. tristella***			**Total**	
		Aster	Fabeae	Various	Aster	Fabeae	Various	Aster	Fabeae	Various	Aster	Fabeae	Various	Aster	Fabeae	Various		
Eggs	2016	-	-	-	9	5	0	3	1	0	6	2	1	2	1	3	33	73
	2017	8	7	1	11	8	1	2	1	1	-	-	-	-	-	-	40	
Larvae	2016	-	-	-	0	0	0	1	1	0	6	5	1	2	2	1	19	34
	2017	2	3	0	4	5	0	1	0	0	-	-	-	-	-	-	15	
Total	2016	-	-	-	9	5	0	4	2	0	12	7	2	4	3	4	52	107
	2017	10	10	1	15	13	1	3	1	1	-	-	-	-	-	-	55	
Total per treatment		10	10	1	24	18	1	7	3	1	12	7	2	4	3	4	107	
Total per species		21			43			11			21			11				

Table 2.2. Sample description for the amino acid analysis of bee pollen provisions collected during the 2016 experiment. Bee species include *Osmia coloradensis* (1), *O. iridis* (3), *O. montana* (3), *O. subaustralis* (3), *O. tersula* (1), and *O. tristella* (1). Bee nests were collected from five sites near the Rocky Mountain Biological Laboratory in Colorado, USA (FT, KC, KP, RP, and VB). Provisions from multiple nests or sites were often combined to obtain enough material for analysis.

		Sample description			
Bee species	**Sample**	**Site(s)**	**Number of Mother bee(s)**	**Number of Nest(s)**	**Plant family**
O. tristella	1	KP; VB	2	2	Boraginaceae Fabaceae Plantaginaceae Violaceae
O. tersula	1	FT; VB	2	2	Boraginaceae Caprifoliaceae Fabaceae
O.iridis	1	FT	3	3	Fabaceae
	2	KC; KP	5	8	
	3	RB	1	1	
O. montana	1	FT	1	1	Asteraceae
	2	KC	2	2	
	3	KC	2	2	
O. subaustralis	1	VB	3	4	Asteraceae
	2	KP	1	2	
	3	KC; KP	3	3	
O. coloradensis	1	FT	1	1	Asteraceae

Table 2.3. Cox PH ratios for bee survival and development across the three pollen provision types (Aster, Fabeae, and Various) are presented for five species including *Osmia coloradensis*, *O. iridis*, *O. montana*, *O. subaustralis*, and *O. tristella*. The host provision type for each species is shaded. For the three developmental stages, hazard ratios less than one indicate improved chances of reaching the stage relative to the reference provision type (bold). However, for overall survival, a hazard ratio greater than one indicates improved chances of surviving until the end of the experiment.

O. coloradensis

	Defecating larval stage		**Spinning larval stage**		**Cocoon stage**		**Overall survival**	
	Aster	Fabeae	Aster	Fabeae	Aster	Fabeae	Aster	Fabeae
Fabeae	**>100 ****		**>100 ****		NA		**0.29 .**[1]	
Various	1.00	**<0.01 ****	1.00	**<0.01 ****	NA	NA	0.78	2.69

O. iridis

	Defecating larval stage		**Spinning larval stage**		**Cocoon stage**		**Overall survival**	
	Fabeae	Aster	Fabeae	Aster	Fabeae	Aster	Fabeae	Aster
Aster	**<0.01 ****		**<0.01 ****		**<0.01 ****		**>100 ****	
Various	0.72	**>100 ****	0.98	**>100 ****	**<0.01 ****	2.32	1.19	**<0.01 ****

O. montana

	Defecating larval stage		**Spinning larval stage**		**Cocoon stage**		**Overall survival**	
	Aster	Fabeae	Aster	Fabeae	Aster	Fabeae	Aster	Fabeae
Fabeae	0.97		NA		NA		**>100 ****	
Various	**<0.01 ****	**<0.01 ****	NA	NA	NA	NA	**>100 ****	0.97

O. subaustralis

	Defecating larval stage		**Spinning larval stage**		**Cocoon stage**		**Overall survival**	
	Aster	Fabeae	Aster	Fabeae	Aster	Fabeae	Aster	Fabeae
Fabeae	0.84		**>100 ****	**<0.01 ****	**>100 ****		1.01	
Various	**9.45 ****	**11.28 ****	**>100 ****	**7.14 .**	1.00	**<0.01 ****	**<0.01 ****	**<0.01 ****

O. tristella

	Defecating larval stage		**Spinning larval stage**		**Cocoon stage**		**Overall survival**	
	Various	Aster	Various	Aster	Various	Aster	Various	Aster
Aster	**<0.01 ****		**<0.01 ****		**<0.01 ****		**>100 ****	
Fabeae	1.35	**>100 ****	1.70	**>100 ****	0.55	**>100 ****	1.00	**<0.01 ****

[1]. $0.05 < P < 0.075$
* $P < 0.05$
** $P < 0.0$

Table 2.4. The Z scores from the binomial generalized linear models to compare the digestive efficiency of (A) bees fed their host pollen and (B) bees provided Fabeae (Fabaceae) provisions. Analyses were conducted on the proportion of pollen grains in the frass samples that were "empty" (i.e., <25%, top-right section of table) and "partially empty" (i.e., <50% full, bottom-left section). The bee species included *Osmia montana*, *O. subaustralis*, *O. iridis*, and *O. tristella* (sample size in brackets). Positive scores indicate that a greater proportion of the pollen grains were less than 25% or 50% full compared to the reference bee species (bold).

(A) Host pollen provisions

		Reference species	*O. montana*	*O. subaustralis*	*O. iridis*	*O. tristella*
Host pollen type	**Aster**	***O. montana*** (5)		**2.74 ****	**20.51 ****	**−3.84 ****
		O. subaustralis (8)	**−4.13 ****		**20.04 ****	**−6.70 ****
	Fabeae	***O. iridis*** (5)	**−10.01 ****	**−9.05 ****		**−22.59 ****
	Various	***O. tristella*** (4)	**−6.21 ****	**−3.10 ****	**7.98 ****	

(B) Fabeae pollen provisions

		Reference species	*O. coloradensis*	*O. montana*	*O. subaustralis*	*O. iridis*	*O. tristella*
Host pollen type	**Aster**	***O. coloradensis*** (3)		**−17.15 ****	**−19.31 ****	−0.299	**−3.71 ****
		O. montana (2)	**7.97 ****		1.70	**18.56 ****	**15.25 ****
		O. subaustralis (5)	**11.38 ****	**2.61 ****		**22.08 ****	**17.73 ****
	Fabeae	***O. iridis*** (5)	**−6.79 ****	**−10.59 ****	**−11.76 ****		**-3.89 ****
	Various	***O. tristella*** (4)	**2.48 ***	**−6.41 ****	**−10.57 ****	**8.09 ****	

* $P < 0.05$
** $P < 0.01$

Table 2.5. Dufrene-Legendre indicator values (indval) are presented for the pollen type with the highest proportional amount of each amino acid (essential amino acids in bold). Indicator values can range from 0 to 1, and higher values indicate that an amino acid made up a larger proportion of the total amino acid content of a specific pollen type and/or that an amino acid was only found in certain pollen types. The pollen provisions of the six bee species were separated into the Aster specialists (*Osmia coloradensis*, *O. montana*, and *O. subaustralis*) and the other bee species (*O. iridis*, *O. tersula*, and *O. tristella*). All of the amino acids that were analyzed were present in the different pollen provisions, and no significant differences among pollen types in the amino acid profiles were detected after adjusting for multiple comparisons.

Bee species provisions	**Amino acid**	**indval**	***P***	**Adjusted *P*** [1]
other bee species	Proline	0.575	0.048	0.86
	Arginine	0.508	0.78	1
	Tyrosine	0.507	0.50	1
	Valine	0.503	0.87	1
	Phenylalanine	0.503	0.94	1
	Glx	0.502	0.93	1
	Aaba	0.502	0.94	1
Asteraceae specialists	**Tryptophan**	0.670	0.57	1
	Histidine	0.575	0.16	1
	Asx	0.555	0.15	1
	Glycine	0.512	0.53	1
	Leucine	0.512	0.63	1
	Alanine	0.507	0.59	1
	Lysine	0.505	0.87	1
	Methionine	0.502	0.90	1
	Threonine	0.502	0.89	1
	Isoleucine	0.502	0.92	1
	Serine	0.500	0.98	1

[1] *P* values were adjusted for multiple comparisons using a Bonferroni correction.

Figures

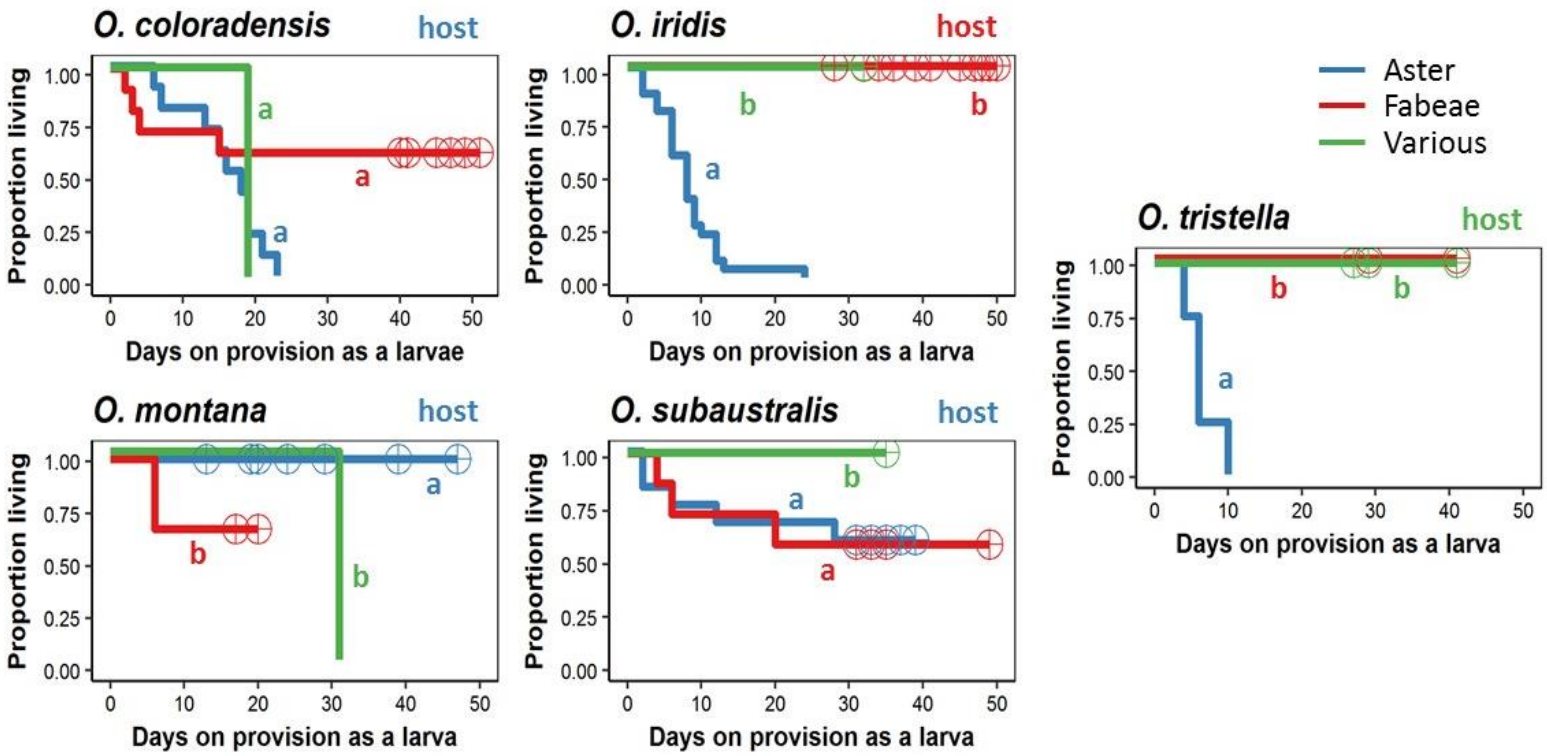

Figure 2.1. Survival curves for larval bees provided Aster, Fabeae, and Various pollen provisions, visualized using the 'survfit' function of the 'survival' package. Bee species included *Osmia coloradensis* (n = 21), *O. iridis* (43), *O. montana* (11), *O. subaustralis* (21), and *O. tristella* (11) and data were collected in Colorado, USA (2016 and 2017). Host labels indicate the pollen provision type of each bee species and letters indicate significant differences between pollen treatments. Bees alive at the end of the experiment are represented by ⊕ symbol. Results were jittered both vertically and horizontally to reduce overlap between the different pollen treatments.

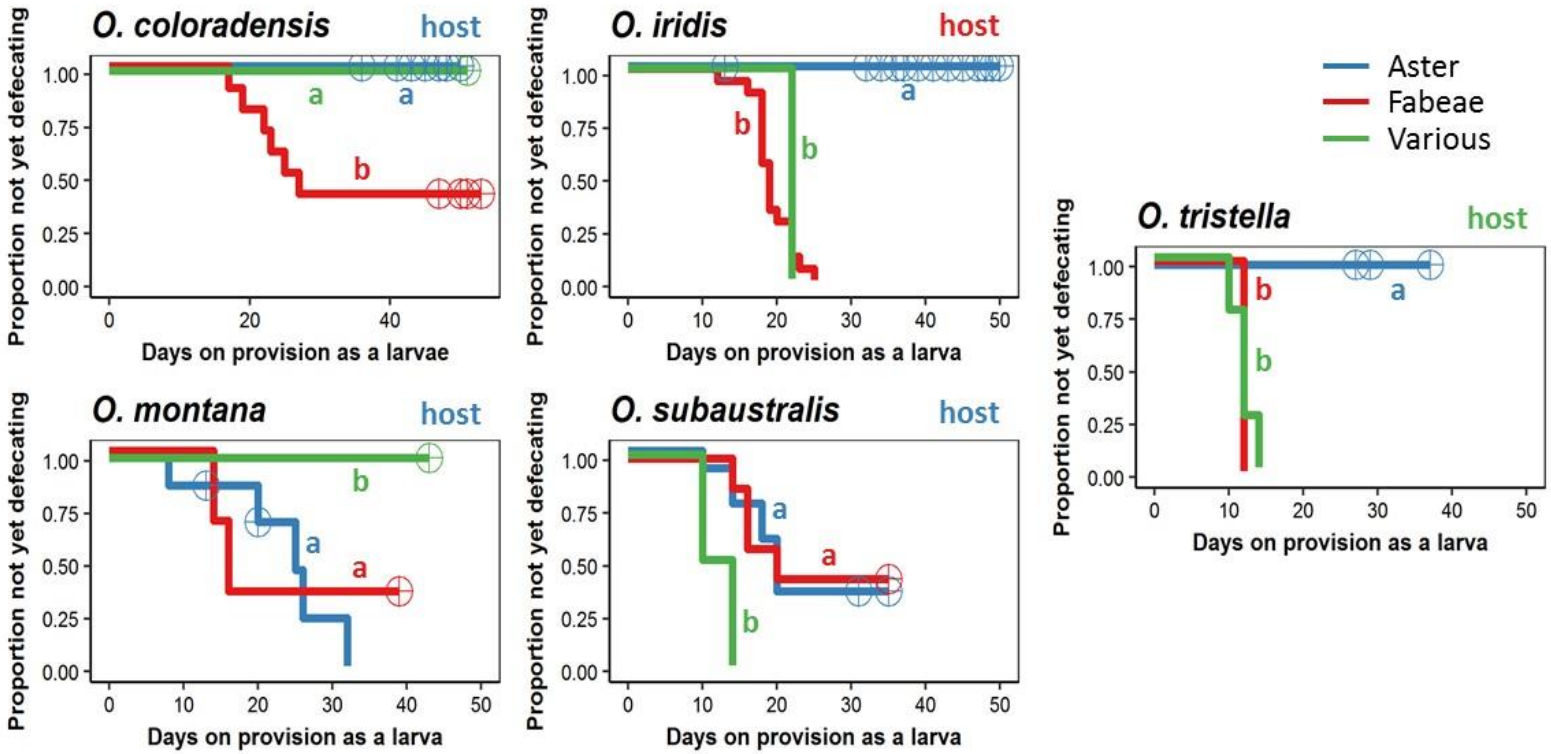

Figure 2.2. Survival curves for development to the defecating larval stage for bees provided Aster, Fabeae, and Various pollen provisions, visualized using the 'survfit' function of the 'survival' package. Bee species included *Osmia coloradensis* (n = 21), *O. iridis* (43), *O. montana* (11), *O. subaustralis* (21), and *O. tristella* (11) and data were collected in Colorado, USA (2016 and 2017). Host labels indicate the pollen provision type of each bee species and letters indicate significant differences between pollen treatments. Bees that died before reaching the defecating stage or were still alive but did not reach the defecating stage by the end of the experiment are indicated by the ⊕ symbol on curves. Results were jittered both vertically and horizontally to reduce overlap between the different pollen treatments.

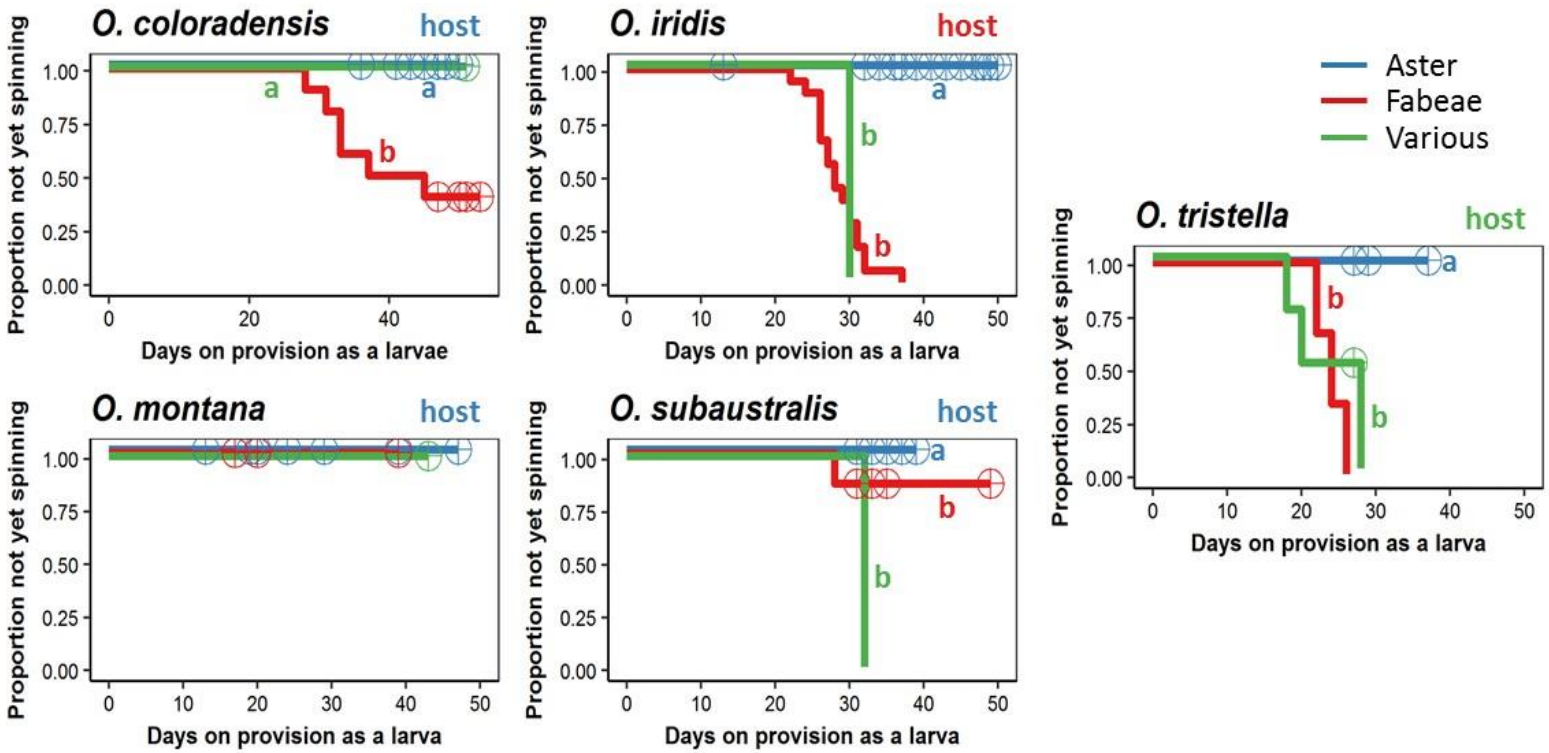

Figure 2.3. Survival curves for development to the spinning larval stage for bees provided Aster, Fabeae, and Various pollen provisions, visualized using the 'survfit' function of the 'survival' package. Bee species included *Osmia coloradensis* (n = 21), *O. iridis* (43), *O. montana* (11), *O. subaustralis* (21), and *O. tristella* (11) and data were collected in Colorado, USA (2016 and 2017). Host labels indicate the pollen provision type of each bee species and letters indicate significant differences between pollen treatments. Bees that died before reaching the spinning stage or were still alive but did not reach the spinning stage by the end of the experiment are indicated by the ⊕ symbol on curves. Results were jittered both vertically and horizontally to reduce overlap between the different pollen treatments.

