How do sunflower pollen mixtures affect survival of queenless microcolonies of bumblebees (*Bombus impatiens*)?

M. K. McAulay (corresponding author)

Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, ON, K1N 6N5 Canada

Email: mmcau067@uottawa.ca

J. R. K. Forrest

Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, ON, K1N 6N5 Canada

Abstract

The high pollen and nectar yield of Asteraceae flowers combined with the abundance and diversity of these plants should make them an important pollen source for both specialist and generalist bees. However, studies have found Asteraceae pollen to be a poor diet for some generalist bees, possibly owing to nutrient deficiencies or the presence of secondary metabolites or digestive barriers that prevent nutrient assimilation. Mixing pollens could allow bees to exploit these unfavourable pollens, if the different pollen sources help to complement nutrient deficiencies or alleviate the effects of toxic secondary metabolites. In our study, we examined how the proportion of sunflower (*Helianthus annuus,* Asteraceae) pollen in the diet of captive-reared bumblebees (*Bombus impatiens*) affects the survival of bees in queenless microcolonies. Bees fed sunflower pollen had significantly shorter lifespans than bees fed broad bean (*Vicia faba*, Fabaceae), rapeseed (*Brassica napus,* Brassicaceae), or Cucurbitaceae pollen. However, survival on mixed pollen diets containing 50% sunflower pollen was as great as that on non-sunflower diets, 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. Our 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.

Keywords: bumblebees, amino acids, protein, nutrition, pollen mixing, sunflower (*Helianthus annuus)*

Electronic supplementary material
The online version of this article contains supplementary material, which is available to authorized users.

Acknowledgements
We would like to thank Natalia Libreros and Shang-Yao Lin for their assistance with microcolony care, Jessamyn Manson for her advice on microcolony set-up and two anonymous reviewers for their helpful comments on the manuscript. We would also like to acknowledge BioBest Canada Ltd (Leamington, ON, Canada) for donating bumblebee colonies. Funding for this project was provided by the Natural Sciences and Engineering Research Council of Canada (MKM and JRKF), Ontario Council on Graduate Studies (MKM), as well as the University of Ottawa (MKM and JRKF).

Introduction

Pollen can be a difficult resource for animals to exploit, yet bees (Hymenoptera: Anthophila) have diversified on a pollen-based diet and most bee species rely on pollen as their sole source of protein (Willmer 2011). However, all pollens are not equivalent as dietary substrates for bees. Several studies have shown that the pollens used by certain specialist (oligolectic) bee species can be inadequate for development of non-specialists (Williams 2003; Praz et al. 2008; Sedivy et al. 2011; Haider et al. 2013; Haider et al. 2014), suggesting that physiological adaptations may be required to tolerate "unfavourable" properties of various pollens. 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). Furthermore, not all pollens collected by bees contain the 10 essential amino acids listed by De Groot (1953) for honeybees; tryptophan and phenylalanine in particular are sometimes absent (reviewed by Roulston and Cane 2000), as in dandelion pollen, *Taraxacum officinale* (Asteraceae; Auclair and Jamieson 1948). Some studies have found that Asteraceae pollen possesses unfavourable properties that render it difficult to utilize for bees that are not specialized on this pollen type (Levin and Haydak 1957; Guirguis and Brindley 1974; Sedivy et al. 2011). This could be the reason that so many generalist bees appear to avoid Asteraceae pollen (Müller 1996; Williams 2003; Müller and Kuhlmann 2008)—despite the fact that species in this family are often abundant, presenting copious floral rewards and supporting numerous oligolectic species (*Helianthus* spp.: Hurd et al. 1980; Asteroideae: Müller and Kuhlmann 2008).

Mixing pollens might be a strategy for generalist bees to exploit unfavourable pollens if the different pollen sources help to complement nutrient deficiencies or alleviate the effects of toxic secondary metabolites (Eckhardt et al. 2014). For example, *Megachile rotundata* (=*M. pacifica*; Megachilidae) larvae developed significantly more quickly on a mixed pollen diet of *Melilotus* and *Medicago sativa* (both Fabaceae) 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 *Sinapis arvensis* (Brassicaceae) and up to 50% *Ranunculus acris* (Ranunculaceae) pollen, whereas a diet of solely *R. acris* pollen was toxic to most *O. cornuta* larvae (Eckhardt et al. 2014). Nevertheless, the nutritional quality of mixed pollen diets for bumblebee health and colony development remains poorly understood (Baloglu and Gurel 2015; Moerman et al. 2016a), despite widespread interest in bee conservation (e.g., Goulson et al. 2015) and pollinator management strategies (e.g., providing wildflower plantings in agroecosystems; reviewed by Williams et al. 2015). Mixed pollen diets could benefit generalist (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).

In this study, we examined how the proportion of sunflower (*Helianthus annuus,* Asteraceae) 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. We were interested in whether mixing sunflower pollen with other pollen species could improve the quality of the pollen diet (e.g., through nutrient complementation or dilution of toxic substances). Our mixed pollen diets contained either 0%, 25%, or 50% sunflower pollen, with an equal mixture of Cucurbitaceae, rapeseed (*Brassica napus*, Brassicaceae) and broad bean (*Vicia faba*, Fabaceae) 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). We assessed the performance of *B. impatiens* microcolonies fed each pollen diet. In queenless bumblebees microcolonies, one worker acts as "queen" by developing oocytes, suppressing ovary development of subordinate workers, and laying male eggs (Cnaani et al. 2002; Cnaani et al. 2007) these microcolonies make excellent systems for testing the nutritive value of different pollen diets (Génissel et al., 2002; Tasei and Aupinel, 2008a; Vanderplanck et al., 2014; Moerman et al., 2016a; Moerman et al., 2017), and the results can be extrapolated to queenright colonies (*B. terrestris*, Tasei and Aupinel, 2008b). We had three response variables: i) worker mortality, ii) initiation of honeypot construction (a normal behaviour of growing colonies and microcolonies), and iii) egg production. We 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 and, therefore, the potential for nutrient complementation among diets.

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 bees 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) was assigned systematically to one of seven pollen treatments (Table 1). 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*], 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).

