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HIGHLIGHTED STUDENT RESEARCH

Understanding pollen specialization in mason bees: a case study of six species

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Abstract

Many bee species are dietary specialists and restrict their pollen foraging to a subset of the available flowers. However, the reasons for specialization—and the reasons certain plant taxa support numerous specialists—are often unclear. Many bees specialize on the plant family Asteraceae, despite evidence its pollen is a poor food for non-specialists. Here, we studied six mason bee (*Osmia*) species, including three Asteraceae specialists, to test whether observed pollen-usage patterns reflect larval nutritional requirements, to investigate what aspects of Asteraceae pollen make it unsuitable for non-specialists, and to understand how Asteraceae specialists tolerate their seemingly low-quality diet. We reared larval bees on host and nonhost pollen and found that Asteraceae specialists could develop on nonhost provisions, but that other bees could not survive on Asteraceae provisions. These effects did not seem related to nutritional deficiencies, since Asteraceae provisions were not amino acid deficient, and we found no consistent differences in digestive efficiency among pollen types. However, Asteraceae specialists completed more foraging flights per larva, generally collected relatively larger provisions, and produced more frass (waste) than the other species, suggesting quantitative compensation for low food quality. Toxins, deficiencies in unmeasured nutrients, or aspects of pollen grain structure might explain poor survival of non-specialists on Asteraceae provisions. Our results suggest that floral host selection by specialist bees is not related to optimizing larval nutrition. We recommend further investigation of host-selection behaviour in adult bees and of pollen digestion in larvae to better understand the evolution of bee–flower associations.

Keywords Amino acids · Nutrition · Oligolecty · *Osmia* (Megachilidae) · Asteraceae

Introduction

Biologists have long wondered why some species specialize on a subset of the available resources while others are generalists (Darwin 1859; Futuyma and Moreno 1988; Simon and Toft 1991; Eby 1998; Forister et al. 2012; Hardy et al. 2020). For example, among herbivorous insects, many species are dietary specialists that restrict their feeding to hosts from three or fewer plant families (Bernays and Graham 1988), despite the availability of numerous other potential food sources in their habitat (Jaenike 1990). The commonness of specialization among insect herbivores has led to several hypotheses about how natural selection shapes plant–insect interactions. For example, specialization could be selectively favoured if it improves aspects of resource-utilization efficiency, such as the speed of decision-making while foraging (Bernays and Funk 1999). Alternatively, specialization may arise if selection favours mother insects that choose only those larval foods that maximize larval fitness. This

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Our study goes beyond past studies of pollen specialization in bees by examining mechanisms underlying bee survival on different pollens and by studying foraging behaviour of the bees in the field.

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hypothesis is known as the 'oviposition preference–offspring performance' or the 'mother-knows-best' hypothesis' (Jaenike 1978; Mayhew 2001). However, 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 searching for the host plants that would be best for their larvae (Scheirs et al. 2000). Finally, selection for enemy-free space could favour mothers that use a narrow range of host plants that confer protection for their offspring from natural enemies (Bernays and Graham 1988, e.g. chemical protection: Denno et al. 1990; Ballabeni et al. 2001, physical protection: Feder 1995). In these cases, the species may have acquired adaptations to physiologically tolerate the "unfavourable" or protective properties of their host plants during larval development, potentially at the cost of a reduced ability to exploit other food sources (see Cornell and Hawkins 2003).

While bees are more often studied in the context of their role as mutualistic pollinators of plants, they also consume plant tissues, such as nectar, and depend—with few exceptions—on pollen (and pollen-born microbes: Steffan et al. 2019) as their protein source for survival and reproduction. For most bee species, pollen is a vital source of protein (Roulston and Cane 2000) and other nutrients (Dobson and Peng 1997) during larval development. Adult bees also consume pollen (Taniguchi 1956; Cane et al. 2017), which can be critical for egg maturation in females (Cane 2016). Female bees harvest pollen which they bind with nectar to create a provision mass for each larva (Roubik 1982). Like phytophagous insects, bees vary greatly in their degree of specialization when foraging for pollen (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). Specialist bees may be more efficient foragers than generalists (e.g. Strickler 1979) or more efficient at digesting their host pollen (e.g. Dobson and Peng 1997); however, the evidence for greater efficiency among specialist bees is limited. Regardless, specialization might be accompanied by a reduced ability to use alternative pollen sources, for example if specialization entailed physiological or behavioural adaptations to exploit host pollen. This could explain why specialist bees sometimes fail to develop on nonhost pollens (reviewed by Rivest and Forrest 2020), or do not develop as well on nonhost pollens as do broadly generalist species (Haider et al. 2014).