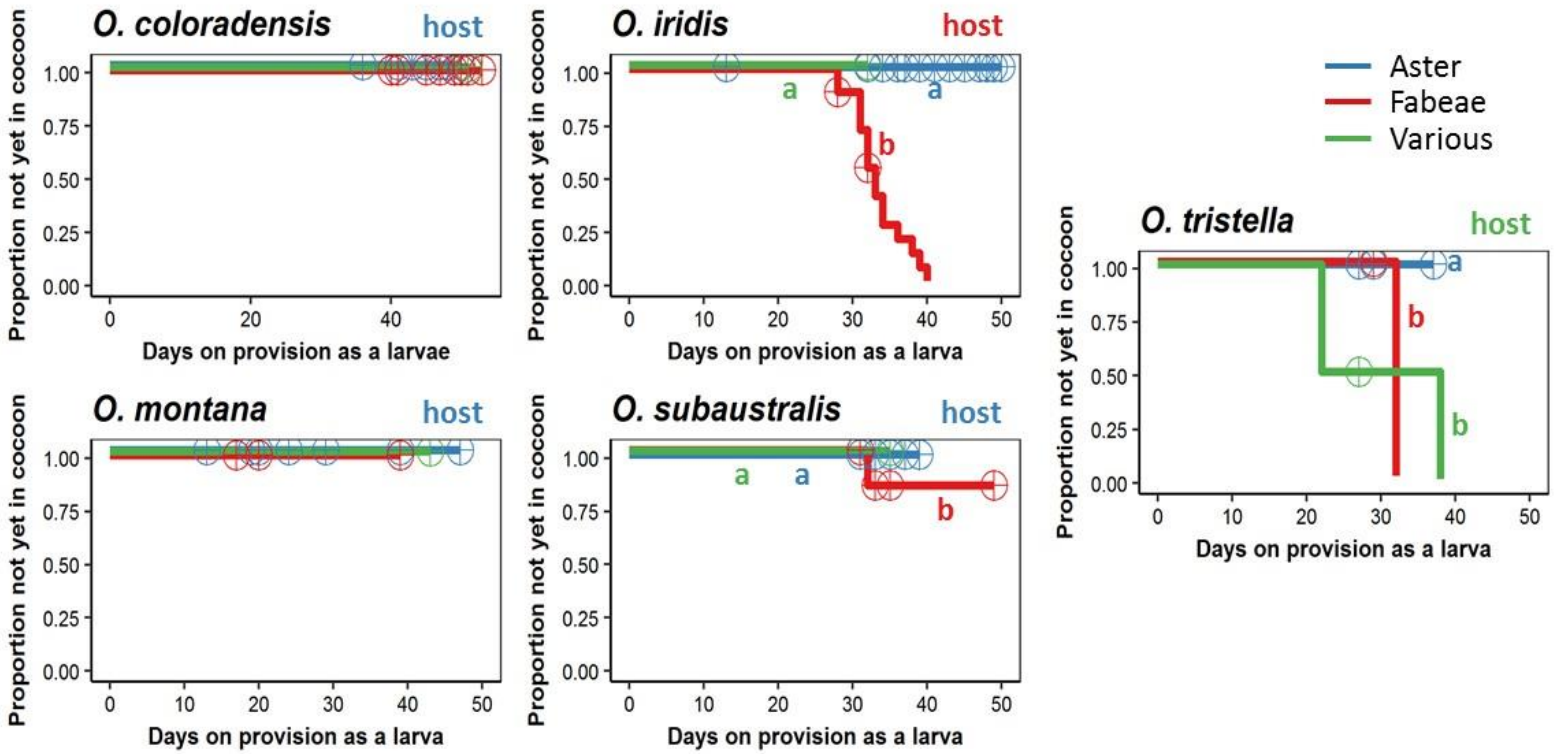

Figure 2.4. Survival curves for development to the cocoon stage for bees provided Aster, Fabeae, and Various pollen provisions visualized using the 'survfit' function of the 'survival' package. Bee species included *Osmia coloradensis* (n = 21), *O. iridis* (43), *O. montana* (11), *O. subaustralis* (21), and *O. tristella* (11) and data were collected in Colorado, USA (2016 and 2017). Host labels indicate the pollen provision type of each bee species and letters indicate significant differences between pollen treatments. Bees that died before reaching the cocoon stage or were still alive but did not reach the cocoon stage by the end of the experiment are represented by the ⊕ symbol on curves. Results were jittered both vertically and horizontally to reduce overlap between the different pollen treatments.

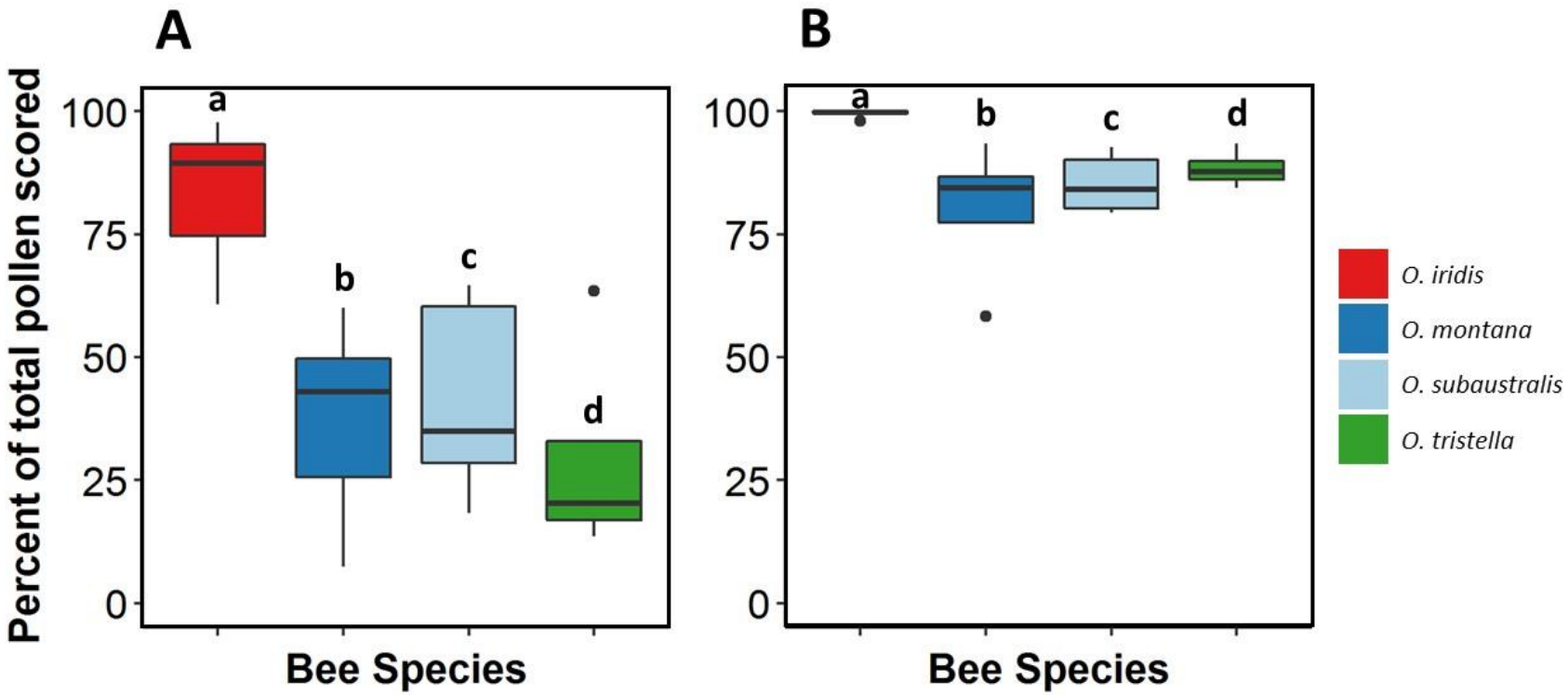

Figure 2.5. The percentage of scored pollen grains that were (A) less than 25% full and (B) less than 50% full in the frass samples of bees fed their host pollen provision during the 2016 experiment. The interquartile range (IQR) extends from the 25th to the 75th percentile and whiskers are ± 1.5 times IQR. Letters indicate significant differences between bee species. Bee species included the specialist of Fabeae (Fabaceae) pollen (*Osmia iridis,* n = 5), two Aster specialists (*O. montana,* 5, and *O. subaustralis,* 8) as well as a generalist species (*O. tristella,* 4).

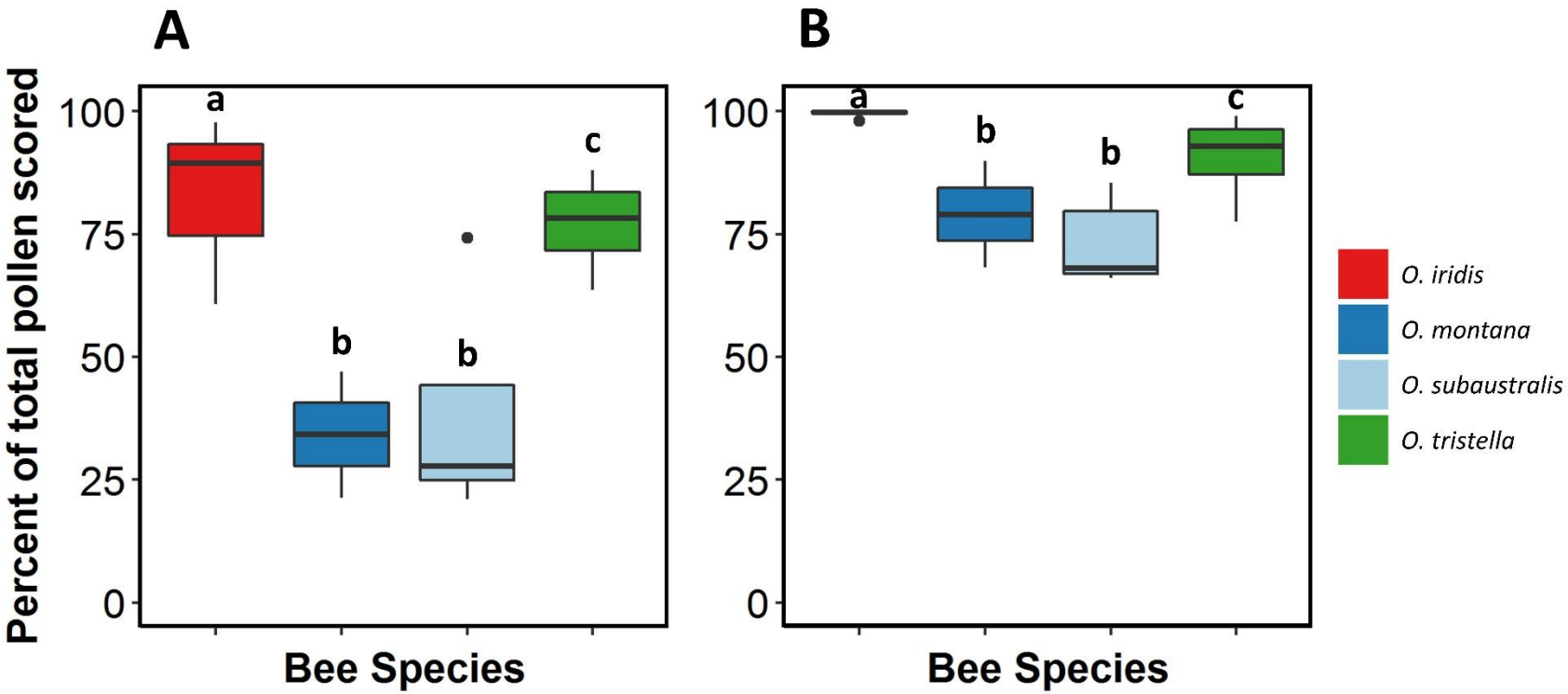

Figure 2.6. The percentage of scored pollen grains that were (A) less than 25% full and (B) less than 50% full in the frass samples of bees fed Fabeae pollen provisions during the 2016 experiment. The interquartile range (IQR) extends from the 25th to the 75th percentile and whiskers are ± 1.5 times IQR. Letters indicate significant differences between bee species. Bee species included the specialist of Fabeae (Fabaceae) pollen (*Osmia iridis,* n = 5), two Aster specialists (*O. montana,* 2, and *O. subaustralis,* 5) as well as a generalist species (*O. tristella,* 4).

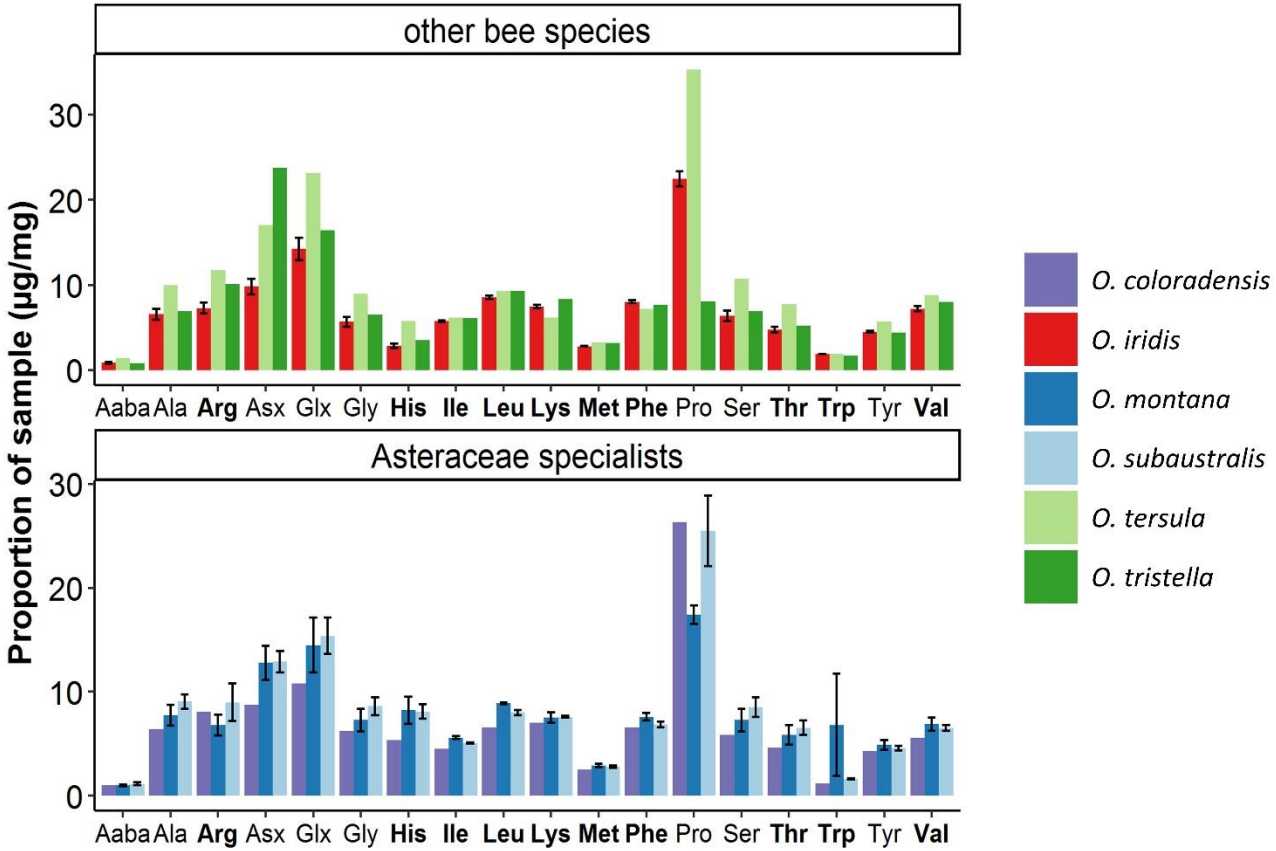

Figure 2.7. The mean percent ± SEM quantities of essential amino acids (EAA) and nonessential amino acids (NAA) (including both protein-bound and free amino acids) in the pollen provisions of six bee species (*O. coloradensis* (n=1), *O. iridis* (3), *O. montana* (3), *O. subaustralis* (3), *O. tersula* (1), and *O. tristella* (1)) as a proportion of the dry mass of the sample (µg/mg of sample). (EAA (bold): Arg = arginine, His = histidine, Ile = isoleucine, Leu = leucine, Lys = lysine, Met = methionine, Phe = phenylalanine, Thr = threonine, Trp = tryptophan, Val = valine; NAA: Aaba = alpha-aminobutyric acid, Ala = alanine, Asx = asparagine + aspartic acid, Glx = glutamine + glutamic acid, Gly= glycine, Pro = proline, Ser = serine, and Tyr = tyrosine)

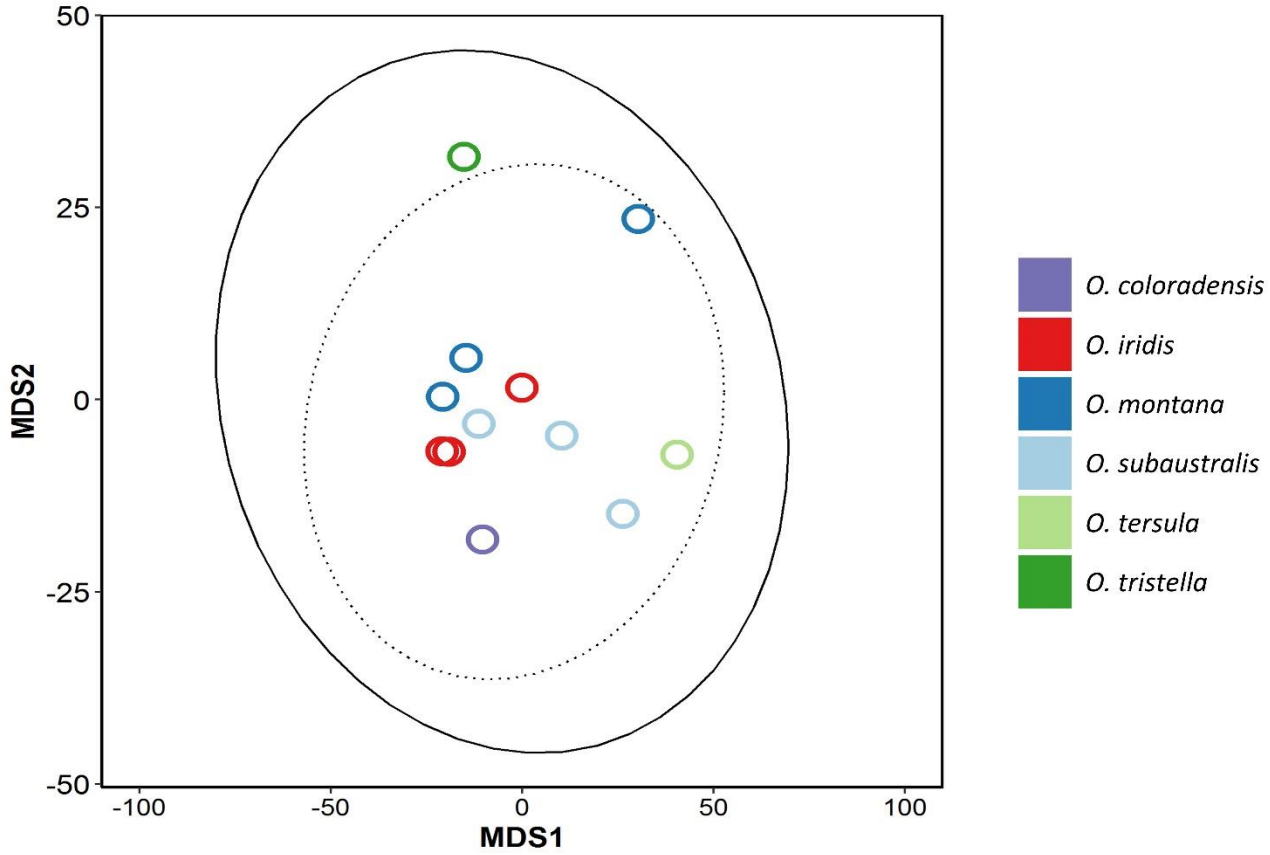

Figure 2.8. nMDS ordination plot based on Manhattan dissimilarity indices for the amino acid profiles of the pollen provisions of six different bee species (*O. coloradensis* (n=1), *O. iridis* (3), *O. montana* (3), *O. subaustralis* (3), *O. tersula* (1), and *O. tristella* (1)). The indices were calculated from absolute values (µg/mg of dried sample) for essential and nonessential amino acids and included both protein-bound and free amino acids. Ellipses (95% confidence) are included for the Asteraceae specialist species (dotted) and the other bee species (solid). Results were jittered (± 100% data resolution for both vertical and horizontal jitter) to better illustrate overlapping data points.