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. Three samples of each pollen type were stained with basic fuchsin and approximately 100 pollen grains were examined under a light microscope to confirm pollen identity and purity (Kearns and Inouye 1993). The samples were all found to be over 95% pure with only trace levels (i.e., < 5%) of other pollen species found in any of the samples. 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). We therefore suspended 20 g of pollen at a time in 800 mL of deionized water and filtered it through 6 µm cellulose filter paper (Little Chalfont, Buckinghamshire, UK) using a vacuum pump 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 1 g or 0.5 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. We do not know if these minor differences in sucrose content affected pollen consumption rates (we did not measure this response variable in our study), but the small differences in the amount of solution added (maximum difference approximately 0.23 mL or 0.081g of sucrose, in

an environment with freely available sugar syrup) make an effect unlikely. The pollen balls were coated in honeybee wax to prevent desiccation and to encourage egg production (cf. Gradish et al. 2013). Pollen balls were stored in the freezer prior to use.

Queenless 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 (Table 1). Microcolonies were housed in clear delicatessen containers (~650 mL, base radius = 4.6 cm, opening radius = 5.5 cm, height = 8.2 cm) that were wrapped in duct tape to keep the bees in darkness and reduce stress (photos of microcolony set-up are provided in Online Resource 1; design is adapted from Gradish et al. 2013). 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 four 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 one week later and replaced every four days over a four-week period. The original 1 g pollen ball was removed if it remained uneaten at least seven 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 six 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 of other causes before the end of the 6-week period. 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 the 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. Although there was variation in the number of microcolonies per

source colony, the only colony that significantly differed from others in terms of bee performance was the one with only three microcolonies, and results were robust to the exclusion of the microcolonies from this source colony.

Amino Acid Analysis

The amino acid profiles 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 (Trp 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 Water Acquity UPLC system was used to analyze the amino acids, and the detection limit for all three analyses was 25 pmol (Bidlingmeyer et al. 1984; Heinrikson and Meredith 1984; Cohen and Strydom 1988). This system consists of a Binary Solvent Manager Module, a Sample Manager Module, a TUV Detector Module and a Waters Acquity UPLC BEH C18 column (2.1 X 100 mm) (Milford, Massachusetts, USA). Prior to analysis, each sample was pulverized and thoroughly mixed with a mortar and pestle. Approximately 0.0100 g of the pollen samples was used for each analysis. For the AA analyses, the samples were hydrolyzed in 6N HCl with 1% phenol for 48 hours at 110°C. The Trp analyses required all samples to be hydrolyzed in 2.4N NaOH for 24 hrs at 110°C. For the FAA analyses, all samples were redissolved in 450 µL of 0.1M HCl. In addition, 50 µL of 25 µmoles/µL of norleucine were added to all samples of all analyses as an internal standard. Samples were dried under vacuum using a centrifugal evaporator (Tomy CC-181 Centrifugal Concentrator with a Sargent-Welch Model 8821 Vacuum pump). The samples were then re-dried, derivatized and dissolved in sample diluent (pH 7.40). An aliquot of the diluted sample was then injected into the column, running on a modified PICO-TAG gradient. Data were collected, stored and processed using Waters Empower 3 Chromatography software.

Statistical Analysis

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

Bee Survival and Microcolony Performance

Cox proportional hazard (henceforth Cox PH) models were used to compare bee survival on the different pollen treatments (‘coxme’ function, ‘coxme’ package; Therneau 2015). Random terms were included in the model for both source colony and microcolony. The data were analyzed first with all data included (model 1) and second (model 2) excluding microcolonies that did not consume visible amounts of pollen (as well as bees that died prior to pollen consumption) and two outlier microcolonies. Specifically, two (of 6) microcolonies (12 bees) from the broad

bean treatment were strong outliers since they contained 3 and 4 bees respectively that survived until the end of the experiment as well as several bees (3 and 2 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 (Schoenfeld residuals; all bees: $\chi^2 = 5.65$, $P = 0.018$; only pollen-consuming bees: $\chi^2 = 16.0$, $P < 0.0001$)—inconsistent with the proportional-hazards assumption. We present here the results of model 1 (including all data), but the results for both models are included in Online Resource 2, and we mention whenever results differed qualitatively between models. A Cox PH model was also used to compare time to honeypot construction across the different pollen treatments, with source colony as a random 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; cf. Vanderplanck et al. 2014). Two NMDS ordinations were performed, both using Manhattan dissimilarity matrices, 2 dimensions, and 20 runs. These parameters were sufficient to reach a stress level less than 0.05 and model convergence. Manhattan distances were used since this dissimilarity metric takes into account the sums of the absolute differences between objects (e.g., pollen samples) across all variables (e.g., amino acids) and does not ignore zero values (e.g., two samples containing zero amino acids of any kind would be considered identical in terms of Manhattan distance) (Quinn and Keogh 2002). 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, we verified homogeneity of group covariances using permutational multivariate homogeneity of group dispersions tests (PERMDISP; 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 ('indval' function, 'labdsv' package; Roberts 2016).

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 any pollen was consumed 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. Both Cox PH models for bee survival revealed significantly shorter lifespans of bees provided 100% sunflower pollen (median lifespan 19.5 d) than those in all other pollen treatments (median 20–27 d; $P < 0.05$) except 25% sunflower mixed pollen (19.5 d; Fig. 1; Table 2;); however, the difference between 100% sunflower and broad bean was only marginally significant in model 2 ($P = 0.089$; Online Resource 2). Bees provided a mixed diet containing 50% sunflower pollen lived approximately 10 days longer than bees in the 100% sunflower treatment. In addition, lifespans of bees provided the 25% sunflower mixed pollen were shorter than those in the 50% sunflower mixed treatment (significantly so only in model 2; $P < 0.021$), the broad bean pollen treatment (model 1 only; $P < 0.028$), and the rapeseed pollen treatment (model 2 only; $P < 0.037$). 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 those provided the 25% sunflower mixed pollen treatment ($P < 0.038$) and marginally more likely to construct honeypots than the 50% sunflower mixed pollen treatment ($P = 0.058$) and the 100% sunflower pollen treatment ($P = 0.095$) (Fig. 2; Table 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 4, Online Resource 3). However, these results could underestimate the total FAA content of naturally occurring pollen since some free amino acids could have been lost during the pollen washing process. Pollen types differed in their absolute amounts of total amino acids (i.e., µg/mg of dry sample; perMANOVA, $R^2 = 0.93$, $F_{3,8} = 36.41$, $P = 0.0020$; Fig. 3, Online Resource 4). We found that 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 4, Online Resource 3). 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. The proportions of the different amino acids were similar across the four pollen types (perMANOVA, $R^2 = 0.36$, $F_{3,8} = 1.52$, $P = 0.21$, Fig. 4), and none of the amino acids was indicative of a particular pollen type (Online Resource 5).