In some cases, however, specialists develop well on nonhost pollens, or even develop better when fed a mixture of host and nonhost pollens than when reared on host pollen alone (Williams 2003). The latter observation, paired with the results of a host-choice experiment wherein mother bees refused to collect some nonhost pollens and ceased nesting when their host plants were unavailable (Williams 2003), fails to support the mother-knows-best hypothesis and suggests that the reasons for specialization are unrelated to physiological trade-offs. Furthermore, the nutritional or digestive mechanisms to explain these differences in performance on nonhost pollens remain largely unknown (e.g. Sedivy et al. 2012).

Many oligolectic bees specialize on pollen from the plant family Asteraceae, and specialization on Asteraceae pollen has apparently evolved repeatedly in numerous bee lineages (Hurd et al. 1980; Müller and Kuhlmann 2008). Interestingly, this pollen, despite being abundant in many habitats and widely used by specialists, is collected in only marginal amounts by many generalists (Müller 1996). Furthermore, past studies have found Asteraceae pollen to be a difficult resource to utilize for bees that are not Asteraceae specialists. For example, Asteraceae pollen is considered a poor diet for honeybees (*Apis mellifera*; Rayner and Langridge 1985) and bumblebees (*Bombus terrestris*: Regali and Rasmont 1995; Tasei and Aupinel 2008; *Bombus impatiens*: McAulay and Forrest 2019). In addition, several broadly polylectic solitary bees also failed to develop, or developed poorly, on diets of pure Asteraceae pollen (Guirguis and Brindley 1974; Levin and Haydak 1957; Sedivy et al. 2011; Williams 2003). Asteraceae pollen could be unfavourable for non-specialist bees for a variety of reasons including nutrient deficiencies (Roulston et al. 2000; Auclair and Jamieson 1948; Herbert et al. 1970) and poor digestibility (e.g. morphological or chemical barriers; Peng et al. 1985; Williams 2003). Thus, the reasons that so many bees have evolved to specialize on Asteraceae pollen are mysterious, although escape from natural enemies (cleptoparasites (*Sapyga* wasps): Spear et al. 2016; gut pathogens (*Crithidia bombi*): Giacomini et al. 2018; Adler et al. 2020) or from competitors (but see Wcislo and Cane 1996) could have been involved.

In the bee genus *Osmia*, specialization on Asteraceae pollen has seemingly evolved at least twice, in the subgenera *Helicosmia* and *Cephalosmia* (although a formal reconstruction of ancestral diets has not yet been conducted). Other members of the genus are polylectic or specialize on other plant taxa (Rust and Clement 1972; Cripps and Rust 1989a, b; Forrest and Chisholm 2017). In this study, we focused on several co-occurring *Osmia* species, including three Asteraceae oligoleges, and investigated factors that might explain (a) why multiple taxa specialize on this plant family while others avoid it, (b) the apparently poor quality of a diet based on Asteraceae pollen, and (c) how Asteraceae oligoleges overcome or compensate for the low quality of their diet. First, we tested the ability of larvae of several

bee species to develop on a diet containing nonhost pollen, thereby testing the mother-knows-best hypothesis and, more specifically, testing whether Asteraceae specialists develop most quickly and successfully on a diet of Asteraceae pollen. Then, because this first experiment showed generally poor survival and development on Asteraceae pollen provisions, we compared the amino acid profiles of the different bee-collected pollen provisions and quantified the digestive efficiencies of bees reared on different pollens to test whether the observed differences in performance were due to nutrient deficiencies or poor digestibility. Finally, we investigated whether Asteraceae specialists invest more in pollen foraging (in terms of pollen provision mass and the number of foraging flights per provision) to potentially compensate for the low quality of their pollen diet.

Materials and methods

Study species

Cavity-nesting bees normally build their nests in tunnels they find in dead trees, but they will also nest in artificial "trap nests" that consist of holes drilled into blocks of wood (Cane et al. 2007). Our study was conducted using trap nests established at several sites around the Rocky Mountain Biological Laboratory, Colorado, USA (38° 57′ 30″ N, 106° 59′ 18″ W, 2900 m above sea level; see Forrest and Chisholm (2017) for trap nest details). Among other species, trap nest occupants at our sites include the solitary mason bee species *Osmia* (*Helicosmia*) *coloradensis*, *O.* (*Hapsidosmia*) *iridis*, *O.* (*Cephalosmia*) *montana*, *O.* (*Cephalosmia*) *subaustralis*, *O.* (*Melanosmia*) *tersula*, and *O.* (*Melanosmia*) *tristella* (Hymenoptera: Megachilidae).