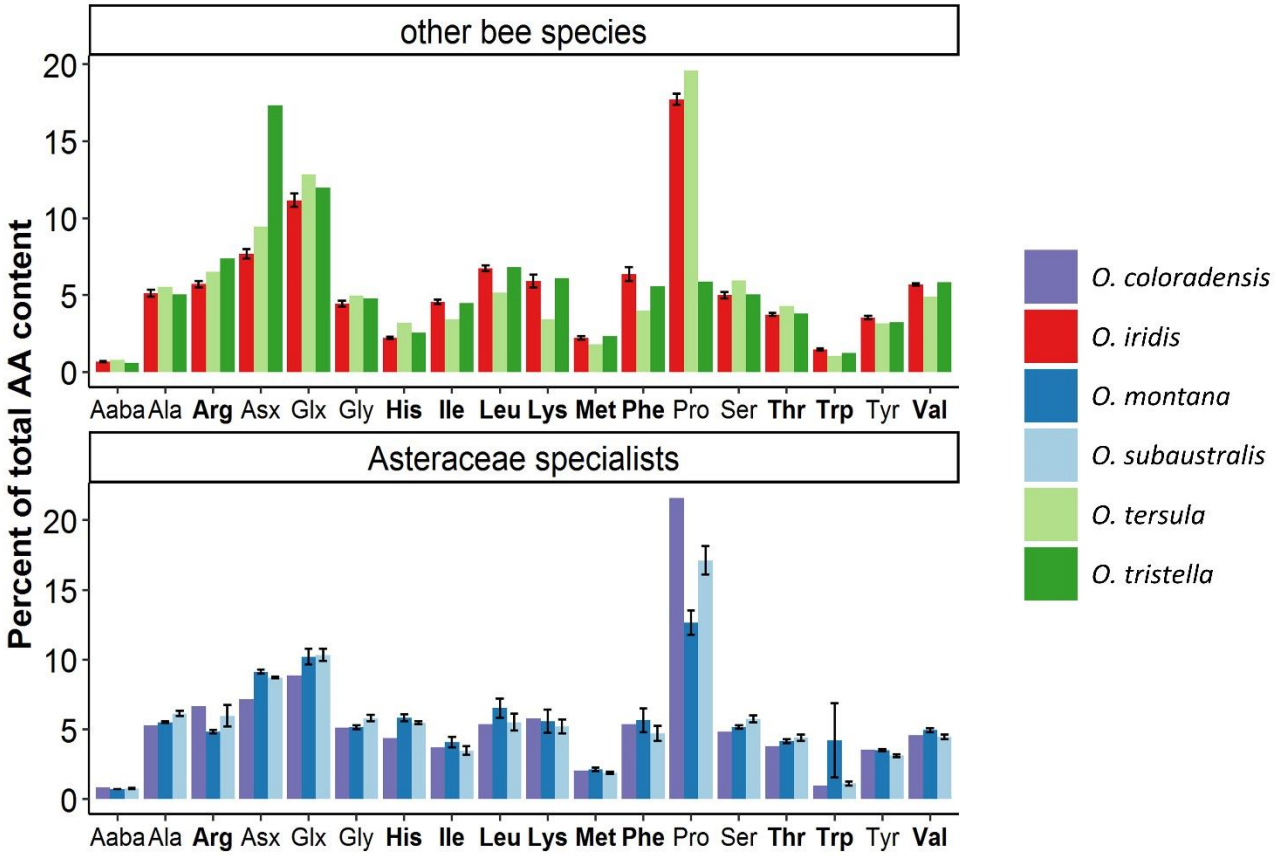

Figure 2.9. The mean percent ± SEM quantities of essential amino acids (EAA) and nonessential amino acids (NAA) (including both protein-bound and free amino acids) in the pollen provisions of six bee species (*O. coloradensis* (n=1), *O. iridis* (3), *O. montana* (3), *O. subaustralis* (3), *O. tersula* (1), and *O. tristella* (1)) as a proportion of the total amino acid content. (EAA (bold): Arg = arginine, His = histidine, Ile = isoleucine, Leu = leucine, Lys = lysine, Met = methionine, Phe = phenylalanine, Thr = threonine, Trp = tryptophan, Val = valine; NAA: Aaba = alpha-aminobutyric acid, Ala = alanine, Asx = asparagine + aspartic acid, Glx = glutamine + glutamic acid, Gly= glycine, Pro = proline, Ser = serine, and Tyr = tyrosine)

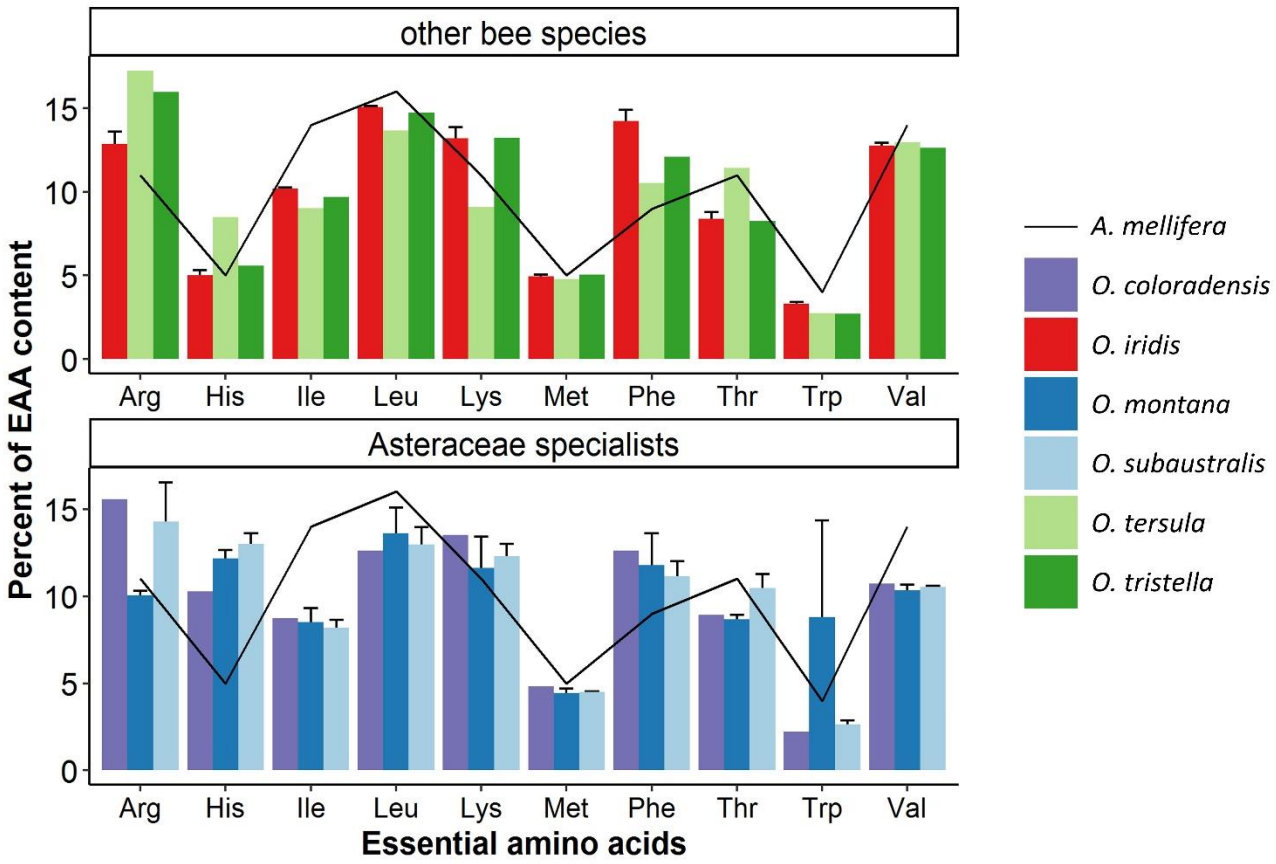

Figure 2.10. The mean percent ± SEM quantities of essential amino acids (EAA) (including both protein-bound and free amino acids) in the pollen provisions of six bee species (*O. coloradensis* (n=1), *O. iridis* (3), *O. montana* (3), *O. subaustralis* (3), *O. tersula* (1), and *O. tristella* (1)) as a proportion of the total amino acid content. The black line represents the EAA requirements for *Apis mellifera* described by De Groot (1953). (Arg = arginine, His = histidine, Ile = isoleucine, Leu = leucine, Lys = lysine, Met = methionine, Phe = phenylalanine, Thr = threonine, Trp = tryptophan, Val = valine)

Chapter 3

How do sunflower pollen mixtures affect survival of queenless microcolonies of bumblebees?

Introduction

The high pollen requirements of bee larvae (Schlindwein *et al.*, 2005; Müller *et al*., 2006) may select for a generalist foraging strategy in order to reduce dependency on individual pollen sources (Eickwort and Ginsberg, 1980). However, exploiting many floral resources requires bees to manipulate different floral morphologies and digest pollen of varying nutritional compositions, which may be costly in that it requires complex neural processing capabilities as well as greater dietary (i.e., physiological) flexibility. For example, limits on memory and learning capacity (Bernays and Wcislo 1994; Dukas and Kamil 2001) could impose neurological constraints on the ability of bees to recognize and handle different flowering species (Strickler 1979; Williams 2003; Praz *et al*., 2008b).

Potentially due to these limits, individual bees of polylectic species will often specialize on a floral resource and collect pollen from one plant species on a foraging bout (Linsley and MacSwain, 1958). However, at least some broadly polylectic bee species are known to mix pollen while foraging and provide their offspring with mixed provisions from unrelated plant species (e.g., Brooks and Roubik, 1983; Williams and Tepedino, 2003; Budde and Lunau, 2007 as cited by Eckhardt *et al.*, 2014; Eckhardt *et al.*, 2014). Pollen mixing might benefit polyleges by increasing both pollen collection and nectar-feeding efficiency (Williams and Tepedino, 2003). Mixing pollens might also be a strategy to exploit unfavourable pollens while still stabilising larval diet quality if the different pollen sources help to complement nutrient deficiencies and alleviate the effects of toxic secondary metabolites (Eckhardt *et al*., 2014). For

example, *Megachile rotundata* (=*M. pacifica*) larvae developed significantly more quickly on a mixed pollen diet of *Melilotus* and *Medicago sativa* than on a diet containing only *Melilotus* pollen (Tasei and Masure, 1978). Similarly, *Osmia cornuta* larvae developed well on mixed pollen provisions containing their normal host plant *Sinapsis arvensis* and up to 50% *Ranunculus acris* pollen, whereas a diet of solely *R. acris* pollen was toxic to most *O. cornuta* larvae (Eckhardt *et al.*, 2014).

The nutritional quality of mixed pollen diets compared to monofloral diets for bumblebee health and colony development remains poorly understood (Baloglu and Gurel, 2015), despite widespread interest in bumblebee conservation and pollinator management strategies (e.g., composition of wildflower plantings). Mixed pollen diets could benefit broadly polylectic bees by minimizing the chances of vitamin, mineral, and/or protein deficiencies while simultaneously diluting the toxic secondary metabolites found in certain pollen species (Eckhardt *et al.*, 2014). Tasei and Aupinel (2008a) reported that the protein efficacy (weight of larvae/protein consumption) was ~4 times greater for queenless microcolonies of *B. terrestris* fed mixed pollen diets than pure pollen diets, suggesting that another pollen constituent besides nitrogen content might affect the nutritive value of the pollen diet for bee development.

In this study, I examined how the proportion of sunflower pollen in the diet of captive-reared bumblebees (*Bombus impatiens*) affects the survival of worker bees. Previous studies have demonstrated that sunflower pollen has deleterious effects on both honeybees (*Apis mellifera*: Schmidt *et al.*, 1995) and a European bumblebee species (*B. terrestris*: Regali and Rasmont, 1995; Tasei and Aupinel, 2008a), although the precise mechanism of effect is unknown. I was interested in whether mixing sunflower pollen with other pollen species could improve the quality of the pollen diet through nutrient complementation or dilution of toxic substances. The

mixed pollen diets contained either 0%, 25%, or 50% sunflower pollen with an equal mixture of Cucurbitaceae, rapeseed (*Brassica napus*) and broad bean (*Vicia faba*) pollen making up the rest of the diet. Each pollen type was also included as a monofloral pollen treatment in the experiment (i.e., broad bean, Cucurbitaceae, rapeseed, and sunflower). Queenless microcolonies of bumblebees are often used for testing the nutritive value of pollen diets for European bumblebee species (Génissel *et al*., 2002; Tasei and Aupinel, 2008a; Vanderplanck *et al*., 2014; Moerman *et al.*, 2016b; Moerman *et al.*, 2017), and the results can be extrapolated to queenright colonies (*B. terrestris*, Tasei and Aupinel, 2008b). Although microcolony designs are often used in *B. impatiens* studies (e.g., Richardson *et al.*, 2015; Schaeffer *et al.*, 2016), to my knowledge this method has not been applied to study pollen quality for *B. impatiens*.

I assessed the performance of *B. impatiens* microcolonies fed each pollen diet using the following three parameters: i) worker mortality, ii) initiation of honeypot construction, and iii) egg production. I also compared the amino acid composition of the four pollen types (i.e., broad bean, Cucurbitaceae, rapeseed, and sunflower) to determine if any diet was deficient in essential amino acids.

Methods

Study Species

Five colonies of captive-reared *Bombus impatiens* Cresson were obtained from Biobest (Leamington, Ontario, Canada) and fed a honeybee-collected, multifloral pollen mixture (Hawkins Honey Bee Pollen, Rockwood, Ontario). The pollen was ground and mixed with deionized water to form pollen balls, and each colony received one pollen ball (~0.5 g) per day. BIOGLUC® (Biobest Biological Systems), a nectar substitute, was included with each colony and they were fed ad libitum on this syrup solution. The colonies were kept at room temperature

(21–22 °C) at the University of Ottawa and were used as sources for the experimental microcolonies (described below).

Pollen Treatments

Each bumblebee microcolony (described below) received one of the seven pollen treatments. The three SFM ("sunflower mixed") diets contained either 0%, 25%, or 50% sunflower pollen (97% *Helianthus annuus* and 3% other Asteraceae species [*Rudbeckia* sp., *Coreopsis* sp., and *Heliopsis* sp.]), with an equal mixture of Cucurbitaceae (30% summer squash [*Cucurbita pepo*] and 70% watermelon [*Citrullus lanatus*]), rapeseed (*Brassica napus*) and broad bean (*Vicia faba*) pollen making up the rest of the diet. Each pollen type was also included as a monofloral pollen treatment in the experiment (i.e., broad bean, Cucurbitaceae, rapeseed, and sunflower). Bumblebee microcolonies were assigned systematically to the different pollen treatments (Table 3.1.).

Honeybee-collected pollen of four types (broad bean, rapeseed, sunflower, and watermelon) was obtained from Changge Ruifenfang Bee Products Company (Changge City, Henan Province, China) and washed to remove nectar residues. Nectar-derived sugars can make up 40% of the dry weight of bee-collected pollen (Todd and Bretherick, 1942; reviewed by Roulston and Cane, 2000), and analyses that ignore the nectar contribution are likely to underestimate the concentration of protein in the pollen (Weiner *et al.*, 2010). Twenty grams of pollen at a time was suspended in 800 mL of deionized water and filtered through 6 µm cellulose filter paper (Little Chalfont, Buckinghamshire, UK) using a vacuum pump. This filtering technique should have been sufficient to remove the nectar residues (see Kitaoka and Nieh, 2009). Pollen was then air-dried before being stored in a −20°C freezer. Pollen from *Rudbeckia* sp., *Coreopsis* sp., *Heliopsis* sp., and summer squash (*Cucurbita* sp.) was hand-collected from greenhouse-grown

plants at the University of Ottawa and added to the commercial pollen after the latter had been washed.

Pollen balls were made by combining either 0.5 or 1 g of washed pollen with 10–15 drops of 35% w/w sucrose solution; variation in the number of drops was due to the fact that different amounts of solution were required to form pollen balls, depending on pollen type. The maximum difference in amount of solution added was approximately 0.23 mL (0.081g of sucrose). The pollen balls were coated in honeybee wax to prevent desiccation and encourage egg production (mentioned by Gradish *et al.*, 2013). Pollen balls were also stored in the freezer.