Discussion

Microcolony Success in Relation to Pollen Protein Content

Bumblebees died sooner in the 100% sunflower treatment than in any other pollen treatment except 25% sunflower mixed pollen. The difference in survival is correlated with both the lower total amino acid content and essential amino acid contents of the sunflower pollen than the other pollen types. These results are 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; Somerville and Nicol 2006; honeybee- and hand-collected pollen: Nicolson and Human 2013). In addition, Tasei and Aupinel (2008a) observed the highest worker mortality for *Bombus terrestris* fed sunflower pollen—higher than for bees fed five other pollen diets including *Castanea* (Fagaceae), *Rubus* (Rosaceae), *Papaver* (Papaveraceae), *Actinidia* (Actinidiaceae), and *Cistus* (Cistaceae) pollen.

In contrast, broad bean pollen had both the highest total and essential amino acid content, which is also 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 our 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 more likely to make honeypots, a normal colony behaviour, than those in the mixed sunflower pollen treatments.

It is unclear why so few microcolonies in our experiment produced eggs and why the few microcolonies that did lay eggs later destroyed them. Egg-eating (oophagy) can occur amongst workers that are competing for reproduction (Michener 1969), and it might also occur if workers lay non-viable eggs (cf. 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, we do not believe the microcolonies in this

study were food-stressed, since they all had constant access to sugar solution and pollen. In addition, rapeseed pollen is a suitable diet for queenless microcolony brood production by *B. terrestris* workers (Regali and Rasmont 1995), and Fabaceae pollen (e.g., broad bean) is an important pollen source for many bumblebee species, as noted above. *Bombus terrestris* queenless 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 *B. terrestris* and our study species, *B. impatiens*, could reflect species-specific requirements for brood production. Gradish et al. (2013) also observed differences between these species, with few males produced by *B. impatiens* queenless microcolonies under experimental conditions designed for *B. terrestris*.

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 overall protein content could have very different nutritional qualities for bees if some are deficient in essential amino acids (Standifer 1967). For example, Tasei and Aupinel (2008a) found that *B. terrestris* queenless microcolonies fed a mixed pollen diet that contained 9% sunflower pollen and had a lower nitrogen content than a 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 diet (Tasei and Aupinel 2008a). Similarly, in our study, the Cucurbitaceae pollen treatment, which had an amino acid content that was only slightly higher than that of 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 latter treatment.

We found that 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. 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). Nevertheless, the pollen of some Asteraceae taxa has also been found to lack or be deficient in certain essential amino acids (Auclair and Jamieson 1948; Herbert et al. 1970; Wille et al. 1985 as cited by Müller and Kuhlmann 2008). 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). In our study, we found that all pollen

types either met or exceeded the minimum requirements for the 10 essential amino acids outlined for honeybees (De Groot 1953). However, it is possible that the proportional amino acid requirements differ slightly between honeybees and bumblebees. For example, 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 also differ among *Bombus* species (Moerman et al. 2016b).

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).

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 at the germinal apertures 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 we 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. These possibilities remain to be tested.

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). We 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 yielded worker survival as high as that of the non-sunflower diets, potentially due to the presence of the other pollens in the diet which may have mitigated any harmful effects of the sunflower pollen. For example, the 50% sunflower pollen treatment had a higher amino acid content than both the sunflower and Cucurbitaceae pollen treatments (296 µg/mg compared to 250 µg/mg and 256 µg/mg, respectively), supporting the idea that one benefit of the mixture may have been its increased nitrogen content. The other pollen mixtures also had reasonably high amino acid content (342 µg/mg and 319 µg/mg for the 0% and 25% sunflower treatments, respectively).

Other studies have 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., *Papaver somniferum*, and *Sinapis* sp. 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). In addition, Tasei and Aupinel (2008a) reported that protein efficacy (weight of larvae/protein consumed) was ~4 times greater for queenless microcolonies of *B. terrestris* fed mixed pollen diets than pure pollen diets. However, it is important to consider that monofloral diets can sometimes be as good as, if not better than, mixed diets for colony performance. For example, Moerman et al. (2017) observed that queenless microcolonies fed monofloral diets of *Cytisus scoparius* (Fabaceae) and *Sorbus aucuparia* (Rosaceae) pollen produced larger larvae than microcolonies given difloral diets containing *Erica* sp. (Ericaceae) pollen, perhaps because the latter contained the harmful sterol δ7-avenasterol, which the former pollens did not (Moerman et al. 2017). Similarly, the 25% sunflower pollen treatment in our study significantly shortened worker lifespan compared to a monofloral diet of broad bean pollen. Finally, bumblebees do not mix pollen randomly but may prefer pollens with a more favourable protein to lipid ratio (Vaudo et al. 2016a, b). In the future, it would be useful to conduct a foraging study to determine whether bumblebees regulate their intake of sunflower pollen when given a choice among pollen types, and if so, what proportion of sunflower pollen they choose to collect.

Conclusion and Implications

Although we 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. At present, we are unable to explain these discrepancies in performance, but future studies using different pollen mixtures may shed light on our results.

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. Our 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 (Vaudo et al. 2015) and how the quality of the available floral resources affects colony health (Leonhardt and Blüthgen 2012). This knowledge could then be integrated into management strategies to support bee populations by providing foraging habitats with high-quality resources.

Reference List

Arnold SEJ, Idrovo MEP, Arias LJL, Belmain SR, Stevenson, PC (2014) Herbivore defence compounds occur in pollen and reduce bumblebee colony fitness. J Chem Ecol 40:878-881. https://doi.org/10.1007/s10886-014-0467-4

Auclair JL, Jamieson CA (1948) A qualitative analysis of amino acids in pollen collected by bees. Science 108:357-358. https://doi.org/10.1126/science.108.2805.357

Baloglu GH, Gurel F (2015) The effects of pollen protein content on colony development of the bumblebee, *Bombus terrestris* L. J Api Sci 59:83-88. https://doi.org/10.1515/jas-2015-0009

Bidlingmeyer BA, Cohen SA, Tarvin TS (1984) Rapid analysis of amino acids using pre-column derivatization. J Chromatogr 336: 93-104.

Cnaani J, Schmid-Hempel R, Schmidt JO (2002) Colony development, larval development and worker reproduction in *Bombus impatiens* Cresson. Insectes Soc 49: 164-170. https://doi.org/0020-1812/02/010164-07

Cnaani J, Wong A, Thomson JD (2007) Effect of Group Size on Ovarian Development in Bumblebee Workers (Hymenoptera: Apidae: Bombus). Entomol Gener 29:305-314. https://doi.org/0171-8177/07/0029-0305

Cohen SA, Strydom DJ (1988) Amino acid analysis utilizing phenylisothiocyanate derivatives. Anal Biochem, 174, 1-16.