Female mason bees collect pollen and nectar to provision their brood. Along the length of the nest 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). The mother bee provisions enough food to suffice for all larval growth of each egg, and she seals each brood cell soon after laying the egg (Michener 2000). She constructs brood cells sequentially such that she completes the wall of the previous cell before gathering pollen and nectar for the following offspring (Michener 2000).

Based on our examination of the pollen contents of hundreds of nests (as described in Spear et al. 2016) as well as published literature (Rust 1974), four of the study species (*O. coloradensis*, *O. iridis*, *O. montana*, and *O. subaustralis*), representing three subgenera, 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 *Vicia americana* at the study site). The other specialists, which we refer to henceforth as the "Aster bees", restrict their foraging to plants from tribes in the Asteraceae family including Cichorieae, Helenieae, Heliantheae, and Senecioneae. We have confirmed through microscopic examination of pollen samples from nests that virtually all pollen (> 99%) in Aster bee nests in our study area is indeed Asteraceae pollen (C. Cahill and J. Forrest, unpublished data from 128–181 brood cells per species). In our study area, *O. tristella* collects pollen from multiple plant families including Boraginaceae, Fabaceae, Lamiaceae, and Plantaginaceae. *Osmia tersula* collects pollen from the families Boraginaceae, Fabaceae, Lamiaceae, Plantaginaceae, Rosaceae, and Violaceae.

Egg-transfer experiment

The egg-transfer experiment was conducted in a 3 × 3 factorial design with three bee species groups, and three corresponding pollen provision types: (1) the Aster bees, *O. coloradensis*, *O. montana*, and *O. subaustralis*; (2) the Fabaceae specialist, *O. iridis*; and (3) the generalist species, *O. tristella* (Online Resource Table A1). The experiment was performed over two consecutive summers (2016: 26 Jun to 27 Aug; 2017: 21 Jun to 15 Aug). *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 were 'Aster' (pollen provisions of the Aster bees *O. coloradensis*, *O. montana*, and *O. subaustralis*, which we treated as interchangeable), 'Fabeae' (pollen provisions of the Fabaceae specialist *O. iridis*) and 'Various' (the majority were provisions of the generalist *O. tristella*, and two were likely *O. tersula* but the mother bee was not identified). Bee eggs were assigned sequentially (as much as possible given the availability of different provision types at the time of transfer) to different treatments (control [= host] or novel provision type [Aster, Fabeae or Various]).

Bee nests were collected from trap nests established in 2016 (38 nests, 5 sites) and 2017 (26 nests, 4 sites). To obtain bee nests from the field, the holes of the trap nests were lined with paper straws that could be 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 10–25 °C ramping diurnal cycle (dark). Eggs were used when possible, but sometimes young larvae (within 15 days of the date on which they were estimated to have been laid) were included.

The experimental protocol for transferring eggs or young larvae (henceforth "eggs") was based on methods employed in previous egg-transfer experiments (Williams 2003; Praz et al. 2008a; Haider et al. 2014; Online Resource Fig. A2). A scalpel and microscissors were used to cut the straw into individual nest cells and expose the egg and pollen provision of each cell. A metal spatula and a pair of forceps were

then used to 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 (~2 cm × 1 cm) of a wood block (~31 cm × 4 cm, with 15 wells per block). A glass coverslip was taped across the top of each well, and the front edge of the well was covered with a coverslip or a strip of paper or wood to minimize disturbance to the bees during observations.

The bees were kept in the growth chamber and assessed every other day to monitor their status (alive/dead) and stage of development. Developmental stages (illustrated in Online Resource Fig. A3) were "pre-defecating" (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" (recognized by the presence of at least one fecal pellet), "spinning" (recognized by the presence of silk threads), and cocoon (defined here as the stage at which the larva could no longer be seen through the silk). However, we consider only development to the defecating larval stage since few bees reached the later developmental stages.

Frass mass and digestive efficiency

For bees that developed in natural conditions (i.e., in trap nests in the field), we used the proportion of a brood cell that consisted of frass (bee fecal pellets) at the end of bee larval development as a simple metric of food quality (reasoning that a greater quantity of waste would be generated from lower-quality food, relative to the initial amount of food ingested; cf. Couture et al. 2016). To compare relative mass of frass among species, we weighed frass from 118 nest cells of bees in the cocoon stage, constructed in 2015 and 2016. We also measured cocoon mass from the same cell used for frass measurement (see Online Resource Table B1 for average cocoon and frass masses per species). Relative frass mass was then calculated for a given cell as frass mass divided by the sum of cocoon mass and frass mass (a proxy for the mass of the total original provision). We excluded from analysis any nest cells in which the pollen provisions had not been completely consumed (e.g. because the larva had died or because it had pupated before consuming the entire provision). Of all the cells we examined containing a bee that had survived to the pupal stage, 16% contained unconsumed pollen. This was observed for all the specialist species but was more common for Aster bees (20–35% in these species vs. only 7% for *O. iridis*).