Microcolony Set-Up and Experimental Design

Pupal clumps of worker-caste bees were collected from source colonies and stored in plastic containers. Bees were placed in microcolonies of 4–6 bees within 24 hours of their emergence. Microcolonies were housed in delicatessen containers (~ 650 mL) that were wrapped in duct tape to keep the bees in darkness and reduce stress (photos of microcolony set up are provided in Appendix E). The base of each container was removed and replaced with either plastic mesh (0.635 cm or 0.4 cm) or 18×16 nylon window-screen mesh. A plastic painter pot (~15 mL) filled with 35% sucrose solution was placed underneath the mesh in a second deli container. A cotton wick (1 cm diameter) was secured in the painter pot through a hole cut in the lid. The wick soaked up the sucrose solution and allowed the bees to access the solution through the mesh base of their deli container. When microcolonies were first established, honey—which is naturally scented—was placed on the tip of cotton wicks to encourage the bees to feed. The sugar solution was changed every other day and the wicks were replaced every 4 days. Within 24 hours of microcolony establishment, bees were given a 1 g pollen ball in a 6.35 cm diameter dish. An additional 0.5 g pollen ball was provided 1 week later and replaced every 4 days over a 4-week

period. The original 1 g pollen ball was removed if it remained uneaten at least 7 days after microcolony establishment to prevent the development of mold.

Microcolonies were assessed every day to note bee deaths, pollen consumption, honeypot initiation, and egg production. No larvae were produced during the experiment. Microcolonies were kept for 6 weeks (42 days), after which all remaining bees were culled. Over the course of the experiment, only 25 bees (10.4%) were culled; thus, the majority died during the experimental treatments. In total, 43 microcolonies (3–13 microcolonies from each source colony) and 242 bees were included in the experiment, but only 237 were included in analysis. Two bees were omitted since they were accidentally killed during microcolony observations; another bee died after she had escaped from her microcolony; and two bees were omitted since they were mistakenly added after each of their microcolonies was established.

Amino Acid Analysis

The amino acid profile of the four pollen types (broad bean, Cucurbitaceae, rapeseed, and sunflower mix; n = 3 of each) were analyzed at the SPARC BioCentre of the Hospital for Sick Children (Toronto, Ontario, CA). Each sample underwent three analyses: a standard amino acid analysis (AA analysis) and a free amino acid analysis (FAA analysis), which both excluded cysteine and tryptophan, as well as a tryptophan analysis, which included both bound and free tryptophan. Cysteine was not quantified since this is not an essential amino acid for bees (De Groot, 1953). The Waters Pico-Tag System was used to analyze the amino acids and the detection limit for all three analyses was 25 pmol (Heinrikson and Meredith, 1984; White *et al.*, 1986). Prior to analysis, each sample was pulverized and thoroughly mixed with a mortar and pestle.

Statistical Analysis

Bee Survival and Microcolony Performance

Cox proportional hazard (henceforth Cox PH) models were used to compare bee survival on the different pollen treatments using the 'coxph' function from the 'survival' package (Therneau, 2015). Cluster terms were included in the model for both source colony and microcolony. These terms are used to compute a robust variance for the model by accounting for nonindependence and correlation among observations. The data were analyzed both including and excluding microcolonies that did not consume visible amounts of pollen (as well as bees that died prior to pollen consumption). Since the broad bean treatment in both Cox PH models violated the assumption of proportional hazards (Schoenfeld residuals; model with all bees: $\chi^2 = 5.65$, $P = 0.018$; model with only pollen-consuming bees: $\chi^2 = 16.0$, $P < 0.0001$), both models were re-run while omitting two (of six) microcolonies (12 bees) from the broad bean treatment to satisfy this assumption. These two microcolonies were strong outliers since they contained three and four bees respectively that survived until the end of the experiment as well as several bees (three and two respectively) that died earlier. Consequently, the proportional risk of death for the bees in these two microcolonies, as well as the hazard risk relative to the other pollen treatments, varied with time—inconsistent with the assumption of proportional hazards. The results of the Cox PH model with all bees is presented in this chapter, and the results for all four models (including and excluding microcolonies that did not consume pollen, both with and without outliers) are included in Appendix F. A Cox PH model was also used to compare time to honeypot construction across the different pollen treatments, with source colony as a cluster term. This model was consistent with the assumption of proportional hazards (Schoenfeld residuals: $\chi^2 = 2.35$, $P = 0.89$), and all 43 microcolonies were included in this analysis.

Pollen Amino Acids

The amino acid profiles (summed across AA and FAA for each amino acid except tryptophan) of the four different pollen types (broad bean, Cucurbitaceae, rapeseed, and sunflower) were compared using non-metric multidimensional scaling (NMDS) with the 'metaMDS' function in the 'vegan' package (Oksanen *et al.*, 2017). Two NMDS ordinations were performed, both using Manhattan dissimilarity matrices, two dimensions, and 20 runs. These parameters were sufficient to reach a stress level less than 0.05 and model convergence. The first NMDS was based on absolute amounts of each amino acid (i.e., µg of each amino acid per mg of dry sample); the second used proportions (i.e., each amino acid expressed as a percent [by mass] of total amino acid content). Permutational multivariate analyses of variance (perMANOVA; 1000 permutations) using the 'adonis' function were then performed to compare pollen types. Pairwise permutation MANOVAs with a Bonferroni correction were also performed because the global test indicated that pollen types differed significantly ('pairwise.perm.manova' function, 'RVAideMemoire' package; Hervé, 2017). Prior to these analyses, I verified homogeneity of group covariances using permutational multivariate homogeneity of group dispersions tests (PERMISP; 1000 permutations) with the 'betadisper' function. To determine if certain amino acids were representative of a particular pollen type, indicator values for each amino acid (based on proportions of total amino acid content) were calculated using the 'indval' function of the labdsv package (Roberts, 2016). Lastly, the proportions of essential amino acids (EAA) for each pollen type were compared to the EAA requirements for honeybees (*Apis mellifera*) outlined by De Groot (1953) (arginine (11%), histidine (5%), isoleucine (14%), leucine (16%), lysine (11%), methionine (5%), phenylalanine (9%), threonine (11%), tryptophan (4%) and valine (14%)) by running an additional NMDS on a Manhattan dissimilarity matrix (2 dimensions and 20 runs) with these requirements included as

an additional “sample”. A perMANOVA and pairwise comparisons (as above) were then performed to compare the pollen types and the EAA requirements for honeybees.

Results

Bee Survival and Microcolony Performance

There was no evidence of pollen consumption in 10 microcolonies (~23% of the total 43 microcolonies) containing 53 bees. In addition, 15 bees died before pollen consumption in the remaining 33 microcolonies. All 25 bees that survived until the end of the 6-week period (42 days) were from microcolonies with pollen consumption. None of the bees in the 100% sunflower pollen treatment survived until the end of the 6-week period. All four Cox PH models for bee survival reported significantly shorter lifespans of bees provided 100% sunflower pollen than those in all other pollen treatments ($P < 0.01$) except 25% sunflower mixed pollen (Figure 3.1.; Table 3.2.; Appendix F). During the experiment, 19 of the 43 microcolonies (44%) constructed honeypots, and microcolonies provided broad bean pollen were significantly more likely to construct honeypots than either the 25% or the 50% sunflower mixed pollen treatments ($P < 0.05$) and marginally more likely to construct honeypots than the 100% sunflower treatment ($P = 0.071$) (Figure 3.2.; Table 3.3.). Egg masses were visible in four microcolonies: one that was provided 0% mix pollen, another that was given Cucurbitaceae pollen, and two that were given broad bean pollen. However, all eggs that were laid were later destroyed or eaten, and no male bees emerged from any of the microcolonies.

Pollen Amino Acids

Free amino acids accounted for 3.49–6.43% of the total amino acid content for the four different pollen types (Table 3.4.; Appendix G). Pollen types differed in their absolute amounts of amino acids (i.e., µg/mg of dry sample; perMANOVA, $R^2 = 0.93$, $F_{3,8} = 36.41$, $P = 0.0020$; Figures 3.3., 3.4.). However, despite the overall difference among pollen types, no single pollen

type differed significantly from any another in pairwise comparisons once corrected for multiple comparisons ($P > 0.6$), likely due to low sample size. Overall, broad bean pollen had both the highest total amino acid and highest essential amino acid content, and sunflower pollen—followed closely by Cucurbitaceae pollen—had the lowest total amino acid and lowest essential amino acid content (Table 3.4.). The proportions of the different amino acids were similar across the four pollen types (perMANOVA, $R^2 = 0.36$, $F_{3,8} = 1.52$, $P = 0.21$, Figure 3.5.), and none of the amino acids was indicative of a particular pollen type (Table 3.5.). Interestingly, the relative proportions of the essential amino acids did differ between the four pollen types and the requirements for honeybees (perMANOVA, $R^2 = 0.58$, $F_{4,8} = 2.75$, $P = 0.027$, Figure 3.6.). Although I lacked the statistical power to detect differences in pairwise comparisons.

Discussion

Bee Survival Across Pollen Treatments

Bumblebees died sooner in the 100% sunflower treatment than in any other pollen treatment except 25% sunflower mixed pollen. The difference in survival was likely not due to the amino acid composition of this pollen type, since all four pollen types had similar amino acid profiles, but could be partially explained by the lower total amino acid content. In a similar study, Tasei and Aupinel (2008a) observed the highest worker mortality for *Bombus terrestris* fed sunflower—higher than for bees fed six other pollen diets including *Castanea*, *Rubus*, *Papaver*, *Actinidia*, and *Cistus* pollen. The same study found that microcolonies fed a mixed pollen diet that contained 9% sunflower pollen and had a lower nitrogen content than the pure sunflower diet produced heavier larvae than microcolonies in the pure sunflower treatment. This result suggests that crude protein content is not the only factor affecting pollen nutritive value and that the addition of other pollens may have improved the nutritional properties of the mixed pollen diet (Tasei and Aupinel, 2008a). Similarly, in my study, the Cucurbitaceae pollen treatment,

which had an amino acid content that was only slightly higher than the 100% sunflower treatment, did not reduce bee lifespan relative to the other pollen treatments, suggesting that another nutritional factor besides protein content was responsible for the poor performance of bees in the 100% sunflower pollen treatment.

Crude protein, estimated from nitrogen content, has traditionally been used as a measure of pollen nutritional quality (reviewed by Roulston and Cane, 2000). However, different pollen diets containing the same protein content could have very different nutritional qualities for bees if they lack or are deficient in essential amino acids (Standifer, 1967). Moreover, pollens could be inferior diets if they lack other key nutrients, but few studies to date have looked at the nutritional requirements of bees for other pollen constituents such as lipids, sterols, vitamins, or minerals (Vaudo *et al*., 2015). Pollen could also contain secondary metabolites, which could have toxic or harmful effects on bees. For example, queenless *B. terrestris* microcolonies produced fewer and smaller males when provided pollen treated with ecologically-relevant concentrations of the secondary metabolite D-lupanine, a quinolizidine alkaloid found in the pollen of *Lupinus* spp. (Fabaceae) (Arnold *et al*., 2014). Interestingly, pollen from other Asteraceae plant species (Senecioneae) has been found to contain pyrrolizidine alkaloids (Reinhard *et al*., 2009). Although adult honeybees did not experience any toxic effects from exposure to these compounds at ecologically relevant concentrations, Reinhard *et al*. (2009) proposed that these compounds could still pose a threat to the bee larvae. However, to my knowledge, it is still unknown whether sunflower pollen contains harmful secondary metabolites.

Digestibility is another factor that could influence the nutritive quality of a pollen for bees (Human *et al.*, 2007). For example, the thickness of the intine layer of pollen grains can affect pollen digestion by *Osmia* bees (Suárez-Cervera *et al.*, 1994). In addition, an experiment on

honeybees (*A. mellifera scutellata*) observed higher extraction efficiency in bees fed aloe pollen (*Aloe greatheadii* var. *davyana*; Asphodelaceae) than those fed sunflower pollen, potentially due to differences in pollen morphology (Human *et al.*, 2007). Aloe pollen grains are large and smooth, whereas sunflower pollen grains are smaller and covered in spines (echinate), which could impede digestion (Human *et al.*, 2007). In addition, the prominent oily coating (pollenkitt) of sunflower pollen must first be digested before the cytoplasm can be extracted; this might further reduce nutrient extraction efficiency (Human *et al.*, 2007). Consequently, it is possible that I observed a shorter bee lifespan in the sunflower pollen treatment due to nutritive factors other than the low amino acid content of this pollen treatment. These factors could include the absence or deficiency of other essential nutrients, the presence of harmful secondary metabolites, or pollen grain structure.

Pollen Amino Acid Content and Bee Nutrition

The relative amounts of amino acids (i.e., percentages of total amino acid content and of total essential amino acid content) were similar across the four pollen types used in this study. This finding is consistent with the results of Vanderplanck *et al.* (2014) and could indicate that the amino acid profile of pollen is generally conserved across plant species (but see Weiner *et al.*, 2010). The pollen types did differ in their absolute amounts of amino acids, but I was unable to detect significant differences in pairwise comparisons due to low sample size. Overall, sunflower pollen had both the lowest total amino acid content and the lowest essential amino acid content, which is consistent with past studies that have found sunflower (*Helianthus annuus*) pollen to be low in protein (honeybee-collected pollen: Kleinschmidt and Kondos, 1976; Pernal and Currie, 2000; honeybee- and hand-collected pollen: Nicolson and Human, 2013).

The pollen of some Asteraceae species has also been found to lack or be deficient in certain essential amino acids (e.g., *Taraxacum officinale*; reviewed by Roulston and Cane, 2000). In

addition, Nicolson and Human (2013) found both hand- and bee-collected sunflower pollen to be deficient in the essential amino acids methionine and tryptophan; however, other studies on bee-collected sunflower pollen have not found essential amino acid deficiencies (Rayner and Langridge, 1985; Somerville and Nicol, 2006—although the latter excluded tryptophan). I did not find sunflower pollen to be deficient in methionine, and none of the four pollen types met the 4% requirement for tryptophan recommended for honeybees (De Groot, 1953). Interestingly, tryptophan was found to be low in more than one third of the 142 plant species from more than 40 families sampled by Weiner *et al.* (2010). All four pollen types used in this study also failed to meet the 14% recommendation for isoleucine (De Groot, 1953). However, it is important to consider that the proportional amino acid requirements might differ slightly between honeybees and bumblebees. Kriesell *et al.* (2017) found low proportions of isoleucine in the pollen loads of seven European bumblebee species, which could indicate that bumblebees require proportionally less of this amino acid. The amino acid requirements for bumblebees still need to be determined and could differ among *Bombus* species (Moerman *et al.*, 2016a).

Overall, broad bean pollen had both the highest total and essential amino acid content, which is consistent with the results of previous studies that have found legume (Fabaceae) pollen to be particularly protein-rich (Hanley *et al.*, 2008) and an important pollen source for bumblebees (Goulson *et al.*, 2005). Although no larvae were produced during my study, two of the four microcolonies that laid eggs were provided broad bean pollen, and another microcolony that produced eggs was given 0% sunflower mixed pollen, which was one-third broad bean pollen. The microcolonies provided broad bean pollen were also significantly more likely to make honeypots, a normal colony behaviour, than those in the mixed sunflower pollen treatments, and

marginally significantly more likely to make honeypots than those in the sunflower pollen treatment.

It is unclear why so few microcolonies produced eggs and the few microcolonies that did lay eggs later destroyed them. Egg-eating or oophagy can occur amongst workers that are competing for reproduction (Michener, 1969), and it might also occur if workers lay non-viable eggs—something that has been observed in other social bee species such as stingless bees (*Trigona* (*Tetragonisca*) *angustula*; Koedam *et al*., 1996). Oophagy may also be a response to a lack of food or poor resource quality, as it allows bees to recycle nutrients for future egg production (mentioned by Génissel *et al.*, 2002). However, I do not believe the microcolonies in this study were food-stressed, since sugar solution was provided ad libitum and pollen was replaced frequently enough that no microcolony was ever devoid of this resource. In addition, rapeseed pollen is a suitable diet for microcolony brood production by *B. terrestris* workers (Regali and Rasmont, 1995), and Fabaceae pollen (e.g., broad bean) is high in protein and an important pollen source for many bumblebee species (Goulson *et al.*, 2005). *Bombus terrestris* microcolonies can even rear brood on sunflower pollen, though fewer and smaller males were produced on this diet than on other pollen diets (Regali and Rasmont, 1995). This difference in performance between the two bumblebee species could reflect species-specific requirements for brood production. Gradish *et al.* (2013) also observed interspecific differences between these species, and few males were produced by *B. impatiens* microcolonies under the experimental conditions designed for *B. terrestris*.

Advantages of a Mixed Pollen Diet

In general, bumblebees are broadly polylectic (dietary generalist) species that can collect pollen from multiple plant species while foraging (Leonhardt and Blüthgen, 2012; Somme *et al.*, 2015). Mixing pollen from different plant species might allow bumblebees to exploit low-quality

resources if the mixed diet compensates for nutrient deficiencies and/or reduces the toxicity of the monofloral diet (Eckhardt *et al.*, 2014). I observed that bees provided sunflower pollen did not live as long as bees in the other pollen treatments. However, a mixed pollen diet containing 50% sunflower pollen did not reduce worker survival, potentially due to the presence of the other pollens in the diet which may have mitigated any harmful effects of the sunflower pollen. Other studies have also observed better bee performance on a mixed pollen diet than a monofloral diet of comparable protein content. For example, Baloglu and Gurel (2015) found that a mixed pollen diet containing equal amounts of *Cistus* spp. (Cistaceae), *Papaver somniferum* (Papaveraceae), and *Sinapsis* sp. (Brassicaceae) was a superior pollen diet for queenright *B. terrestris* colonies than *P. somniferum* pollen alone, even though the monofloral diet had a higher protein content than the polyfloral diet (21.4% and 18.5%, respectively). However, it is important to consider that monofloral diets can sometimes be as good as, if not better than, mixed diets for colony performance (Moerman *et al.*, 2017). For example, larger larvae were produced by microcolonies fed monofloral diets of *Cytisus scoparius* and *Sorbus aucuparia* pollen, potentially due to the higher amino acid content and more favourable sterol profiles of these pollens, compared to microcolonies given difloral diets containing *Erica* sp. pollen, which has a lower amino acid content and contains the harmful sterol δ7-avenasterol (Moerman *et al.*, 2017). Bumblebees also do not mix pollen randomly but have been found to prefer pollens with a higher protein content (Goulson *et al.*, 2005; Hanley *et al.*, 2008; Kitaoka and Nieh, 2009, Leonhardt and Blüthgen, 2012) or a more favourable protein to lipid ratio (Vaudo *et al.*, 2016a,b).

Conclusion and Implications

In this study, *B. impatiens* workers fed 100% sunflower pollen lived approximately 19.5 days (median lifespan), which was on average 7 days less than bees in the broad bean (26.5 days), Cucurbitaceae (26 days), and rapeseed (27 days) pollen treatments but comparable to bees in the

25% (19.5 days) and 0% mixed treatments (20 days). Bees provided a mixed diet containing 50% sunflower pollen lived approximately 10 days longer than bees in the 100% sunflower treatment, which suggests that the other pollens were able to compensate for the low nutritive quality of the sunflower pollen. Part of the poor quality of the sunflower pollen may be a consequence of the low concentration of amino acids, particularly the low essential amino acid content. However, other pollen constituents such as other macronutrients, micronutrients, and secondary metabolites, as well as the digestibility and nutrient assimilation from the pollen grains, might have also contributed to the poorer quality of this pollen.

Although I did observe improved survival of bees offered sunflower mix pollen diets (relative to the 100% sunflower diet), additional trials with a greater variety of sunflower mixed diets (potentially with the addition of other pollen species) will need to be conducted to determine what proportion of sunflower pollen is acceptable—particularly considering the observed discrepancies in performance between the 25% and 50% sunflower mixed pollen treatments as well as the relatively low performance on the 0% mixed treatment.