Eckhardt M, Haider M, Dorn S, Müller A (2014) Pollen mixing in pollen generalist solitary bees: a possible strategy to complement or mitigate unfavourable pollen properties? J Anim Ecol 83:588-597. https://doi.org/10.1111/1365-2656.12168

Génissel A, Aupinel P, Bressac C, Tasei J-N, Chevrier C (2002) Influence of pollen origin on performance of *Bombus terrestris* micro-colonies. Entomol Exp Appl 104:329-336. https://doi.org/10.1046/j.1570-7458.2002.01019.x

Goulson D, Hanley ME, Darvill B, Ellis JS, Knight ME (2005) Causes of rarity in bumblebees. Biol Cons 122:1-8. https://doi.org/10.1016/j.biocon.2004.06.017

Goulson D, Nicholls E, Botías C, Rotheray EL (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347:1255957. https://10.1126/science.1255957

Gradish AE, Scott-Dupree CD, McFarlane AD, Frewin AJ (2013) Too much work, not enough tarsi: group size influences queen-less reproduction in bumble bees (*Bombus impatiens*), with implications for sub-lethal toxicity assessments. J Econ Entomol 106:552-557. https://doi.org/10.1603/EC12154

De Groot AP (1953) Protein and amino acid requirements of the honey bee. Physiol Comp Oecol 3:197-285

Guirguis GN, Brindley WA (1974) Insecticidal susceptibility and response to selected pollens of larval alfalfa leafcutting bees, *Megachile pacifica* (Panzer) (Hymenoptera: Megachilidae). Environ Entomol 3:691-694. https://doi.org/10.1093/ee/3.4.691

Haider M, Dorn S, Müller A (2013) Intra- and interpopulational variation in the ability of a solitary bee species to develop on non-host pollen: implications for host range expansion. Funct Ecol 27:255-263. https://doi.org/10.1111/1365-2435.12021

Haider M, Dorn S, Müller A (2014) Better safe than sorry? A Fabaceae species exhibits unfavourable pollen properties for developing bee larvae despite its hidden anthers. Arth-Plant Int 8:221-231. https://doi.org/10.1007/s11829-014-9299-y

Hanley ME, Franco M, Pichon S, Darvill B, Goulson D (2008) Breeding system, pollinator choice and variation in pollen quality in British herbaceous plants. Funct Ecol 22:592-598. https://doi.org/10.1111/j.1365-2435.2008.01415.x

Heinrikson RL, Meredith SC (1984) Amino acid analysis by reverse-phase high-performance liquid chromatography: precolumn derivatization with phenylisothiocyanate. Anal Biochem 136:65-74. https://doi.org/10.1016/0003-2697(84)90307-5

Herbert EW, Bickley WE, Shimanuki H (1970) The brood-rearing capability of caged honey bees fed dandelion and mixed pollen diets. J Econ Entomol 63:215–218. https://doi.org/10.1093/jee/63.1.215

Hervé M (2017) RVAideMemoire: Testing and Plotting Procedures for Biostatistics. R package version 0.9-68. URL https://CRAN.R-project.org/package=RVAideMemoire [accessed on 30 December 2017]

Human H, Nicolson SW, Strauss K, Pirk CWW, Dietemann V (2007) Influence of pollen quality on ovarian development in honeybee workers (*Apis mellifera scutellata*). J Insect Physiol 53:649-655. https://doi.org/10.1016/j.jinsphys.2007.04.002

Hurd PD Jr, LaBerge WE, Linsley EG (1980) Principal sunflower bees of North America with emphasis on the south-western United States (Hymenoptera: Apoidea). Smithson Contrib Zool 310:1-158. https://doi.org/10.5479/si.00810282.310

Kearns CA, Inouye DW (1993) Techniques for pollination biologists. University Press of Colorado, Colorado.

Kitaoka TK, Nieh JC (2009) Bumble bee pollen foraging regulation: role of pollen quality, storage levels, and odor. Behav Ecol Sociobiol 63:501-510. http://dx.doi.org/10.1007/s00265-008-0684-3.

Kleinschmidt GJ, Kondos AC (1976) The influence of crude protein levels on colony production. Australian Beekeep 78:36-39.

Koedam D, Velthausz PH, Krift TVD, Dohmen MR, Sommeijer MJ (1996) Morphology of reproductive and trophic eggs and their controlled release by workers in *Trigona* (Tetragonisca) *angustula* Illiger (Apidae, Meliponinae). Physiol Entomol 21:289-296. http://dx.doi.org/10.1111/j.1365-3032.1996.tb00867.x

Kriesell L, Hilpert A, Leonhardt SD (2017) Different but the same: bumblebee species collect pollen of different plant sources but similar amino acid profiles. Apidologie 48:102-116. http://dx.doi.org/10.1007/s13592-016-0454-6

Leonhardt SD, Blüthgen N (2012) The same, but different: pollen foraging in honeybee and bumblebee colonies. Apidologie 43:449-464. http://dx.doi.org/10.1007/s13592-011-0112-y

Levin MD, Haydak MH (1957) Comparative value of different pollens in the nutrition of *Osmia lignaria*. Bee World 38:221-226. https://doi.org/10.1080/0005772X.1957.11095007

Michener CD (1969) Comparative social behavior of bees. Annu Rev Entomol 14:299-342. https://doi.org/10.1146/annurev.en.14.010169.001503

438 Moerman R, Roger N, De Jonghe R, Michez D, Vanderplanck M (2016b) Interspecific variation in bumblebee
439 performance on pollen diet: new insights for mitigation strategies. PLoS ONE 11:e0168462.
440 https://doi.org/10.1371/journal.pone.0168462

441 Moerman R, Vanderplanck M, Fournier D, Jacquemart A-L, Michez D (2017) Pollen nutrients better explain
442 bumblebee colony development than pollen diversity. Insect Conserv Divers 10:171-179.
443 https://doi.org/10.1111/icad.12213

444 Moerman R, Vanderplanck M, Roger N, Declèves S, Wathelet B, Rasmont P, Fournier D, Michez D (2016a)
445 Growth rate of bumblebee larvae is related to pollen amino acids. J Econ Entomol 109:25-30.
446 https://doi.org/10.1093/jee/tov279

447 Morandin LA, Kremen C (2013) Hedgerow restoration promotes pollinator populations and exports native bees to
448 adjacent fields. Ecol Appl 23:829-839. https://doi.org/10.1890/12-1051.1