For bees reared in the laboratory (in the egg-transfer experiment), frass samples were taken in August 2016 (for *O. montana*, *O. subaustralis*, *O. iridis*, and *O. tristella*) and August 2017 (for *O. coloradensis*) to measure the extent to which pollen grains had been effectively digested by larvae. Frass was sampled from bees that had reached the defecating larval stage and was stored in a freezer until microscope slides could be prepared. 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 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 400 × magnification and scored as "empty" (< 25% protoplasm), "partially empty" (25–50% protoplasm), "partially full" (50–80% protoplasm) and "full" (> 80% protoplasm; Online Resource Fig. C1). To account for natural rates of pollen grain abortion, pollen samples from the dominant plant species used by *Osmia* spp. at each site were collected in 2017 and approximately 300 grains were scored using the same method as the frass samples (Online Resource Table D1). Separate values for the proportion of empty and partially empty pollen grains were calculated for each plant sample. These values were then used to determine mean values for the plant taxa used by the Aster bees (*O. coloradensis*, *O. montana*, and *O. subaustralis*), the Fabaceae specialist (*O. iridis*), and the generalist (*O. tristella*), as listed in Online Resource Table D1. The percent of empty pollen grains in the frass samples was then corrected for the percent of aborted pollen grains using the following formula:

$$\text{Corrected percent empty pollen grains} = \left[\frac{\text{percent empty in frass sample} - \text{percent empty in pollen sample}}{100 - \text{percent empty in pollen sample}} \right] \times 100.$$

An analogous formula was used to obtain a corrected percent of partially empty pollen grains (i.e., < 50% full; includes pollen grains scored as "empty" and "partially empty").

Bee provision amino acid analysis

We analyzed the amino acid profiles of pollen provisions from the Aster bees (*O. coloradensis* [$n = 1$ sample], *O. montana* [3], *O. subaustralis* [3]), the Fabaceae specialist (*O. iridis* [3]), and the two generalist species (*O. tersula* [1] and *O. tristella* [1]) to test for nutrient deficiencies in the Aster provisions. Our samples were analyzed at the SPARC Bio-Centre of the Sick Kids Hospital (Toronto, ON, CA) (Online Resource Table E1, pollen provision description). Each sample underwent three analyses: a standard amino acid analysis and a free amino acid 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) or other animals (Nation 2008). 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 the Aster bees, which were solid and dry, were pulverized with a mortar and pestle; samples from the other bee species, which were more viscous, were thoroughly mixed.

Provision mass

To compare pollen foraging investment among bee species, we weighed contents of 200 completed brood cells constructed in 2016 and 2017. The wet mass of pollen provisions within brood cells (including eggs) was measured by excising individual cells from paper straw nests and weighing them (contained within their section of paper straw, with mud or leaf wall removed). Mass of an equal length of paper straw of the same diameter was subtracted. To control for differences in average bee size among species, we estimated the dry weight of each species based on average intertegular distances (ITD) of 8–10 specimens for each species. These measurements were obtained from female specimens collected from the Rocky Mountain Biological Laboratory (RMBL) and housed at RMBL or the University of Ottawa. We then used the R package 'Pollimetry' (Kendall et al. 2019) to convert these ITD averages to weight estimates. These estimates ranged by ~ 2.4-fold across the six species (Aster bees: *O. coloradensis* [0.0136 g], *O. montana* [0.0217 g] and *O. subaustralis* [0.0194 g], Fabaceae specialist: *O. iridis* [0.0188 g], and generalist species: *O. tersula* [0.0169 g] and *O. tristella* [0.00894 g]; see Online Resource Table B1).