Host selection by bees is a multifaceted process that is influenced not only by reward quality but also by the spatiotemporal availability of floral resources and the accessibility of floral rewards. As a consequence of agricultural intensification and a loss of wildflowers, farmland monocultures can be important floral resources for bumblebees (Westphal *et al.*, 2003). However, a monotonous diet could have adverse effects on bumblebee colony health, particularly if the floral rewards of the crop are of low nutritional value. Schmidt *et al.* (1995) noted that commercial honeybee colonies established on or near sunflower crops, with little access to other floral resources during sunflower bloom, might experience both colony stress and a shorter worker lifespan, which could in turn reduce pollination services. My results suggest that

providing alternative floral resources of high nutritive quality could help mitigate the potential harmful effects of a monofloral diet of sunflower pollen on bumblebees. These alternative resources could be provided as hedgerows (Morandin and Kremen, 2013) or wildflower plantings along field margins (Williams *et al.*, 2015) that ideally sustain flowering throughout the growing season. To support bee populations, we need to improve our understanding of both the nutritional requirements of different bee species and how the quality of the available floral resources affects colony health (Vaudo *et al*., 2015). This knowledge could then be integrated into management strategies to support bee populations by providing foraging habitats with high-quality resources.

Tables

Table 3.1. Pollen treatment allocation for the 43 microcolonies (MC) of *B. impatiens* used in the experiment. Each microcolony was exposed to one of seven pollen diets, which included 0%, 25%, and 50% sunflower mixed pollen (SFM) (97% *Helianthus annuus* and 3% other Asteraceae species [*Rudbeckia* sp., *Coreopsis* sp., and *Heliopsis* sp.]) with an equal mixture of broad bean (*Vicia faba*), Cucurbitaceae (30% summer squash [*Cucurbita pepo*] and 70% watermelon [*Citrullus lanatus*]), and rapeseed (*Brassica napus*) making up the rest of the diet. The remaining four diets contained only one kind of pollen (broad bean, Cucurbitaceae, rapeseed, or sunflower). There was no pollen consumption in 10 of the microcolonies (~23%) included in the experiment. The numbers of bees in microcolonies that did and did not consume pollen are in brackets. Bees that died before pollen consumption are included with the microcolonies with no pollen consumption.

	Pollen Treatments							**Total MC**
	0% SFM	**25% SFM**	**50% SFM**	**Broad Bean**	**Cucurbitaceae**	**Rapeseed**	**Sunflower**	
MC with pollen consumption	4 (21)	6 (28)	4 (20)	5 (28)	5 (25)	5 (27)	4 (20)	**33 (169)**
MC with no pollen consumption	1 (8)	0 (4)	2 (11)	1 (6)	2 (14)	2 (14)	2 (11)	**10 (68)**
Total MC	5 (29)	6 (32)	6 (31)	6 (34)	7 (39)	7 (41)	6 (31)	**43 (237)**

Table 3.2. Cox PH ratios for bee survival across the seven pollen treatments (0% sunflower mixed (SFM), 25% SFM, 50% SFM, broad bean, Cucurbitaceae, rapeseed, and sunflower). Sample size is included in brackets. Bees that did and did not consume pollen were included in this analysis. Results of analyses that omitted outliers and bees that did not consume pollen are provided in Appendix F. Hazard ratios less than one indicate higher chances of survival, and values greater than one indicate reduced chances of survival, relative to the reference pollen treatment. Sunflower pollen significantly reduced survival compared to all other pollen treatments except 25% SFM pollen.

Model 1: All bees included

	Reference pollen treatment						
	0% SFM	**25% SFM**	**50% SFM**	**Broad bean**	**Cucurbitaceae**	**Rapeseed**	**Sunflower**
0% SFM (29)		0.78	1.25	1.65	1.10	1.23	**0.44 ****
25% SFM 32)	1.28		1.61	**2.12 .**[1]	1.41	1.57	0.57
50% SFM (31)	0.80	0.62		1.32	0.88	0.98	**0.35 ****
Broad bean (34)	0.61	**0.47 .**	0.76		0.67	0.74	**0.27 ****
Cucurbitaceae (39)	0.91	0.71	1.14	1.50		1.11	**0.40 ****
Rapeseed (41)	0.82	0.64	1.02	1.35	0.90		**0.36 ****
Sunflower (31)	**2.26 ****	1.76	**2.82 ****	**3.73 ****	**2.49 ****	**2.76 ****	

[1]. $0.05 < P < 0.075$

* $P < 0.05$

** $P < 0.01$

Table 3.3. Cox PH ratios for microcolony honeypot construction across the seven pollen treatments (0% sunflower mixed (SFM) (n = 5 microcolonies), 25% SFM (6), 50% SFM (6), broad bean (6), Cucurbitaceae (7), rapeseed (7), and sunflower (6)). All microcolonies were included in this analysis. Hazard ratios less than one indicate reduced chances of honeypot construction, and values greater than one indicate greater chances of honeypot construction, relative to the reference pollen treatment. Microcolonies provided broad bean pollen were significantly more likely to construct honeypots than the 25% and 50% SFM pollen treatments, and marginally significantly more likely to make honeypots than those in the 100% sunflower pollen treatment.

	Honeypot construction						
	Reference pollen treatment						
	0% SFM	**25% SFM**	**50% SFM**	**Broad bean**	**Cucurbitaceae**	**Rapeseed**	**Sunflower**
0% SFM (5)		4.91	2.53	0.51	1.71	1.50	2.08
25% SFM (6)	**0.20 .**[1]		0.51	**0.10 ***	0.35	0.31	0.42
50% SFM (6)	0.40	1.94		**0.20 ***	0.68	0.60	0.82
Broad bean (6)	1.98	**9.72 ***	**5.00 ***		3.39	2.98	**4.11 .**
Cucurbitaceae (7)	0.58	2.87	1.48	0.30		0.88	1.21
Rapeseed (7)	0.66	3.26	1.68	0.34	1.14		1.38
Sunflower (6)	0.48	2.36	1.22	**0.24 .**	0.82	0.72	

[1]. $0.05 < P < 0.075$
* $P < 0.05$
** $P < 0.01$

Table 3.4. Mean ± SEM for total amino acid (AA), essential amino acid (EAA) and percent free amino acid content (% FAA) for the four pollen types (broad bean (*Vicia faba*), Cucurbitaceae (30% summer squash [*Cucurbita pepo*] and 70% watermelon [*Citrullus lanatus*]), rapeseed (*Brassica napus*), and sunflower (97% *Helianthus annuus* and 3% other Asteraceae species [*Rudbeckia* sp., *Coreopsis* sp., and *Heliopsis* sp.]).

Pollen Treatments	AA (µg/mg of sample)	EAA (µg/mg of sample)	% FAA[1]
Broad Bean	420.89 ±16.28	216.29 ± 4.20	6.06 ± 0.41
Cucurbitaceae	255.99 ± 12.51	129.47 ± 2.25	6.43 ± 0.27
Rapeseed	350.45 ± 3.27	180.72 ± 1.91	4.50 ± 0.24
Sunflower	249.61 ± 3.56	127.56 ± 1.33	3.49 ± 0.21

[1]The proportion of free tryptophan could not be determined since this amino acid was analyzed in a separate analysis which did not distinguish between protein-bound and free amino acid. As a result, this amino acid was omitted for the % FAA calculation.

Table 3.5. Dufrene-Legendre indicator values (indval) are presented for the pollen type with the highest proportional amount of each amino acid (essential amino acids in bold). Indicator values can range from 0 to 1, and higher values indicate that an amino acid made up a larger proportion of the total amino acid content of a specific pollen type and/or that an amino acid was only found in certain pollen types. The four pollen types included broad bean, Cucurbitaceae, rapeseed, and sunflower. All of the amino acids were present in the different pollen types, and no significant differences among pollen types in the amino acid profiles were detected after adjusting for multiple comparisons.

Pollen treatment	**Amino acid**	**indval**	***P***	**Adjusted *P*** [1]
Broad bean	Asparagine + Aspartic acid	0.258	0.29	1.00
	Valine	0.258	0.022	0.40
	Isoleucine	0.256	0.56	1.00
Cucurbitaceae	**Methionine**	0.271	0.017	0.31
	Glycine	0.263	0.23	1.00
	Glutamine + Glutamic acid	0.256	0.65	1.00
	Histidine	0.253	0.80	1.00
Rapeseed	**Tryptophan**	0.279	0.31	1.00
	Proline	0.263	0.17	1.00
	Tyrosine	0.263	0.014	0.25
	Threonine	0.260	0.14	1.00
	Alanine	0.259	0.036	0.65
	Phenylalanine	0.253	0.98	1.00
Sunflower	Alpha-aminobutyric acid	0.297	0.025	0.45
	Arginine	0.267	0.016	0.29
	Serine	0.265	0.058	1.00
	Lysine	0.264	0.81	1.00
	Leucine	0.255	0.60	1.00

[1]*P* values were adjusted for multiple comparisons using a Bonferroni correction.

Figures

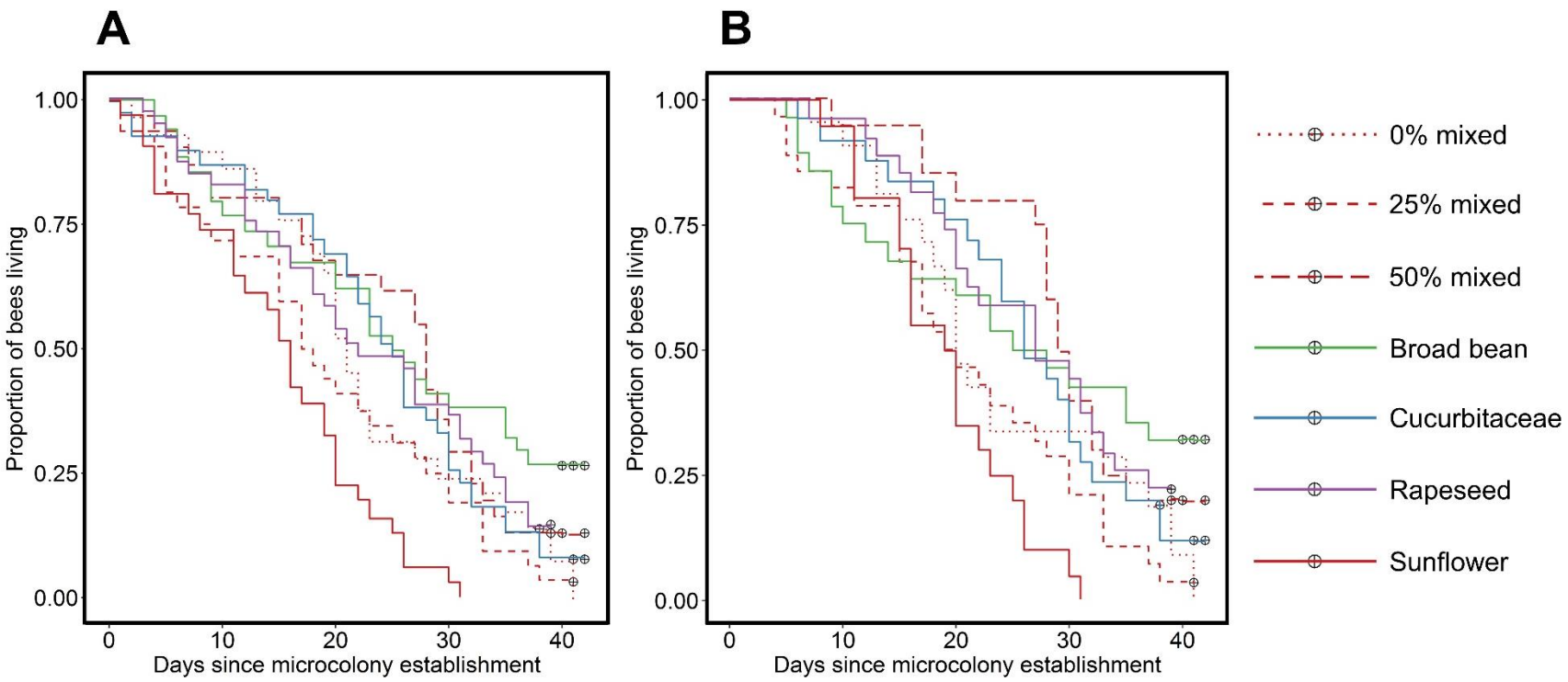

Figure 3.1. Bee survival over time on the seven pollen treatments visualized using the 'survfit' function of the 'survival' package. Panel (A) contains all bees and (B) contains only the bees from microcolonies with pollen consumption. The seven pollen diets included 0% sunflower mixed (SFM) pollen diet (A: n = 29 bees; B: n = 21 bees), 25% SFM (33; 27), and 50% SFM (31; 20), broad bean (34; 28), Cucurbitaceae (39; 25), rapeseed (41; 27), and sunflower (31; 20). Survival was significantly reduced on sunflower pollen relative to all pollen treatments except 25% SFM pollen. Bees were censored at the end of the 6 weeks (42 days) (⊕ symbol on curves). Results were jittered both vertically and horizontally to reduce overlap between the different pollen treatments.

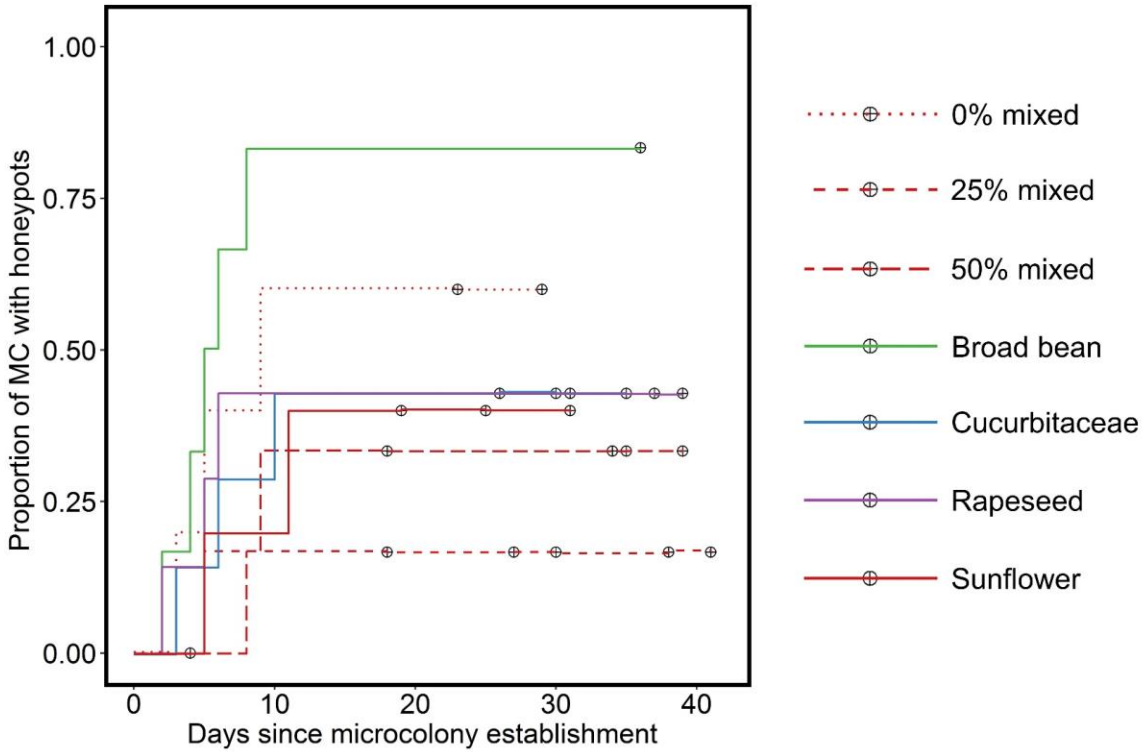

Figure 3.2. Honeypot construction over time on the seven pollen treatments visualized using the 'survfit' function of the 'survival' package. All microcolonies were included in this analysis. The seven pollen diets included 0% sunflower mixed (SFM) pollen diet (n = 5 microcolonies), 25% SFM (6), and 50% SFM (6), broad bean (6), Cucurbitaceae (7), rapeseed (7), and sunflower pollen (6). Microcolonies provided broad bean pollen were significantly more likely to construct honeypots than the 25% and 50% SFM pollen treatments, and marginally significantly more likely to make honeypots than those in the 100% sunflower pollen treatment. Microcolonies were censored at the end of the 6 weeks (42 days) (⊕ symbol on curves). Results were jittered both vertically and horizontally to reduce overlap between the different pollen treatments.

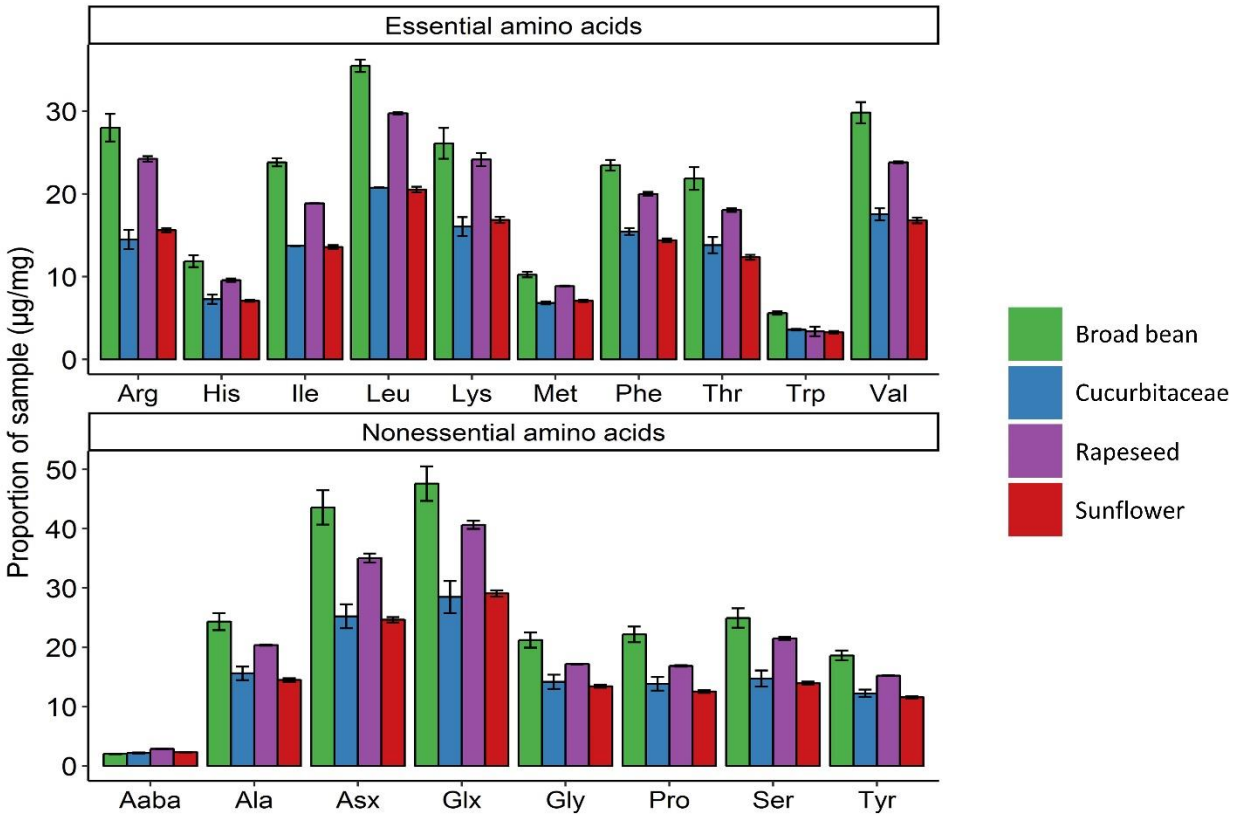

Figure 3.3. The mean ± SEM of essential amino acids (EAA) and nonessential amino acids (NAA) (including both protein-bound and free amino acids) in the four pollen types (broad bean (*Vicia faba*), Cucurbitaceae (30% summer squash [*Cucurbita pepo*] and 70% watermelon [*Citrullus lanatus*]), rapeseed (*Brassica napus*), and sunflower (97% *Helianthus annuus* and 3% other Asteraceae species [*Rudbeckia* sp., *Coreopsis* sp., and *Heliopsis* sp.]); n=3) as a proportion of the dry mass of the sample (µg/mg of sample). (EAA: Arg = arginine, His = histidine, Ile = isoleucine, Leu = leucine, Lys = lysine, Met = methionine, Phe = phenylalanine, Thr = threonine, Trp = tryptophan, Val = valine; NAA: Aaba = alpha-aminobutyric acid, Ala = alanine, Asx = asparagine + aspartic acid, Glx = glutamine + glutamic acid, Gly= glycine, Pro = proline, Ser = serine and Tyr = tyrosine)