449 Müller A (1996) Host-plant specialization in western palearctic anthidiine bees (Hymenoptera: Apoidea:
450 Megachilidae). Ecol Monograph 66:235-257. https://doi.org/10.2307/2963476

451 Müller A, Kuhlmann M (2008) Pollen hosts of western palaearctic bees of the genus *Colletes* (Hymenoptera:
452 Colletidae): the Asteraceae paradox. Biol J Linnean Soc 95:719-733. https://doi.org/10.1111/j.1095-
453 8312.2008.01113.x

454 Nicolson SW, Human H (2013) Chemical composition of the 'low quality' pollen of sunflower (*Helianthus annuus*,
455 Asteraceae). Apidologie 44:144-152. https://doi.org/10.1007/s13592-012-0166-5

456 Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL,
457 Solymos P, Stevens MHH, Szoecs E, Wagner H (2017) vegan: Community Ecology Package. R package
458 version 2.4-3. URL https://CRAN.R project.org/package= vegan [accessed on 28 January 2018]

459 Pernal SF, Currie RW (2000) Pollen quality of fresh and 1-year-old single pollen diets for worker honey bees (*Apis*
460 *mellifera* L.). Apidologie 31:387-409. https://doi.org/10.1051/apido:2000130

461 Praz CJ, Müller A, Dorn S (2008) Specialized bees fail to develop on non-host pollen: do plants chemically protect
462 their pollen? Ecology 89:795-804. https://doi.org/10.1890/07-0751.1

463 Quinn GP and Keough MJ (2002) Experimental Design and Data Analysis for Biologists. Cambridge University
464 Press, Cambridge.

465 R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical
466 Computing, Vienna, Austria. URL https://www.R-project.org/ [accessed on 28 January 2018]

467 Rayner CJ, Langridge DF (1985) Amino acids in bee-collected pollens from Australian indigenous and exotic
468 plants. Aust J Exp Agri 25:722-726. https://doi.org/10.1071/EA9850722

469 Regali A, Rasmont P (1995) Nouvelles méthodes de test pour l'évaluation du régime alimentaire chez des colonies
470 orphelines de *Bombus terrestris* (L) (Hymenoptera, Apidae). Apidologie 26:273-281.
471 https://doi.org/10.1051/apido:19950401 [In French]

472 Roberts DW (2016) labdsv: Ordination and Multivariate Analysis for Ecology. R package version 1.8-0. URL
473 https://CRAN.R-project.org/package=labdsv [accessed on 30 December 2017]

474 Roulston TH, Cane JH (2000) Pollen nutritional content and digestibility for animals. Plant Syst Evol 222:187-209.
475 https://doi.org/10.1007/BF00984102

476 Roulston TH, Cane JH (2002) The effect of pollen protein concentration on body size in the sweat bee *Lasioglossum*
477 *zephyrum* (Hymenoptera: Apiformes). Evol Ecol 16:49-65. https://doi.org/10.1023/A:1016048526475

478 Schmidt LS, Schmidt JO, Rao H, Wang W, Xu L (1995) Feeding preference and survival of young worker honey
479 bees (Hymenoptera: Apidae) fed rape, sesame, and sunflower pollen. J Econ Entomol 88:1591-1595.
480 https://doi.org/10.1093/jee/88.6.1591

481 Schmidt JO, Thoenes SC, Levin MD (1987) Survival of honey bees, *Apis mellifera* (Hymenoptera: Apidae), fed
482 various pollen sources. Ann Entomol Soc Am 80:176-183. https://doi.org/10.1093/aesa/80.2.176

483 Sedivy C, Müller A, Dorn S (2011) Closely related pollen generalist bees differ in their ability to develop on the
484 same pollen diet: evidence for physiological adaptations to digest pollen. Funct Ecol 25:718-725.
485 https://doi.org/10.1111/j.1365-2435.2010.01828.x

Somerville DC, Nicol HI (2006) Crude protein and amino acid composition of honey bee-collected pollen pellets from south-east Australia and a note on laboratory disparity. Aust J Exp Agri 46:141-149. https://doi.org/10.1071/EA03188

Somme L, Vanderplanck M, Michez D, Lombarde I, Moerman R, Wathelet B, Wattiez R, Lognay G, Jacquemart A-L (2015) Pollen and nectar quality drive the major and minor floral choices of bumble bees. Apidologie 46:92-106. https://doi.org/10.1007/s13592-014-0307-0

Standifer LN (1967) A comparison of the protein quality of pollens for growth-stimulation of the hypopharyngeal glands and longevity of honey bees, *Apis mellifera* L. (Hymenoptera, Apidae). Insectes Soc 14:415-426. https://doi.org/10.1007/BF02223687

Suárez-Cervera M, Marquez J, Bosch J, Seoane-Camba J (1994) An ultrastructural study of pollen grains consumed by larvae of *Osmia* bees (Hymenoptera, Megachilidae). Grana 33:191-204. https://doi.org/10.1080/00173139409429000

Tasei J-N, Aupinel P (2008a) Nutritive value of 15 single pollens and pollen mixes tested on larvae produced by bumblebee workers (*Bombus terrestris*, Hymenoptera: Apidae). Apidologie 39:397-409. https://doi.org/10.1051/apido:2008017

Tasei J-N, Aupinel P (2008b). Validation of a method using queenless *Bombus terrestris* micro-colonies for testing the nutritive value of commercial pollen mixes by comparison with queenright colonies. J Econ Entomol 101, 1737–1742. https://doi.org/10.1603/0022-0493-101.6.1737

Tasei J-N, Masure MM (1978) Sur quelques facteurs influençant le dévelopment de *Megachile pacifica* Panz (Hymenoptera, Megachilidae). Apidologie 9:273-290. https://doi.org/10.1051/apido:19780402 [In French]

Therneau T (2015) coxme: Mixed Effects Cox Models. R package version 2.2-5. URL https://CRAN.R-project.org/package=coxme [accessed on 30 December 2017]

Todd FE, Bretherick O (1942) The composition of pollens. J Econ Entomol 35:312-317.