Foraging flights

To assess the energetic cost of provisioning brood cells, we observed the number of foraging flights per completed brood cell for four *Osmia* species occupying trap nests. We observed the Aster bees *O. coloradensis*, *O. montana*, and *O. subaustralis* and the Fabaceae specialist *O. iridis*. We chose to observe only the specialist *Osmia* that occupy our trap nests because specialists may generally be more efficient than generalists in pollen collection (e.g. Strickler 1979). Observations of foraging flights were made at several established field sites where nesting was most active in June and July of 2016 and 2017. We attempted to begin observations with the first foraging flight for each cell and continue observations until the cell was complete. Where this was not possible, we recorded foraging flights for the portion of the brood cell that was observed being constructed. We were able to observe construction of 42 brood cells, 24 of these until cell completion. Cell completion was determined by observation of egg laying in nests of *O. iridis* and *O. coloradensis*, which lay their eggs on top of the provision, or excavation of the pollen-and-nectar provision in nests of *O. montana* and *O. subaustralis*, which bury their eggs within the pollen mass after completion of provisioning (Torchio 1989).

Statistical analysis

All statistical analyses were performed in R (version 3.6.1; R Core Team 2019).

Egg-transfer experiment

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 2019). The Aster bees were pooled together for both the survival and development analyses due to low sample sizes. Hazard ratios were calculated for each bee species (or species group for the Aster bees) and represent the proportional risk of either dying (for the bee survival analysis) or reaching the defecating stage of development (for the development analysis) for bees reared on different provision types. Cluster terms were included in the model for nest identity. These terms are used to compute a robust variance for the model by accounting for non-independence and correlation among observations. Additional fixed (categorical) factors were initially included in the models for experiment year (2016 or 2017; included for Aster bees and *O. iridis* since these bees were sampled in both years), transfer status (egg or larva; included for all bees) and bee species (*O. coloradensis*, *O. montana*, or *O. subaustralis*; Aster bees), but these terms were later removed since none of them were significant (all $P > 0.1$). Several models showed some deviation from the assumption of proportional hazards (Therneau 2019); hazard ratios should, therefore,

be interpreted as weighted averages of the true (possibly time varying) hazard ratios over the entire experiment period (Stensrud and Hernán 2020).

Frass mass and digestive efficiency

We constructed linear mixed-effect models (LME) to compare frass mass among bee species. We used the 'lme4' package in R (Bates et al. 2015) to build this model and we included nest identity as a random factor to address the lack of independence among brood cells from the same nest. We excluded *O. tristella* from the analysis due to low sample size. The dependent variable (frass mass as a proportion of total cell mass) was square-root-transformed to meet assumptions of normality. Replicates were the measurements of different nest cells for each species. The significance of the fixed factor, 'bee species', was determined using the 'anova' function in the package 'lmerTest' (Kuznetsova et al. 2017). We examined differences between bee species using multiple comparisons (function: 'glht', Hothorn et al. 2008).

We compared the proportion of digested pollen grains in frass samples using binomial generalized linear models with bee species as an explanatory variable. We used the 'glm' function from the 'stats' package to compare the proportion of pollen grains in frass that were "empty" (i.e., containing < 25% protoplasm) or "partially empty" (i.e., containing < 50% protoplasm; includes pollen grains scored as "empty" and "partially empty") between the Aster bees (i.e., *O. montana* and *O. subaustralis*), the Fabaceae specialist *O. iridis*, and the generalist *O. tristella* fed their host provisions. The Aster bee *Osmia coloradensis*, was omitted from these analyses since none of the bees fed host pollen reached the defecating larval stage. The two remaining Aster bees were pooled due to small sample sizes.

Two additional binomial generalized linear models were run for the bees fed Fabeae pollen provisions to compare the proportions of digested pollen grains (i.e., those < 25% and < 50% full) among the same species with the addition of the Aster bee *O. coloradensis*. Due to small sample sizes, the Aster bees were also pooled for these analyses. All four models were run using the quasibinomial family to address overdispersion in the data.

The results for the proportion of "empty" (i.e. < 25% full) pollen grains are presented both for bees fed host provisions and bees fed Fabeae provisions. Comparisons of the proportion of "partially empty" (i.e. < 50% full) pollen grains for bees fed both host and Fabeae pollen provisions and the Z scores from all models are provided in Online Resource Fig. G1 and Table G2, respectively.

Amino acid analysis

The amino acid profiles (bound plus free amino acids) for the pollen provisions of the six bee species were compared using non-metric multidimensional scaling (NMDS) with the 'metaMDS' function in the 'vegan' package (Oksanen et al. 2019). Two NMDS ordinations were performed, both using Manhattan dissimilarity matrices, two dimensions, and 20 runs—parameters sufficient to reach a stress level < 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 bees (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 sample sizes. Prior to these analyses, we verified homogeneity of group covariances using permutational multivariate homogeneity of group dispersions tests (PERMISP; 1000 permutations) with the 'betadisper' function. 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) 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 2020; Bonferroni-corrected) were then performed to compare each group with the EAA requirements for honeybees.