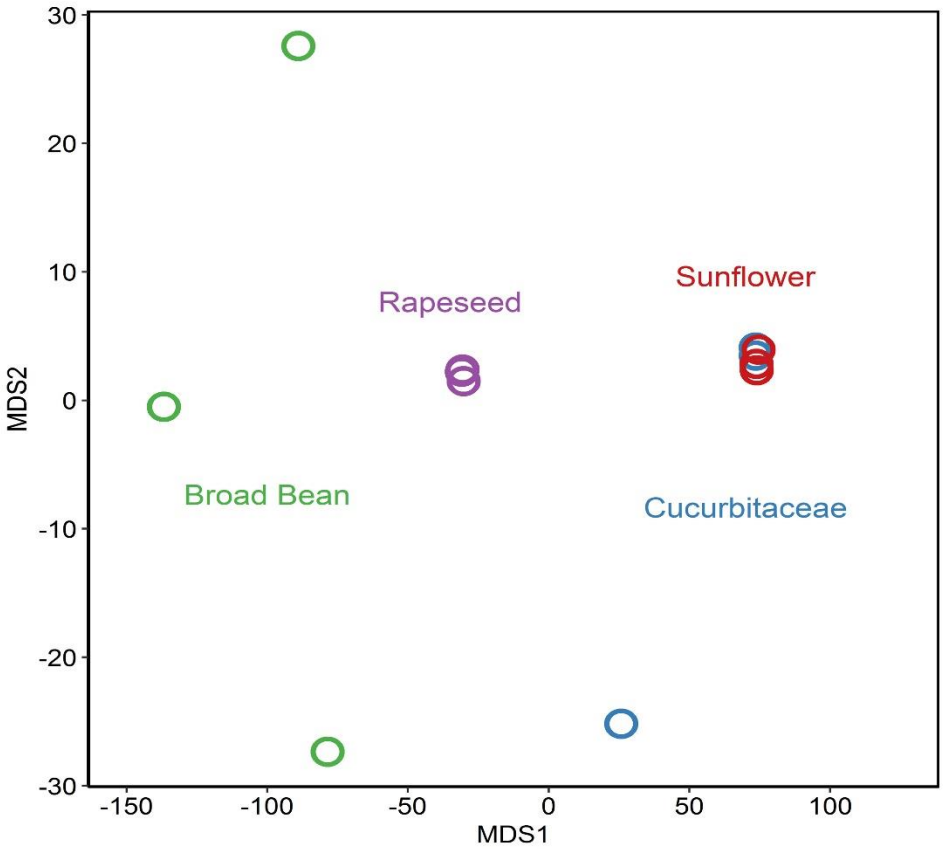

Figure 3.4. nMDS ordination plot based on Manhattan dissimilarity indices for the amino acid profiles of the four pollen types (broad bean (*Vicia faba*), Cucurbitaceae (30% summer squash [*Cucurbita pepo*] and 70% watermelon [*Citrullus lanatus*]), rapeseed (*Brassica napus*), and sunflower (97% *Helianthus annuus* and 3% other Asteraceae species [*Rudbeckia* sp., *Coreopsis* sp., and *Heliopsis* sp.]); n=3). The indices were calculated from absolute values (µg/mg of dried sample) for essential and nonessential amino acids and included both protein-bound and free amino acids. Results were jittered (± 100% data resolution for both vertical and horizontal jitter) to better illustrate overlapping data points.

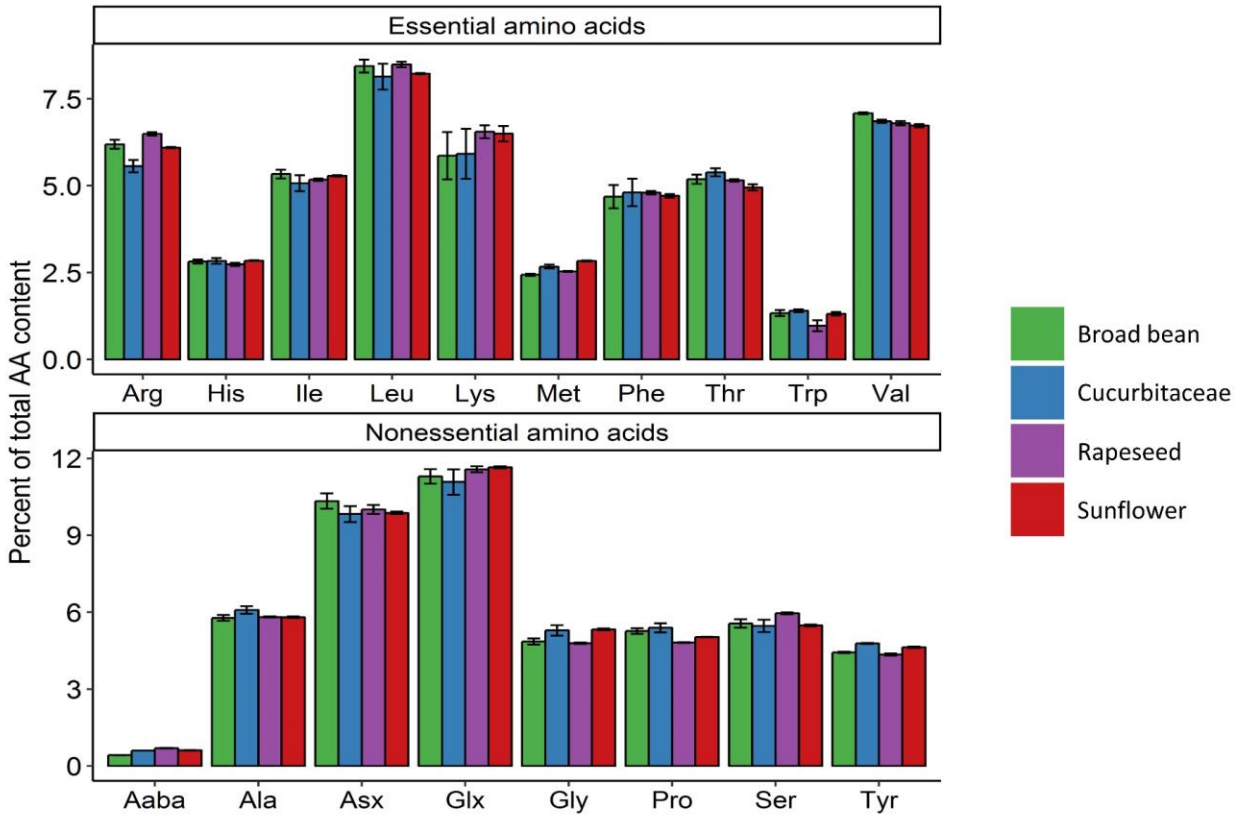

Figure 3.5. The mean percent ± SEM of essential amino acids (EAA) and nonessential amino acids (NAA) (including both protein-bound and free amino acids) in the four pollen types (broad bean (*Vicia faba*), Cucurbitaceae (30% summer squash [*Cucurbita pepo*] and 70% watermelon [*Citrullus lanatus*]), rapeseed (*Brassica napus*), and sunflower (97% *Helianthus annuus* and 3% other Asteraceae species [*Rudbeckia* sp., *Coreopsis* sp., and *Heliopsis* sp.]); n=3) as a proportion of the total amino acid content. (EAA: Arg = arginine, His = histidine, Ile = isoleucine, Leu = leucine, Lys = lysine, Met = methionine, Phe = phenylalanine, Thr = threonine, Trp = tryptophan, Val = valine; NAA: Aaba = alpha-aminobutyric acid, Ala = alanine, Asx = asparagine + aspartic acid, Glx = glutamine + glutamic acid, Gly= glycine, Pro = proline, Ser = serine and Tyr = tyrosine)

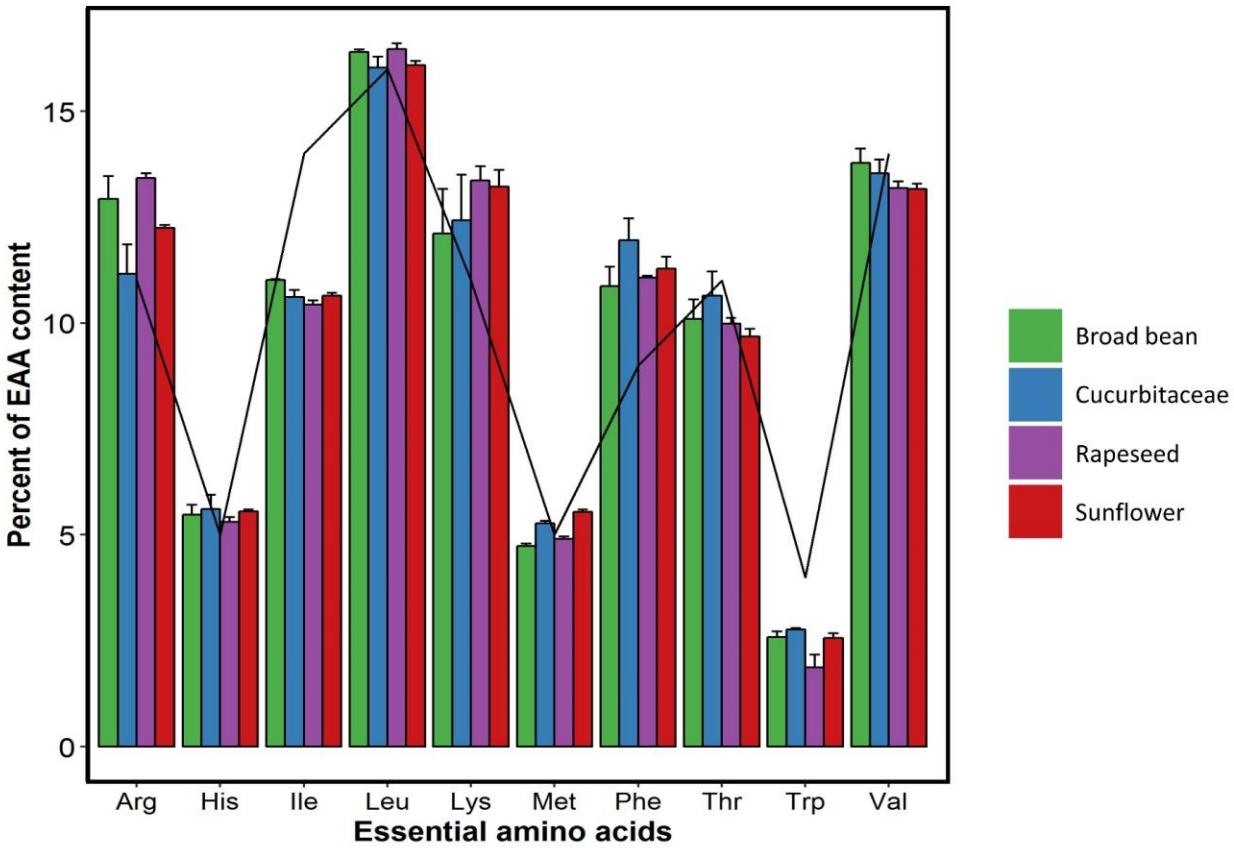

Figure 3.6. The mean percent + SEM of essential amino acids (EAA) (including both protein-bound and free amino acids) in the four pollen types (broad bean (*Vicia faba*), Cucurbitaceae (30% summer squash [*Cucurbita pepo*] and 70% watermelon [*Citrullus lanatus*]), rapeseed (*Brassica napus*), and sunflower (97% *Helianthus annuus* and 3% other Asteraceae species [*Rudbeckia* sp., *Coreopsis* sp., and *Heliopsis* sp.]); n=3) as a proportion of the total EAA content. The black line represents the EAA requirements for *Apis mellifera* described by De Groot (1953). (Arg = arginine, His = histidine, Ile = isoleucine, Leu = leucine, Lys = lysine, Met = methionine, Phe = phenylalanine, Thr = threonine, Trp = tryptophan, Val = valine)

Chapter 4

General Conclusion

In my thesis, I demonstrate that both pollen specialization and pollen mixing could be effective strategies to exploit low-quality pollen resources. In my first experiment (Chapter 2), I found that the Aster specialists could develop on both host and nonhost provisions. However, Aster provisions were unsuitable larval diets for bee species that do not collect Asteraceae pollen (i.e., *Osmia iridis* and *O. tristella*). These results indicate that the Aster specialists are physiologically adapted to the chemistry of their host pollen, but have not lost the ability to tolerate other pollens. *Osmia iridis* (a Fabaceae specialist) may also be physiologically adapted to its host pollen, but this species could still survive on *O. tristella* provisions (which contain a mix of pollen species), despite its poor performance on Aster provisions. My findings are inconsistent with the physiological-efficiency hypothesis but are consistent with past studies on Asteraceae-specialist bees (e.g., Williams, 2003; Praz *et al.*, 2008a). They are also consistent with most studies conducted on species from multiple insect orders (reviewed by Jaenike, 1990), which have not observed an association between increased performance on hosts and decreased performance on nonhosts (reviewed by Joshi and Thompson, 1995; but see Via, 1991). In addition, my results provide mixed support for the preference–performance hypothesis, since the non-Aster specialist bees could not tolerate Asteraceae provisions, which is consistent with the hypothesis. However, the Aster-specialists could survive and develop on nonhost provisions, and certain species even appeared to develop faster on nonhost provisions—though the small sample size means that further research will be required.

In Chapter 2, I found that the Aster provisions were not deficient in amino acids, and that Aster specialists could digest their host pollen as efficiently as the generalist species, *O. tristella*. Deficiencies in other essential nutrients or the presence of toxic compounds could be the reason the Aster provisions were a low-quality larval diet for *O. iridis* and *O. tristella*. It is also possible that these species were unable to digest and assimilate nutrients from Aster provisions; however, I was unable to assess their digestive efficiency since the larvae died before reaching the defecating stage. In the future, it would be ideal if larval digestive efficiency for these two species could be assessed by examining serial sections of the midgut and hindgut (e.g., Peng *et al.*, 1985; Dobson and Peng, 1997).

In Chapter 3, I investigated how the proportion of sunflower pollen in the diet affects the survival of bumblebee microcolonies. Previous studies have demonstrated that sunflower pollen negatively affects both honeybees and bumblebees. Mixing pollen could be a strategy for broadly polylectic species to utilize unfavourable pollens while maintaining diet quality through nutrient complementation and the dilution of harmful secondary metabolites. I found that worker lifespan for bees fed sunflower pollen was shorter than that of bees fed broad bean, rapeseed, or Cucurbitaceae pollen. Sunflower pollen had both the lowest total amino acid content and the lowest essential amino acid content of any tested pollen type. However, the proportions of different amino acids (i.e., percentages of total amino acid content and of total essential amino acid content) were similar across pollen types. Therefore, the shorter lifespan of bees fed sunflower pollen was most likely not due to the amino acid composition of this pollen type, since all pollen types had similar amino acid profiles, but might be partially explained by the lower total amino acid content. However, since the amino acid content of the Cucurbitaceae pollen treatment was only slightly higher than the 100% sunflower treatment, another nutritional factor

was probably also responsible for the poor performance of bees in the 100% sunflower pollen treatment.

Survival on mixed diets with 50% sunflower pollen was significantly higher than survival in the 100% sunflower pollen treatment and comparable to the broad bean, Cucurbitaceae, and rapeseed treatments. The enhanced performance of bees fed 50% sunflower mixed diet suggests that the addition of pollens fully mitigated the unfavourable qualities of the sunflower pollen. Similarly, multiple studies on dietary mixing in generalist herbivorous insects have reported better performance on mixed diets due to either nutrient complementation (Unsicker *et al*., 2008) or dilution of toxic compounds (Singer *et al.*, 2002), or a combination of the two (Bernays *et al.*, 1994). Mysteriously, however, in my experiment, survival was almost as low in mixed diets containing little to no sunflower as in the 100% sunflower treatment. The reasons for this finding remain unclear and require further experimentation.

A caveat of my microcolony experiment was that reproduction could not be compared amongst treatments since so few microcolonies produced eggs, and the few microcolonies that did lay eggs later destroyed them. It is possible to create queenless microcolonies by splitting queenright colonies instead of collecting newly emerged bees from pupal clumps (e.g., Tasei *et al*., 2000; Manson and Thomson, 2009). Arnold *et al.* (2014) had better success forming queenless *B. terrestris* microcolonies from split-colonies than worker-founded microcolonies with no brood. The split-colonies also tended to produce new brood more quickly. It would be ideal if a similar study could be conducted with split-colonies in order to test whether the mixed sunflower diets not only increase worker longevity but also improve reproduction compared to the 100% sunflower pollen treatment.

Pollen is evidently not an easy resource for bees to utilize, and it can exhibit a variety of unfavourable properties including nutrient deficiencies, barriers to digestion, and even the presence of toxins. Both specialist and generalist bees appear to be able to exploit low-quality pollens by either adapting to tolerate unfavourable pollen properties (in the former case) or overcoming the neurological and cognitive challenges of manipulating different floral species (in the latter). As a result, floral specialization and pollen mixing could be viewed as alternative strategies to exploit pollens of variable quality. The former could require physiological specialization to the pollen chemistry of a particular host, potentially at the cost of a reduced ability to exploit other pollens, though my results provide little support for this hypothesized cost. The second strategy requires both the neurological capacity to recognize as well as the cognitive ability to handle flowers with different morphologies. Integrative studies comparing phylogenetic relationships of bees and patterns of host-plant use will provide further insight on the evolution of different foraging strategies. Future preference assays, consisting of feeding studies paired with host-selection experiments, are needed to improve our understanding of the potential host range and nutritional requirements of different bee species. This information could improve pollinator management practices and help predict future host shifts as habitat destruction and agriculture intensification continue to alter host-plant availability.