509 Vanderplanck M, Moerman R, Rasmont P, Lognay G, Wathelet B, Wattiez R, Michez D (2014) How does pollen
510 chemistry impact development and feeding behavior of polylectic bees? PLoS ONE 9:e86209.
511 https://doi.org/10.1371/journal.pone.0086209

512 Vaudo AD, Patch HM, Mortensen DA, Tooker JF, Grozinger CM (2016a) Macronutrient ratios in pollen shape
513 bumble bee (*Bombus impatiens*) foraging strategies and floral preferences. Proc Natl Acad Sci USA
514 113:E4035-E4042. https://doi.org/10.1073/pnas.1606101113

515 Vaudo AD, Stabler D, Patch HM, Tooker JF, Grozinger CM, Wright GA (2016b) Bumble bees regulate their intake
516 of essential protein and lipid pollen macronutrients. J Exp Biol 219:3962-3970.
517 https://doi.org/10.1242/jeb.140772

518 Vaudo AD, Tooker JF, Grozinger CM, Patch HM (2015) Bee nutrition and floral resource restoration. Curr Opin
519 Insect Sci 10:133-141. https://doi.org/10.1016/j.cois.2015.05.008

520 Weiner CN, Hilpert A, Werner M, Linsenmair KE, Blüthgen N (2010) Pollen amino acids and flower specialisation
521 in solitary bees. Apidologie 41:476-487. https://doi.org/10.1051/apido/2009083

522 Westphal C, Steffan-Dewenter I, Tscharntke T (2003) Mass flowering crops enhance pollinator densities at a
523 landscape scale. Ecol Lett 6:961-965. https://doi.org/10.1046/j.1461-0248.2003.00523.x

524 Wille H, Wille M, Kilchenmann V, Imdorf A, Bühlmann G (1985) Pollenernte und Massenwechsel von drei *Apis*
525 *mellifera*-Völkern auf demselben Bienenstand in zwei aufeinanderfolgenden Jahren. Rev. suisse zool
526 92:897–914. https://doi.org/10.5962/bhl.part.81921 [In German]

527 Williams NM (2003) Use of novel pollen species by specialist and generalist solitary bees (Hymenoptera:
528 Megachilidae). Oecologia 134:228-237. https://doi.org/10.1007/s00442-002-1104-4

529 Williams NM, Ward KL, Pope N, Isaacs R, Wilson J, May EA, Ellis J, Daniels J, Pence A, Ullmann K, Peters J
530 (2015) Native wildflower plantings support wild bee abundance and diversity in agricultural landscapes
531 across the United States. Ecol Appl 25:2119-2131. https://doi.org/10.1890/14-1748.1

532 Willmer P (2011) Pollination and floral ecology. Princeton University Press, New Jersey (USA)

Table 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*], 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 ten 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**
	0% SFM	**25% SFM**	**50% SFM**	**Broad Bean**	**Cucurbitaceae**	**Rapeseed**	**Sunflower**	**MC (bees)**
MC (bees) with pollen consumption	4 (21)	6 (28)	4 (20)	5 (28)	5 (25)	5 (27)	4 (20)	**33 (169)**
MC (bees) 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 2 Cox PH ratios for bee survival across the seven pollen treatments 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*], 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). Sample size (number of bees) is included in brackets. Bees that did and did not consume pollen were included in this analysis. Results of the analysis that omitted outliers and bees that did not consume pollen (model 2) are provided in Online Resource 2. 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

	Reference pollen treatment						
	0% SFM	**25% SFM**	**50% SFM**	**Broad bean**	**Cucurbitaceae**	**Rapeseed**	**Sunflower**
0% SFM (29)		0.66	1.11	1.63	1.03	1.13	**0.35***
25% SFM (32)	1.51[a]		1.67	**2.47***	1.56	1.72	0.53
50% SFM (31)	0.90	0.60		1.47	0.93	1.02	**0.32****
Broad bean (34)	0.61	**0.40***	0.68		0.63	0.69	**0.22****
Cucurbitaceae (39)	0.97	0.64	1.07	1.58		1.10	**0.34****
Rapeseed (41)	0.88	0.58	0.98	1.44	0.91		**0.31****
Sunflower (31)	**2.85***	1.88	**3.15****	**4.64****	**2.49****	**3.23****	

*$P < 0.05$

**$P < 0.01$

[a]Hazard ratios above the diagonal are reciprocals of the corresponding values below the diagonal.

Table 3 Cox PH ratios for microcolony honeypot construction across the seven pollen treatments 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*], 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). Sample size (number of microcolonies) is provided in brackets and 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% SFM pollen treatments, and marginally significantly more likely to make honeypots than the 50% SFM pollen treatment and 100% sunflower pollen treatment

	Reference pollen treatment						
	0% SFM	**25% SFM**	**50% SFM**	**Broad bean**	**Cucurbitaceae**	**Rapeseed**	**Sunflower**
0% SFM (5)		5.30	2.66	0.54	1.72	1.45	2.22
25% SFM (6)	0.19[a]		0.50	**0.10***	0.32	0.27	0.42
50% SFM (6)	0.38	1.99		**0.20**[§]	0.65	0.55	0.83
Broad bean (6)	1.87	**9.89***	**4.97**[§]		3.21	2.71	**4.14**[§]
Cucurbitaceae (7)	0.58	3.08	1.55	0.31		0.85	1.29
Rapeseed (7)	0.69	3.64	1.83	0.37	1.18		1.52
Sunflower (6)	0.45	2.39	1.20	**0.24**[§]	0.78	0.66	