Provision mass

We constructed linear mixed-effect models (LME) to compare provision mass among bee species (see "Frass mass and digestive efficiency" for model description). We excluded *O. tersula* from the analysis due to low sample size. The dependent variable (wet pollen-provision mass) was square-root-transformed to meet assumptions of normality. Replicates were individual pollen provisions for each species. Differences between bee species were examined as for frass mass.

Foraging flights

We compared the number of pollen foraging flights until brood cell completion between the Aster bees (*O. coloradensis*, *O. montana*, and *O. subaustralis*) and the Fabaceae specialist *O. iridis* species using a Cox proportional hazard model. This method allowed us to include brood cells that were not completed during field observations (i.e., censored data). Replicates were observations of brood cell completion by individual mother bees. We again included a cluster term to account for multiple brood cells constructed by the same bee.

Results

Egg-transfer experiment

During the study, 178 bees were transferred (131 eggs, 47 larvae) and 106 (72 eggs, 34 larvae) survived the transfer and were included in the analysis. One bee that was apparently killed by a parasite during development was excluded. The mean age of bees at transfer differed by no more than 3.1 days among species and did not differ among provision treatments. None of the bees that died before cocoon construction ran out of food, suggesting that differences in survival between pollen treatments cannot be attributed to a lack of available food.

Aster bees

For the Aster bees, there was no difference in survival across pollen treatments (Fig. 1, Online Resource Fig. F1 and Table F2). In total, 28 (of 52) Aster bees survived until the end of the experiment: 13 (of 27) bees on Aster, 12 (of 20) bees on Fabeae, and 3 (of 5) bees on Various pollen provisions. In addition, there was no difference in development to the defecating larval stage across pollen treatments, with 12 (of 27) bees on Aster, 12 (of 20) bees on Fabeae, and 2 (of 5) bees on Various pollen provisions reaching the defecating larval stage. Although there was no significant effect of bee species in either model, *O. coloradensis* only survived and developed on Fabeae pollen provisions, while *O. montana* and *O. subaustralis* survived on all three pollen types and developed on both host and nonhost provisions.

Other bee species

Survival and development for *O. iridis* and *O. tristella* were significantly reduced on Aster provisions (0 of 24 bees and 0 of 4, respectively) compared to Fabeae (18 of 18 bees, 3 of 3) and Various provisions (4 of 4 for *O. tristella*; Fig. 1, Online Resource Fig. F1 and Table F2). The Various pollen treatment was excluded from the analysis for *O. iridis* due to low sample size, but the single bee in this treatment survived and reached the defecating stage. Survival was comparable on Fabeae and Various provisions for *O. tristella*, but development was significantly greater on Fabeae provisions, though the effect size was rather small (hazard ratio = 1.35).

Frass mass and digestive efficiency

Mass of frass as a proportion of total cell mass differed significantly among species ($F_{4,\ 15.7} = 22.50$, $P < 0.001$, Fig. 2a). Pairwise comparisons showed that the three Aster