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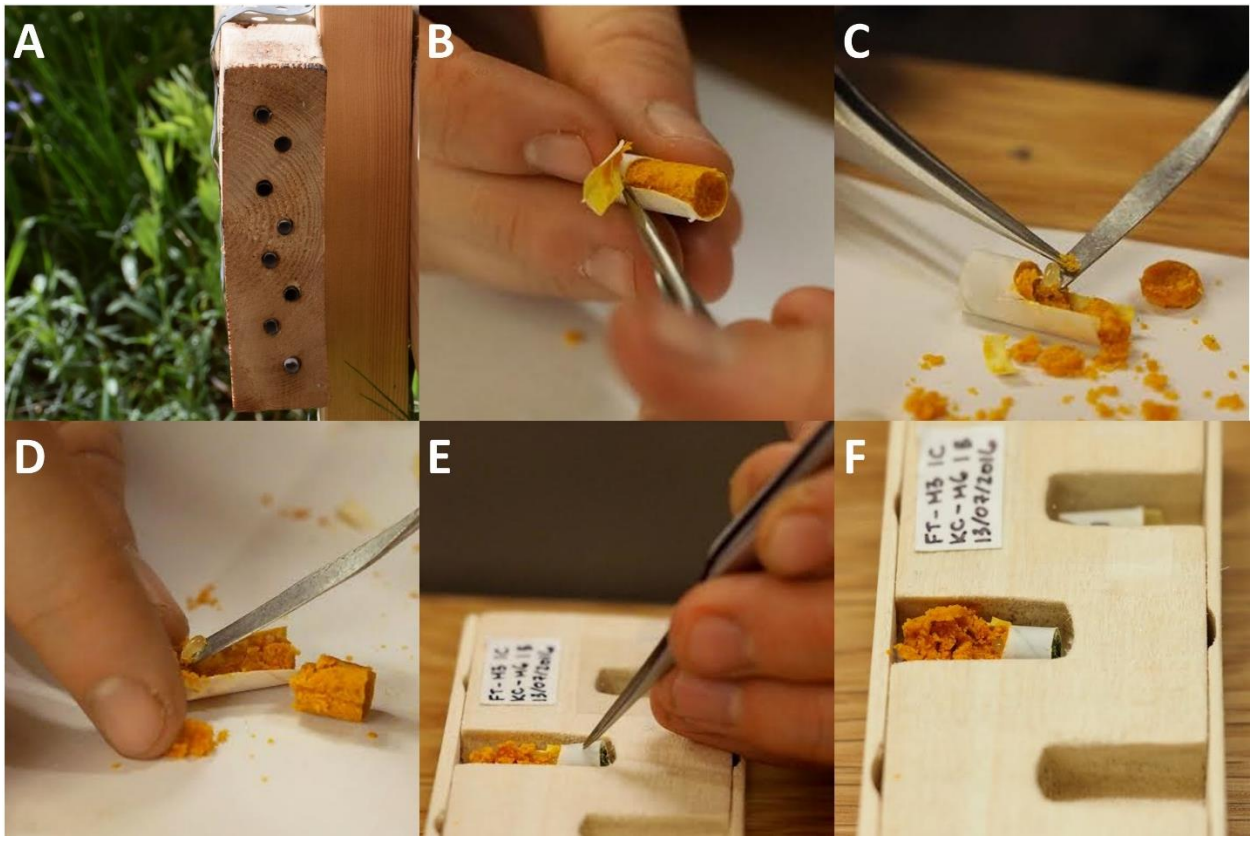
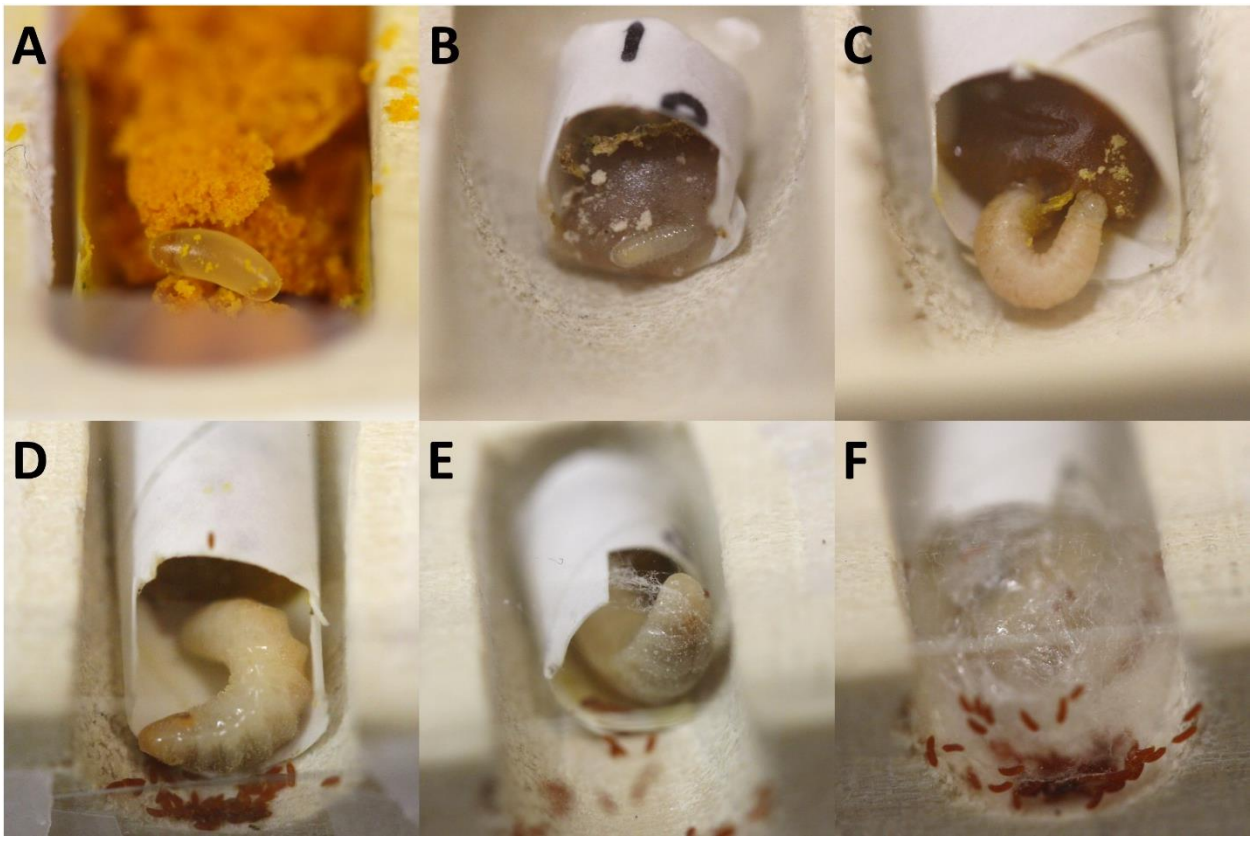
Appendix A



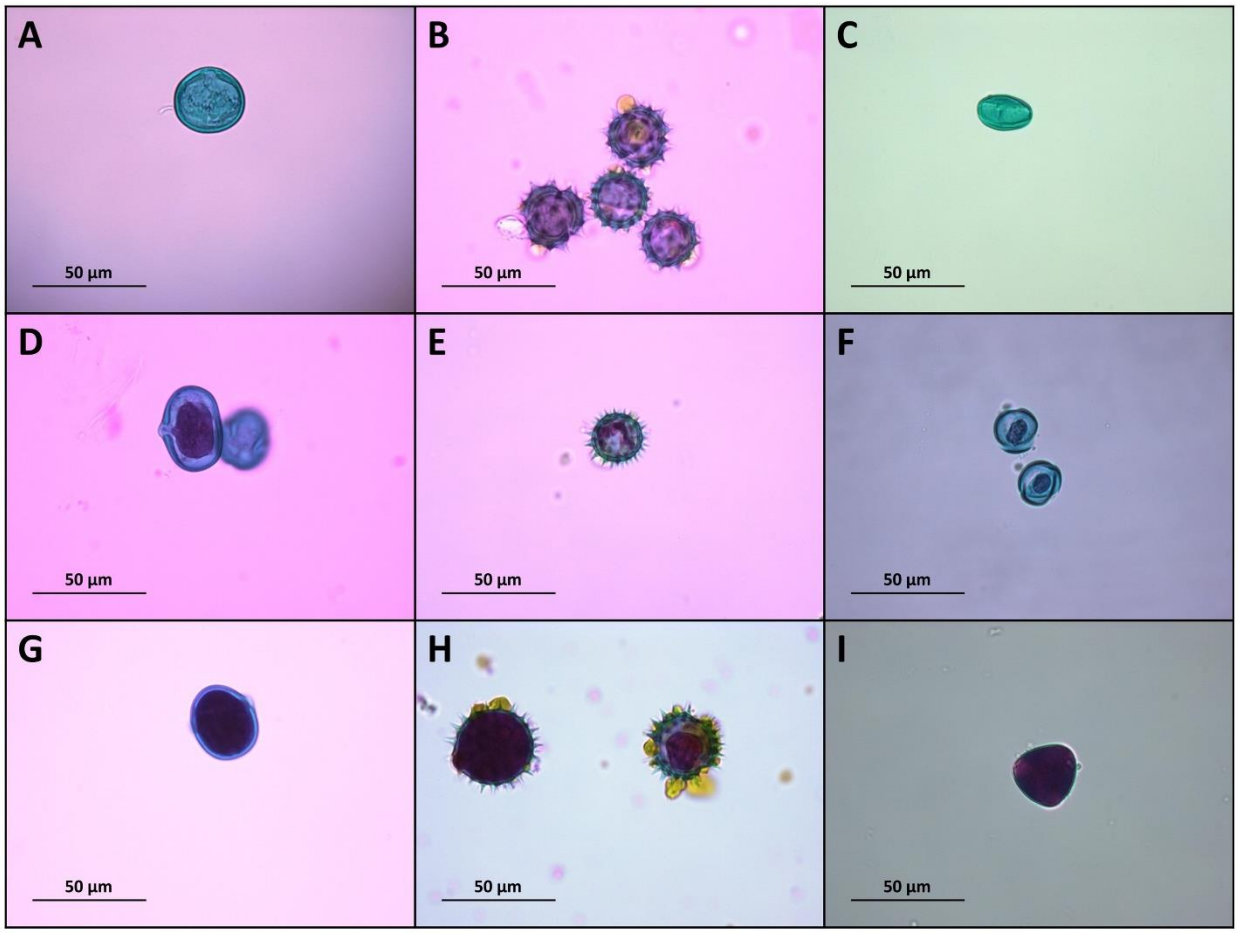
Photos of the egg transfer method. A) Completed straws were collected from trapnests near the Rocky Mountain Biological Laboratory in Colorado, USA; B) nest cells were separated using a scalpel and microscissors; C) forceps and a metal spatula were used to extract eggs; D) eggs were placed on novel pollen provisions; E) pollen provisions were secured in wells of the wood blocks; and F) wood blocks were stored in a growth chamber on a 10°C to 25°C ramping diurnal cycle (dark). Credit: Shang-Yao Peter Lin (photos B, C, D, E, and F)

Appendix B



Photos of bee developmental stages. A) *Osmia montana* egg on an *O. montana* pollen provision; B) early-stage *O. tristella* larva feeding on an *O. tristella* pollen provision (gut contents are visible in this image); C) *O. subaustralis* larva feeding on an *O. iridis* pollen provision; D) defecating *O. iridis* larva on an *O. iridis* provision; E) spinning *O. iridis* larva on an *O. iridis* provision; and F) *O. iridis* cocoon after larva consumed *O. iridis* pollen provision.

Appendix C



Photos of digested pollen grains from 2016. Pollen grains were scored as empty (<25% protoplasm), partially-empty (25–50%), partially-full (50–80%), and full (>80%). Fabaceae (Fabeae) pollen grains: A) empty, D) partially-full, G) full; Asteraceae pollen grains: B) empty, E) partially-empty, H) pollen grains >50% full; Plantaginaceae (*Penstemon*) pollen grains: C) empty, F) partially-empty, I) full.

Appendix D

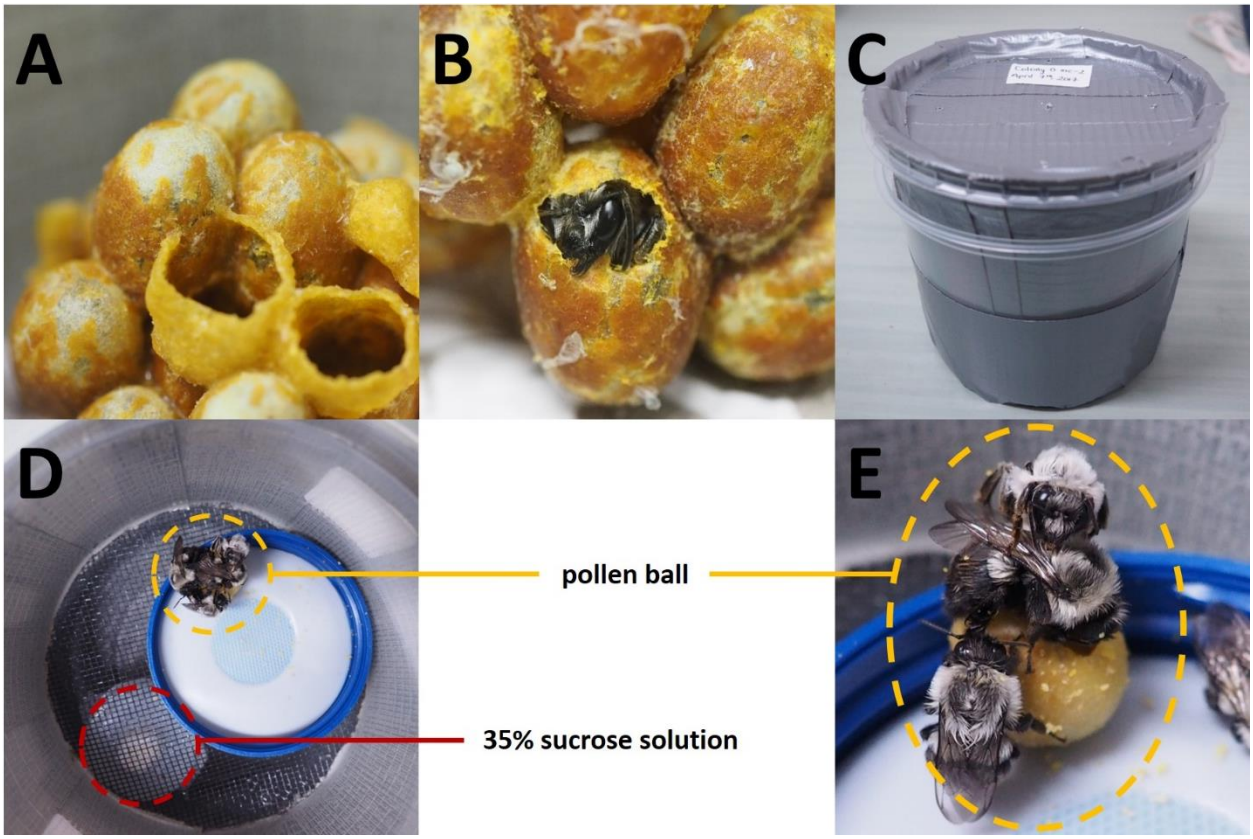
Amino acid analysis results for the pollen provisions of the six bee species (*Osmia coloradensis* (n = 1 sample), *O. iridis* (3), *O. montana* (3), *O. subaustralis* (3), *O. tersula* (1), and *O. tristella* (1)). Mean ± SEM are presented for concentration of bound plus free amino acids (µg/mg dry sample), percent of total amino acid (AA) content (with percent of total essential amino acids (EAA) in brackets), and percent of the total AA content made up of free amino acids (FAA) for each pollen type. Essential amino acids are in bold.

Amino acids	**Bee species**					
	O. coloradensis			***O. iridis***		
	Concentration	**Percent**	**% FAA**	**Concentration**	**Percent**	**% FAA**
α aminobutyric acid	1.01	0.83	0.11	0.85 ± 0.081	0.67 ± 0.029	0.075 ± 0.0062
Alanine	6.44	5.29	0.30	6.55 ± 0.63	5.14 ± 0.23	0.21 ± 0.018
Arginine	**8.10**	**6.65 (15.56)**	**0.71**	**7.28 ± 0.64**	**5.72 ± 0.20 (12.85 ± 0.76)**	**0.025 ± 0.0061**
Asparagine + Aspartic acid	8.75	7.18	0.24	9.80 ± 0.90	7.69 ± 0.30	0.13 ± 0.0056
Glutamine + Glutamic acid	10.82	8.89	0.074	14.22 ± 1.32	11.17 ± 0.45	0.31 ± 0.017
Glycine	6.25	5.13	0.026	5.66 ± 0.55	4.44 ± 0.20	0.033 ± 0.0025
Histidine	**5.34**	**4.38 (10.26)**	**0.75**	**2.84 ± 0.26**	**2.23 ± 0.087 (5.00 ± 0.31)**	**0.037 ± 0.0070**
Isoleucine	**4.55**	**3.73 (8.74)**	**0.15**	**5.75 ± 0.098**	**4.54 ± 0.15 (10.18 ± 0.095)**	**0.12 ± 0.0055**
Leucine	**6.57**	**5.39 (12.62)**	**0.11**	**8.52± 0.20**	**6.73 ± 0.18 (15.08 ± 0.051)**	**0.0052 ± 0.0052**
Lysine	**7.03**	**5.77 (13.51)**	**0.14**	**7.44± 0.19**	**5.91 ± 0.43 (13.22 ± 0.70)**	**0.044 ± 0.0051**
Methionine	**2.51**	**2.06 (4.82)**	**0.018**	**2.80 ± 0.028**	**2.22 ± 0.097 (4.96 ± 0.099)**	**0.011 ± 0.0053**
Phenylalanine	**6.56**	**5.39 (12.61)**	**1.50**	**8.02 ± 0.17**	**6.37 ± 0.44 (14.24 ± 0.67)**	**1.26 ± 0.063**
Proline	26.31	21.60	7.53	22.45 ± 0.87	17.73 ± 0.35	6.15 ± 0.41
Serine	5.88	4.83	0.090	6.36 ± 0.61	5.00 ± 0.21	0.064 ± 0.0054
Threonine	**4.65**	**3.81 (8.92)**	**0.056**	**4.74 ± 0.36**	**3.73 ± 0.091 (8.38 ± 0.41)**	**0.025 ± 0.00034**
Tryptophan	**1.17**	**0.96 (2.24)**	**NA**	**1.87 ± 0.0061**	**1.48 ± 0.073 (3.31 ± 0.087)**	**NA**
Tyrosine	4.30	3.53	0.29	4.48 ± 0.11	3.54 ± 0.10	0.22 ± 0.031
Valine	**5.59**	**4.58 (10.73)**	**0.10**	**7.22 ± 0.29**	**5.69 ± 0.074 (12.77 ± 0.18)**	**0.13 ± 0.012**
AA Sum	121.83	100	12.19	126.85 ± 6.48	100	8.84 ± 0.47
EAA SUM	52.06	42.73	3.53	56.47 ± 1.50	44.63 ± 1.08	1.66 ± 0.064