[§] $0.05 < P < 0.075$

* $P < 0.05$

** $P < 0.01$

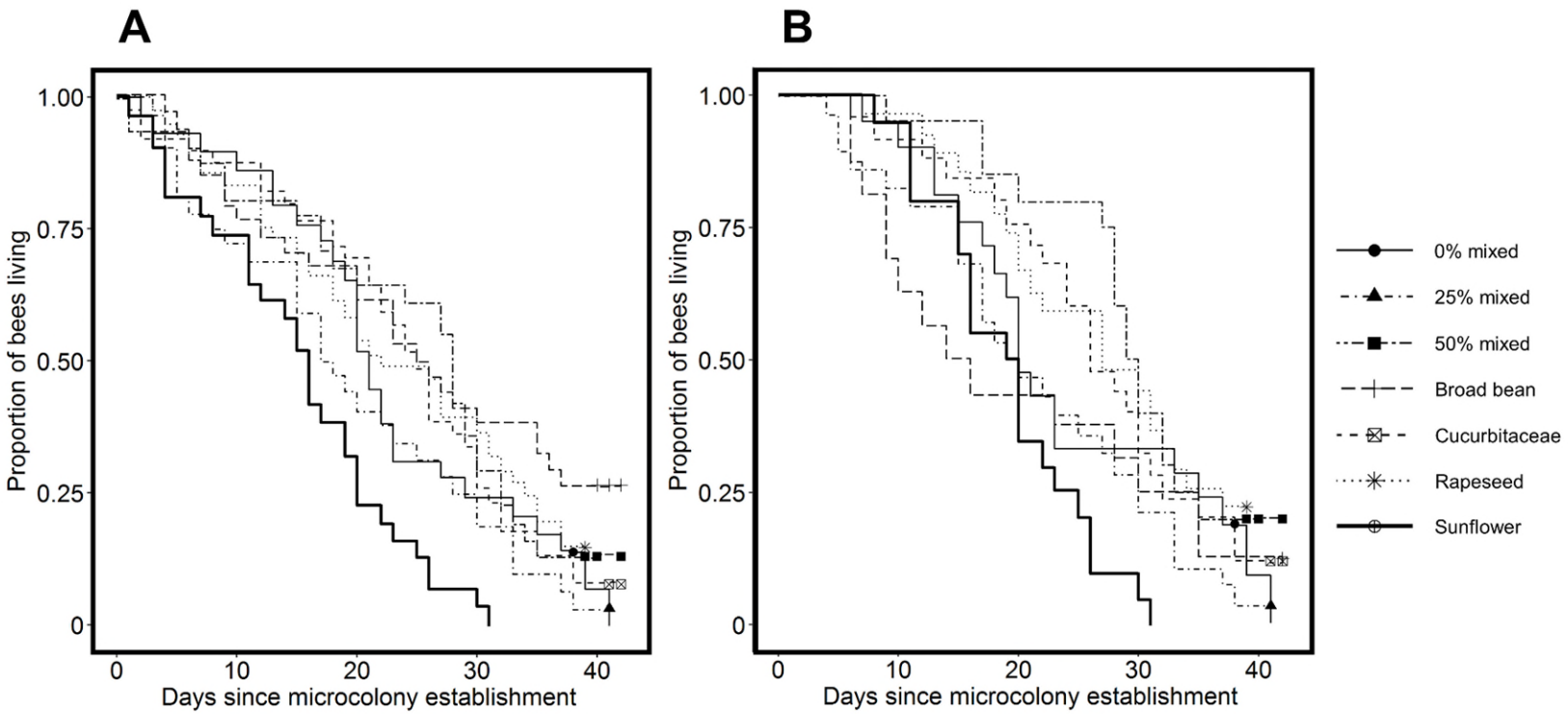
[a] Hazard ratios above the diagonal are reciprocals of the corresponding values below the diagonal.

Table 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*], 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)[a]	EAA (µg/mg of sample)	% FAA[b]
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

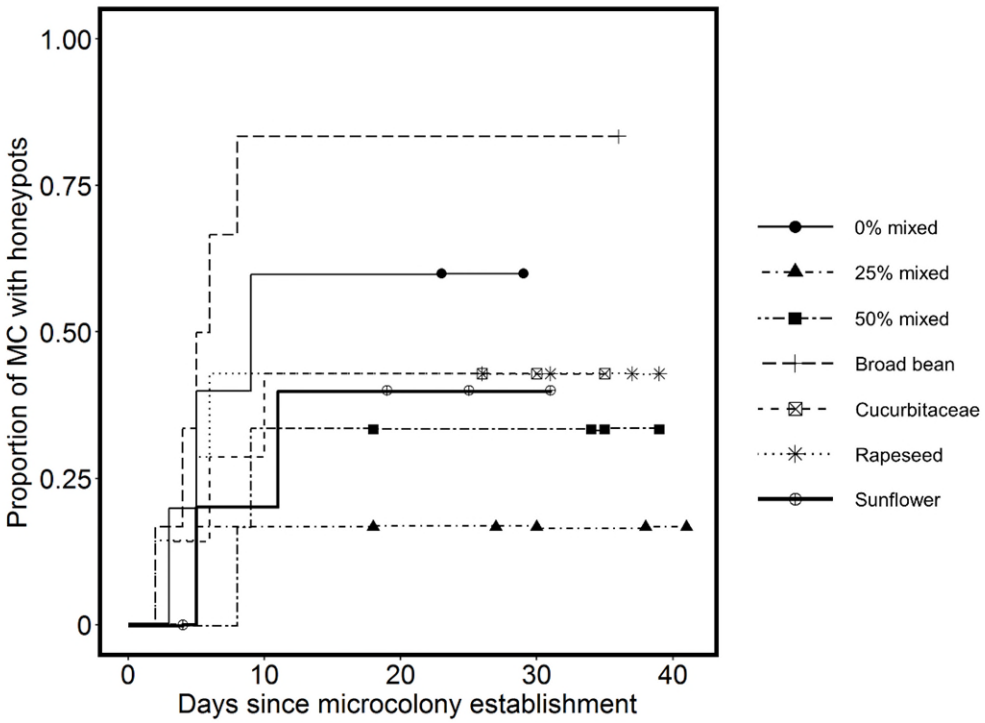
[a]Sample weights are the dry weights of the pollen.
[b]The proportion of free tryptophan could not be determined since this amino acid was analyzed in a separate analysis which did not distinguish between protein-bound and free amino acid. As a result, this amino acid was omitted for the % FAA calculation.

579 **Figures**



580

581 **Fig. 1** Bee survival over time on the seven pollen treatments visualized using the 'survfit' function of the 'survival' package. **A** contains all bees (as in Table 2;
582 model 1) and **B** contains only the bees from microcolonies with pollen consumption and omits two outlier microcolonies from the broad bean treatment (12 bees)
583 (as in Online Resource 2, model 2). The seven pollen diets included 0% sunflower mixed (SFM) pollen diet (A: n = 29 bees; B: n = 21 bees), 25% SFM (33; 28),
584 and 50% SFM (31; 20), broad bean (34; 16), Cucurbitaceae (39; 25), rapeseed (41; 27), and sunflower (31; 20). Survival was significantly reduced on sunflower
585 pollen relative to all pollen treatments except 25% SFM pollen. Results were jittered both vertically and horizontally to reduce overlap between the different
586 pollen treatments. Bees that were censored until the end of the 6-week period (42 days) are represented by the symbols on the curves



587

588 **Fig. 2** Honeypot construction over time on the seven pollen treatments visualized using the 'survfit' function of the
589 'survival' package. All microcolonies ("MC") were included in this analysis. The seven pollen diets included 0%
590 sunflower mixed (SFM) pollen diet (n = 5 microcolonies), 25% SFM (6), and 50% SFM (6), broad bean (6),
591 Cucurbitaceae (7), rapeseed (7), and sunflower pollen (6). Microcolonies provided broad bean pollen were
592 significantly more likely to construct honeypots than the 25% SFM pollen treatments, and marginally significantly
593 more likely to make honeypots than the 50% SFM pollen treatment and 100% sunflower pollen treatment. Results
594 were jittered both vertically and horizontally to reduce overlap between the different pollen treatments.
595 Microcolonies without honeypot construction are represented by the symbols on the curves