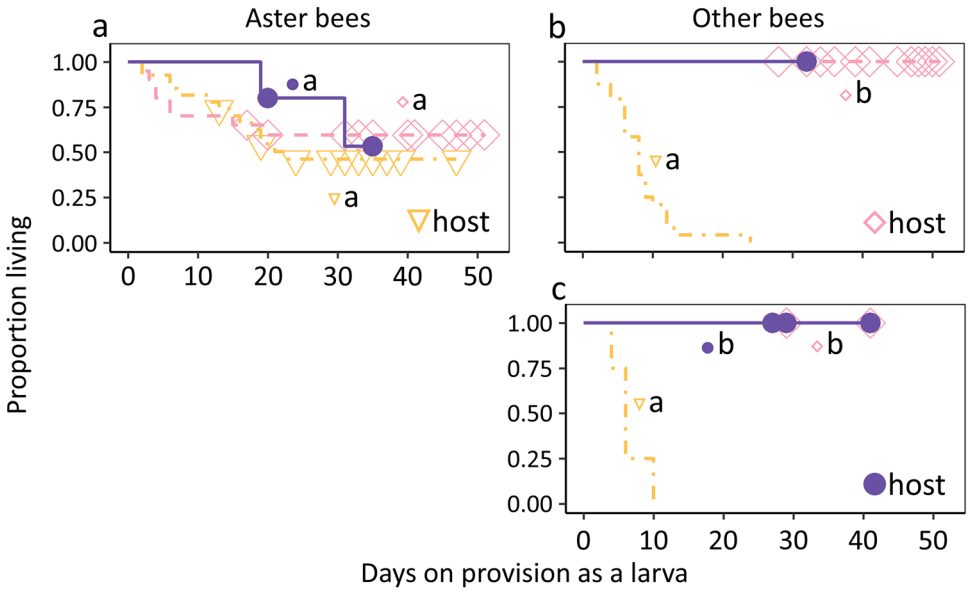

Fig. 1 Survival curves for larval bees provided Aster ("open triangles"), Fabeae ("empty diamond") and generalist ('Various'; "filled circles") pollen provisions. Bee species include the Aster bees **a** ($n = 52$): *Osmia coloradensis* (21), *O. montana* (11), and *O. subaustralis* (20), and the other bee species **b** *O. iridis* (43) and **c** *O. tristella* (11). Host labels indicate the pollen provision type of each bee species and letters indicate significant differences between pollen treatments. The Various pollen treatment was excluded from the analysis for *O. iridis* due to low sample size ($n = 1$). Bees alive at the end of the experiment are represented by the symbols. Colour figure available in the online version

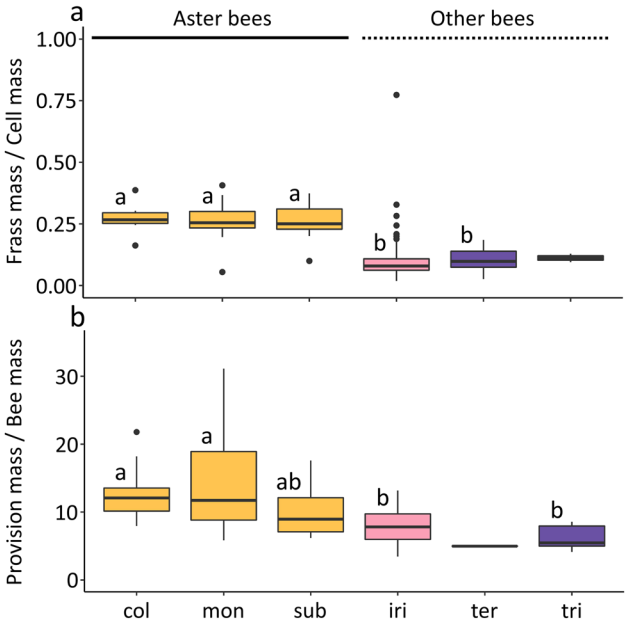

Fig. 2 The relative mass of **a** frass produced by larval bees and **b** the pollen provision for six bee species [Aster bees: "col" = *O. coloradensis* ($n = 8$, $n = 49$), "mon" = *O. montana* (25, 13), and "sub" = *O. subaustralis* (17, 18) and other bee species: "iri" = *O. iridis* (56, 108), "ter" = *O. tersula* (10, 2), and "tri" = *O. tristella* (2, 9)]. To control for interspecific differences in adult body sizes, frass mass is expressed as a proportion of the total mass of the nest cell (i.e. mass of the cocoon and frass combined). The mass of the pollen provision is expressed relative to the estimated dry weights for adults of each species (see text). 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. The generalist species were each omitted from one analysis due to low sample size. Colour figure available in the online version

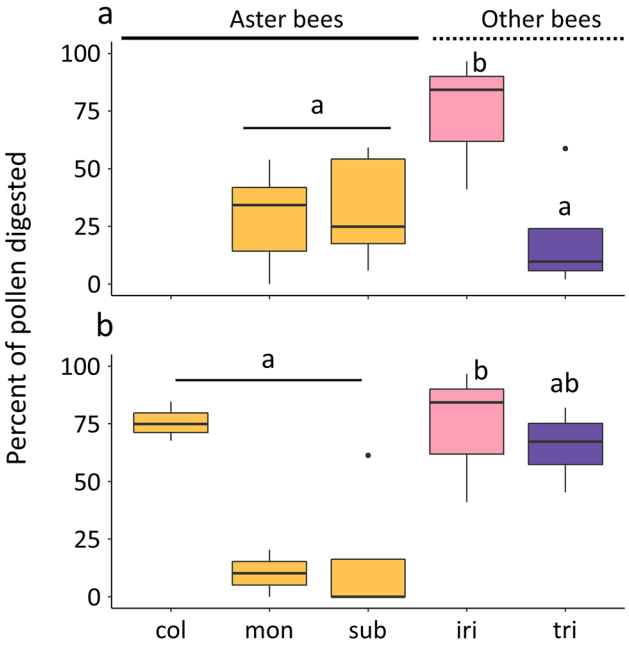

Fig. 3 The percentage of digested pollen grains (i.e., that were less than 25% full) in the frass samples of bees fed **a** their host pollen provision and **b** Fabeae pollen provisions. Bee species include the three Aster bees: "col" = *O. coloradensis* (0, 3), "mon" = *O. montana* (5, 2), and "sub" = *O. subaustralis* (8, 5) and the two other bee species: "iri" = the Fabeae (Fabaceae) specialist *Osmia iridis* (5, 5) and "tri" = the generalist species *O. tristella* (4, 4). None of the *O. coloradensis* bees on host pollen reached the defecating larval stage. Boxes and whiskers as in Fig. 2. Letters indicate significant differences between bee species (the Aster bees were pooled together in the analysis). Colour figure available in the online version