Amino acids	Bee species					
	O. montana			*O. subaustralis*		
	Concentration	**Percent**	**% FAA**	**Concentration**	**Percent**	**% FAA**
α aminobutyric acid	1.00 ± 0.096	0.72 ± 0.018	0.054 ± 0.0058	1.14 ± 0.15	0.77 ± 0.040	0.060 ± 0.0029
Alanine	7.74 ± 1.02	5.52 ± 0.044	0.20 ± 0.021	9.07 ± 0.70	6.14 ± 0.20	0.22 ± 0.017
Arginine	**6.79 ± 1.01**	**4.82 ± 0.12 (10.063 ± 0.25)**	**0.028 ± 0.0057**	**9.00 ± 1.82**	**5.97 ± 0.76 (14.28 ± 2.25)**	**0.27 ± 0.16**
Asparagine + Aspartic acid	12.79 ± 1.65	9.13 ± 0.14	0.83 ± 0.17	12.89 ± 1.02	8.72 ± 0.049	0.45 ± 0.14
Glutamine + Glutamic acid	14.48 ± 2.64	10.20 ± 0.56	0.31 ± 0.030	15.37 ± 1.75	10.34 ± 0.43	0.14 ± 0.025
Glycine	7.28± 1.10	5.16 ± 0.14	0.015 ± 0.0019	8.62 ± 0.88	5.81 ± 0.22	0.021 ± 0.0023
Histidine	**8.22 ± 1.29**	**5.83 ± 0.24 (12.16 ± 0.49)**	**1.50 ± 0.11**	8.10 ± 0.71	**5.47 ± 0.097 (13.00 ± 0.60)**	**1.18 ± 0.093**
Isoleucine	**5.58 ± 0.14**	**4.08 ± 0.37 (8.51 ± 0.79)**	**0.11 ± 0.014**	**5.07 ± 0.045**	**3.47 ± 0.31 (8.20 ± 0.44)**	**0.13 ± 0.013**
Leucine	**8.89 ± 0.066**	**6.52 ± 0.70 (13.61 ± 1.47)**	**0.037 ± 0.0064**	**8.00 ± 0.27**	**5.51 ± 0.60 (12.97 ± 1.00)**	**0.061 ± 0.0071**
Lysine	**7.53 ± 0.50**	**5.57 ± 0.84 (11.63 ± 1.77)**	**0.15 ± 0.020**	**7.59 ± 0.093**	**5.21± 0.48 (12.29 ± 0.72)**	**0.18 ± 0.018**
Methionine	**2.93 ± 0.17**	**2.13 ± 0.13 (4.43 ± 0.27)**	**0.014 ± 0.0017**	**2.79 ± 0.11**	**1.90 ± 0.085 (4.49 ± 0.052)**	**0.016 ± 0.0012**
Phenylalanine	**7.61 ± 0.36**	**5.65 ± 0.87 (11.79 ± 1.83)**	**1.19 ± 0.19**	**6.86 ± 0.26**	**4.72 ± 0.55 (11.12 ± 0.89)**	**1.11 ± 0.089**
Proline	17.42 ± 0.89	12.66 ± 0.88	3.40 ± 0.66	25.49 ± 3.39	17.12 ± 1.01	4.85 ± 0.42
Serine	7.28 ± 1.08	5.17 ± 0.12	0.060 ± 0.0011	8.54 ± 0.94	5.75 ± 0.25	0.078 ± 0.0037
Threonine	**5.86 ± 0.93**	**4.15 ± 0.14 (8.66 ± 0.28)**	**0.037 ± 0.0051**	**6.54 ± 0.71**	**4.41 ± 0.23 (10.48 ± 0.78)**	**0.050 ± 0.0040**
Tryptophan	**6.83 ± 4.90**	**4.23 ± 2.66 (8.80 ± 5.54)**	**NA**	**1.63 ± 0.064**	**1.13 ± 0.14 (2.65 ± 0.23)**	**NA**
Tyrosine	4.89 ± 0.50	3.51 ± 0.073	0.18 ± 0.052	4.59 ± 0.24	3.12 ± 0.094	0.087 ± 0.024
Valine	**6.89 ± 0.63**	**4.96 ± 0.15 (10.34± 0.32)**	**0.079 ± 0.0079**	**6.53 ± 0.27**	**4.44 ± 0.17 (10.52 ± 0.079)**	**0.11 ± 0.013**
AA Sum	140.01 ± 17.30	100	8.18 ± 1.08	147.82 ± 11.60	100	9.02 ± 0.73
EAA SUM	67.14 ± 8.40	47.93 ± 0.097	3.14 ± 0.25	62.10 ± 2.85	42.24 ± 1.44	3.11 ± 0.22

Amino acids	*O. tersula*			*O. tristella*		
	Concentration	**Percent**	**% FAA**	**Concentration**	**Percent**	**% FAA**
α aminobutyric acid	1.42	0.79	0.048	0.84	0.61	0.054
Alanine	9.98	5.54	0.25	6.90	5.04	0.14
Arginine	**11.71**	**6.50 (17.24)**	**0.18**	**10.09**	**7.37 (15.99)**	**0.73**
Asparagine + Aspartic acid	17.05	9.47	0.36	23.72	17.32	4.75
Glutamine + Glutamic acid	23.14	12.85	0.31	16.43	12.00	0.50
Glycine	8.92	4.95	0.036	6.54	4.78	0.038
Histidine	**5.78**	**3.21 (8.51)**	**0.35**	**3.52**	**2.57 (5.58)**	**0.19**
Isoleucine	**6.14**	**3.41 (9.04)**	**0.080**	**6.13**	**4.47 (9.71)**	**0.12**
Leucine	**9.29**	**5.16 (13.67)**	**0.020**	**9.31**	**6.80 (14.75)**	**0.034**
Lysine	**6.18**	**3.43 (9.09)**	**0.049**	**8.35**	**6.09 (13.22)**	**0.085**
Methionine	**3.24**	**1.80 (4.77)**	**0.012**	**3.18**	**2.32 (5.03)**	**0.018**
Phenylalanine	**7.16**	**3.98 (10.54)**	**0.87**	**7.63**	**5.57 (12.09)**	**1.13**
Proline	35.25	19.57	5.49	8.06	5.89	0.99
Serine	10.70	5.94	0.091	6.90	5.04	0.12
Threonine	**7.76**	**4.31 (11.42)**	**0.059**	**5.22**	**3.81 (8.27)**	**0.070**
Tryptophan	**1.87**	**1.04 (2.75)**	**NA**	**1.72**	**1.25 (2.72)**	**NA**
Tyrosine	5.68	3.15	0.14	4.42	3.23	0.16
Valine	**8.82**	**4.90 (12.98)**	**0.11**	**7.98**	**5.82 (12.64)**	**0.14**
AA Sum	180.09	100	8.46	136.94	100	9.30
EAA SUM	67.96	37.73	1.73	63.12	46.10	2.53

Appendix E



Photos of microcolony set-up taken in 2017. A) pupal clumps of worker-caste bees were removed from source colonies; B) bees were placed in microcolonies of 4–6 bees within 24 hours of their emergence; C) microcolonies were housed in delicatessen containers wrapped in duct tape; D) bees had access to a cotton wick soaked in 35% sugar solution through the mesh floor of their container; and E) a 1 g pollen ball coated in honeybee wax was provided within 24 hours of microcolony establishment. Credit: Shang-Yao Peter Lin

Appendix F

Cox proportional hazard models for bee survival across pollen treatments (0% sunflower mixed (SFM), 25% SFM, 50% SFM, broad bean, Cucurbitaceae, rapeseed, and sunflower). Sample size is indicated in brackets. Hazard ratios less than one indicate higher chances of survival, and values greater than one indicate reduced chances of survival, relative to the reference pollen treatment.

Model 1: All bees included

	Reference pollen treatment						
	0% SFM	**25% SFM**	**50% SFM**	**Broad bean**	**Cucurbitaceae**	**Rapeseed**	**Sunflower**
0% SFM (29)		0.78	1.25	1.65	1.10	1.23	**0.44 ****
25% SFM 32)	1.28		1.61	**2.12 .**[1]	1.41	1.57	0.57
50% SFM (31)	0.80	0.62		1.32	0.88	0.98	**0.35 ****
Broad bean (34)	0.61	**0.47 .**	0.76		0.67	0.74	**0.27 ****
Cucurbitaceae (39)	0.91	0.71	1.14	1.50		1.11	**0.40 ****
Rapeseed (41)	0.82	0.64	1.02	1.35	0.90		**0.36 ****
Sunflower (31)	**2.26 ****	1.76	**2.82 ****	**3.73 ****	**2.49 ****	**2.76 ****	

Model 2: MC outliers omitted

	Reference pollen treatment						
	0% SFM	**25% SFM**	**50% SFM**	**Broad bean**	**Cucurbitaceae**	**Rapeseed**	**Sunflower**
0% SFM (29)		0.77	1.25	0.97	1.10	1.23	**0.43 ****
25% SFM (32)	1.29		1.61	1.26	1.42	1.58	0.56
50% SFM (31)	0.80	0.62		0.78	0.88	0.98	**0.35 ****
Broad bean (22)	1.03	0.80	1.28		1.13	1.26	**0.45 ****
Cucurbitaceae (39)	0.91	0.71	1.14	0.89		1.12	**0.40 ****
Rapeseed (41)	0.81	0.63	1.02	0.79	0.89		**0.35 ****
Sunflower (31)	**2.31 ****	1.79	**2.88 ****	**2.25 ****	**2.53 ****	**2.83 ****	

Model 3: Only bees that consumed pollen included

	Reference pollen treatment						
	0% SFM	**25% SFM**	**50% SFM**	**Broad bean**	**Cucurbitaceae**	**Rapeseed**	**Sunflower**
0% SFM (21)		0.75	**1.52 ***	1.64	1.18	1.41	**0.48 ****
25% SFM (28)	1.34		**2.03 ***	**2.19 .**	1.57	1.88	0.65
50% SFM (20)	**0.66 ***	**0.49 ***		1.08	0.77	0.93	**0.32 ****
Broad bean (28)	0.61	**0.46 .**	0.93		0.72	0.86	**0.30 ****
Cucurbitaceae (25)	0.85	0.64	1.29	1.39		1.20	**0.41 ****
Rapeseed (27)	0.71	0.53	1.08	1.16	0.83		**0.34 ****
Sunflower (20)	**2.06 ****	1.55	**3.14 ****	**3.38 ****	**2.43 ****	**2.91 ****	

Model 4: As in Model 3, but MC outliers omitted

	Reference pollen treatment						
	0% SFM	**25% SFM**	**50% SFM**	**Broad bean**	**Cucurbitaceae**	**Rapeseed**	**Sunflower**
0% SFM (21)		0.74	**1.51 ***	0.85	1.17	1.41	**0.47 ****
25% SFM (28)	1.35		**2.04 ***	1.14	1.58	1.90	0.63
50% SFM (20)	**0.66 ***	**0.49 ***		**0.56 ****	0.77	0.93	**0.31 ****
Broad bean (16)	1.18	0.88	**1.79 ****		1.38	1.66	**0.56 ****
Cucurbitaceae (25)	0.86	0.63	1.29	0.72		1.21	**0.40 ****
Rapeseed (27)	0.71	0.53	1.07	0.60	0.83		**0.33 ****
Sunflower (20)	**2.13 ****	1.58	**3.22 ****	**1.80 ****	**2.48 ****	**2.99 ****	

[1]. $0.05 < P < 0.075$

* $P < 0.05$

** $P < 0.01$

Appendix G

Amino acid analysis results for the four pollen types (broad bean, Cucurbitaceae, rapeseed, and sunflower; n = 3 each). Mean ± SEM are presented for concentration of bound plus free amino acids (µg/mg dry sample), percent of total amino acid (AA) content (with percent of total essential amino acids (EAA) in brackets), and percent of the total AA content made up of free amino acids (FAA) for each pollen type. Essential amino acids are in bold.

	Pollen type					
	Broad bean			**Cucurbitaceae**		
Amino acids	**Concentration**	**Percent**	**% FAA**	**Concentration**	**Percent**	**% FAA**
α aminobutyric acid	2.04 ± 0.04	0.49 ± 0.011	0.065 ± 0.0041	2.18 ± 0.078	0.85 ± 0.016	0.25 ± 0.015
Alanine	24.34 ± 1.43	5.78 ± 0.12	0.41 ± 0.032	15.61 ± 1.15	6.08 ± 0.15	0.49 ± 0.022
Arginine	**28.01 ± 1.65**	**6.64 ± 0.14 (12.93 ± 0.53)**	**0.46 ± 0.037**	**14.48 ± 1.16**	**5.64 ± 0.17 (11.16 ± 0.69)**	**0.078 ± 0.0082**
Asparagine + Aspartic acid	43.58 ± 2.91	10.33 ± 0.30	0.47 ± 0.040	25.24 ± 2.03	9.83 ± 0.31	0.63 ± 0.050
Glutamine + Glutamic acid	47.62 ± 2.92	11.30 ± 0.28	0.23 ± 0.014	28.48 ± 2.71	11.08 ± 0.50	0.12 ± 0.0050
Glycine	21.23 ± 1.28	5.04 ± 0.12	0.18 ± 0.015	14.18 ± 1.21	5.52 ± 0.19	0.23 ± 0.0079
Histidine	**11.87 ± 0.71**	**2.82 ± 0.059 (5.48 ± 0.23)**	**0.16 ± 0.010**	**7.27 ± 0.57**	**2.83 ± 0.080 (5.60 ± 0.34)**	**0.19 ± 0.0071**
Isoleucine	**23.82 ± 0.50**	**5.67 ± 0.12 (11.01 ± 0.025)**	**0.33 ± 0.026**	**13.73 ± 0.015**	**5.39 ± 0.25 (10.61 ± 0.18)**	**0.32 ± 0.020**
Leucine	**35.48 ± 0.76**	**8.44 ± 0.18 (16.40 ± 0.055)**	**0.50 ± 0.044**	**20.74 ± 0.026**	**8.14 ± 0.37 (16.03 ± 0.26)**	**0.47 ± 0.027**
Lysine	**26.12 ± 1.86**	**6.26 ± 0.68 (12.11 ± 1.05)**	**0.39 ± 0.031**	**16.05 ± 1.15**	**6.34 ± 0.73 (12.43 ± 1.08)**	**0.42 ± 0.010**
Methionine	**10.24 ± 0.33**	**2.44 ± 0.030 (4.73 ± 0.062)**	**0.13 ± 0.015**	**6.82 ± 0.18**	**2.67 ± 0.057 (5.27 ± 0.060)**	**0.16 ± 0.012**
Phenylalanine	**23.47 ± 0.65**	**5.60 ± 0.36 (10.86 ± 0.46)**	**0.92 ± 0.034**	**15.46 ± 0.41**	**6.08 ± 0.44 (11.96 ± 0.51)**	**1.28 ± 0.044**
Proline	22.19 ± 1.32	5.26 ± 0.11	0.57 ± 0.032	13.84 ± 1.15	5.39 ± 0.18	0.46 ± 0.018
Serine	24.94 ± 1.65	5.91 ± 0.17	0.35 ± 0.028	14.74 ± 1.37	5.74 ± 0.24	0.27 ± 0.0076
Threonine	**21.86 ± 1.37**	**5.19 ± 0.13 (10.09 ± 0.46)**	**0.24 ± 0.020**	**13.81 ± 0.98**	**5.38 ± 0.12 (10.65 ± 0.57)**	**0.30 ± 0.0099**
Tryptophan	**5.59 ± 0.19**	**1.33 ± 0.088 (2.59 ± 0.13)**	**NA**	**3.58 ± 0.093**	**1.40 ± 0.041 (2.76 ± 0.033)**	**NA**
Tyrosine	18.64 ± 0.81	4.43 ± 0.026	0.25 ± 0.021	12.24 ± 0.61	4.78 ± 0.018	0.38 ± 0.015
Valine	**29.83 ± 1.27**	**7.09 ± 0.029 (13.78 ± 0.34)**	**0.41 ± 0.031**	**17.53 ± 0.73**	**6.85 ± 0.049 (13.53 ± 0.33)**	**0.37 ± 0.022**
AA Sum	420.89 ±16.28	100 **(100)**	6.06 ± 0.41	255.99 ± 12.51	100 **(100)**	6.43 ± 0.27
EAA SUM	216.29 ± 4.20	51.47 ± 1.09	3.54 ± 0.23	129.47 ± 2.25	50.73 ± 1.56	3.60 ± 0.16

	Pollen type					
	Rapeseed			Sunflower		
Amino acids	**Concentration**	**Percent**	**% FAA**	**Concentration**	**Percent**	**% FAA**
α aminobutyric acid	2.86 ± 0.040	0.82 ± 0.010	0.13 ± 0.0041	2.31 ± 0.045	0.93 ± 0.0053	0.32 ± 0.0056
Alanine	20.37 ± 0.11	5.81 ± 0.023	0.30 ± 0.022	14.49 ± 0.28	5.80 ± 0.030	0.16 ± 0.0015
Arginine	**24.25 ± 0.33**	**6.92 ± 0.033 (13.42 ± 0.12)**	**0.43 ± 0.021**	**15.62 ± 0.25**	**6.26 ± 0.018 (12.24 ± 0.075)**	**0.16 ± 0.0071**
Asparagine + Aspartic acid	35.07 ± 0.76	10.01 ± 0.17	0.23 ± 0.014	24.65 ± 0.47	9.87 ± 0.050	0.14 ± 0.0032
Glutamine + Glutamic acid	40.65 ± 0.67	11.60 ± 0.11	0.17 ± 0.010	29.08 ± 0.53	11.65 ± 0.045	0.075 ± 0.0043
Glycine	17.17 ± 0.061	4.90 ± 0.031	0.11 ± 0.0075	13.45 ± 0.26	5.39 ± 0.030	0.058 ± 0.0021
Histidine	**9.58 ± 0.20**	**2.73 ± 0.045 (5.30 ± 0.11)**	**0.094 ± 0.0012**	**7.09 ± 0.12**	**2.84 ± 0.010 (5.56 ± 0.037)**	**0.14 ± 0.015**
Isoleucine	**18.86 ± 0.025**	**5.38 ± 0.043 (10.44 ± 0.098)**	**0.21 ± 0.015**	**13.58 ± 0.23**	**5.44 ± 0.021 (10.64 ± 0.074)**	**0.16 ± 0.0039**
Leucine	**29.74 ± 0.12**	**8.49 ± 0.078 (16.46 ± 0.13)**	**0.52 ± 0.057**	**20.53 ± 0.34**	**8.22 ± 0.019 (16.09 ± 0.099)**	**0.22 ± 0.0037**
Lysine	**24.15 ± 0.80**	**6.89 ± 0.21 (13.36± 0.34)**	**0.34 ± 0.022**	**16.86 ± 0.37**	**6.76 ± 0.22 (13.22 ± 0.39)**	**0.26 ± 0.0035**
Methionine	**8.87 ± 0.044**	**2.53 ± 0.016 (4.91 ± 0.048)**	**0.13 ± 0.0083**	**7.08 ± 0.14**	**2.83 ± 0.015 (5.55 ± 0.050)**	**0.084 ± 0.0022**
Phenylalanine	**20.00 ± 0.23**	**5.71 ± 0.052 (11.07 ± 0.048)**	**0.91 ± 0.0063**	**14.39 ± 0.24**	**5.77 ± 0.16 (11.28 ± 0.28)**	**1.06 ± 0.14**
Proline	16.87 ± 0.12	4.81 ± 0.017	0.17 ± 0.012	12.55 ± 0.22	5.03 ± 0.017	0.20 ± 0.014
Serine	21.50 ± 0.25	6.14 ± 0.024	0.18 ± 0.011	13.96 ± 0.27	5.59 ± 0.034	0.11 ± 0.0042
Threonine	**18.05 ± 0.22**	**5.15 ± 0.037 (9.99± 0.12)**	**0.15 ± 0.0092**	**12.35 ± 0.30**	**4.95 ± 0.089 (9.68 ± 0.18)**	**0.089 ± 0.0025**
Tryptophan	**3.39 ± 0.58**	**0.97 ± 0.16 (1.87 ± 0.30)**	**NA**	**3.28 ± 0.16**	**1.31 ± 0.052 (2.57 ± 0.11)**	**NA**
Tyrosine	15.24 ± 0.056	4.35 ± 0.045	0.18 ± 0.011	11.57 ± 0.19	4.64 ± 0.030	0.11 ± 0.0051
Valine	**23.82 ± 0.11**	**6.80 ± 0.060 (13.18 ± 0.16)**	**0.26 ± 0.026**	**16.79 ± 0.34**	**6.73 ± 0.042 (13.16 ± 0.13)**	**0.14 ± 0.0023**
AA Sum	350.45 ± 3.27	100 **(100)**	4.50 ± 0.24	249.61 ± 3.56	100 **(100)**	3.49 ± 0.21
EAA SUM	180.72 ± 1.91	51.57 ± 0.27	3.04 ± 0.16	127.56 ± 1.33	51.11 ± 0.20	2.32 ± 0.17