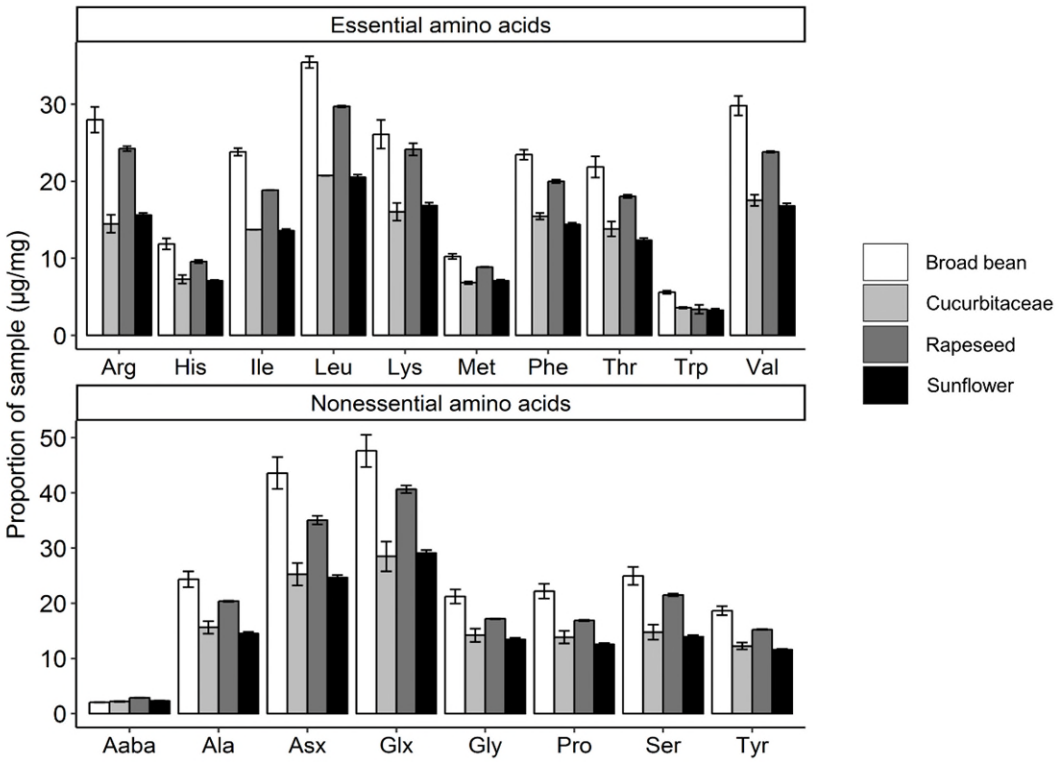


Fig. 3 The mean ± SEM of essential amino acids (EAA) and nonessential amino acids (NAA) (including both protein-bound and free amino acids) in the four pollen types (broad bean (*Vicia faba*), Cucurbitaceae (30% summer squash [*Cucurbita pepo*], 70% watermelon [*Citrullus lanatus*]), rapeseed (*Brassica 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)

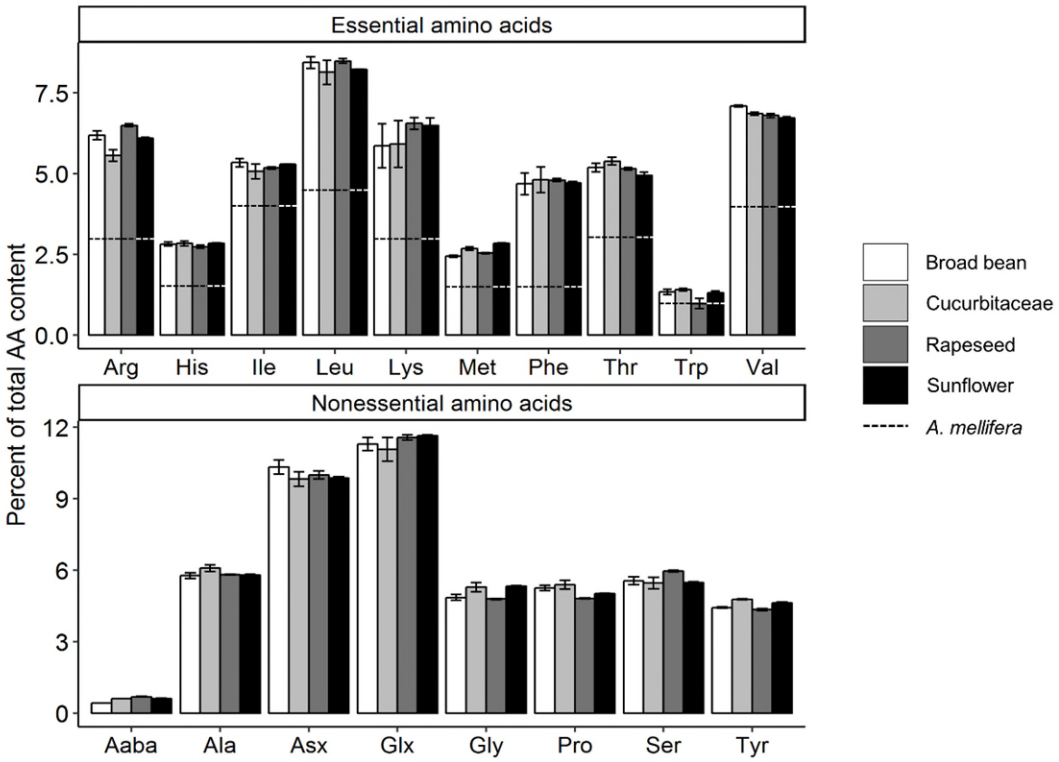


Fig. 4 The mean ± SEM of essential amino acids (EAA) and nonessential amino acids (NAA) (including both protein-bound and free amino acids) in the four pollen types (broad bean (*Vicia faba*), Cucurbitaceae (30% summer squash [*Cucurbita pepo*], 70% watermelon [*Citrullus lanatus*]), rapeseed (*Brassica 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. The dashed lines represent the EAA requirements for *Apis mellifera* described by De Groot (1953). (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)