bees (*O. coloradensis*, *O. montana*, and *O. subaustralis*) all produced significantly more frass (relative to cocoon mass) than the generalist species (*O. tersula*) and the Fabaceae specialist (*O. iridis*). There were no significant differences in mass of frass among the Aster bees or among the other bee species (Fig. 2a). *Osmia tristella* was omitted from the analysis due to low sample size, but its relative frass mass was similar to that of the other generalist species, *O. tersula* (Fig. 2a).

Frass samples from the Fabaceae specialist, *O. iridis*, had significantly more "empty" pollen grains (i.e. < 25% full) than those of the Aster bees (*O. montana* and *O. subaustralis*) and the generalist, *O. tristella*, when all species were fed their host provisions (Fig. 3a, Online Resource Table G2). There was no difference in the proportion of "empty" pollen grains in the frass between *O. tristella* and the Aster bees.

In a comparison of bee species reared on Fabeae (Fabaceae) pollen provisions, the frass samples of *O. iridis* (the Fabeae specialist) contained significantly more "empty" pollen grains than did frass from the Aster bees (*O. coloradensis*, *O. montana* and *O. subaustralis*; Fig. 3b, Online Resource Table G2). No difference in the proportion of empty pollen grains was observed between frass of *O. tristella* and *O. iridis* or frass of *O. tristella* and the Aster bees.

Pollen provision amino acids

The pollen provision samples from every bee species contained all the essential and nonessential amino acids that were assessed. There were no differences between the provisions of the Aster bees and those of the other bee species in terms of absolute amounts of amino acids (i.e., µg/mg of dry sample; perMANOVA, $R^2 = 0.10$, $F_{1, 10} = 1.14$, $P = 0.33$; Fig. 4, Online Resource Table H1 and Fig. H2) or their relative amounts (perMANOVA, $R^2 = 0.13$, $F_{1, 10} = 1.55$, $P = 0.19$; Online Resource Fig. H3). There were marginal differences in the relative amounts of essential amino acids (perMANOVA, $R^2 = 0.43$, $F_{2, 10} = 3.79$, $P = 0.047$; Online Resource Fig. H4), but only between the Aster bees and other bee species (pairwise comparisons, $P = 0.042$); neither provision type differed significantly from the essential

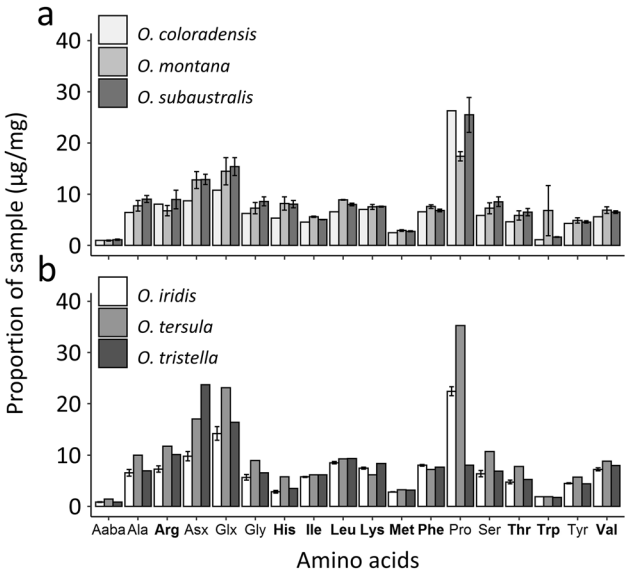

Fig. 4 The mean percent ± SEM quantities of essential amino acids (EAA) and nonessential amino acids (NAA) (including both protein-bound and free amino acids) in the pollen provisions of six bee species **a** Aster bees: *O. coloradensis* ($n = 1$), *O. montana* (3), and *O. subaustralis* (3); **b** other bee species: *O. iridis* (3), *O. tersula* (1), and *O. tristella* (1) 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, *Tyr* tyrosine)

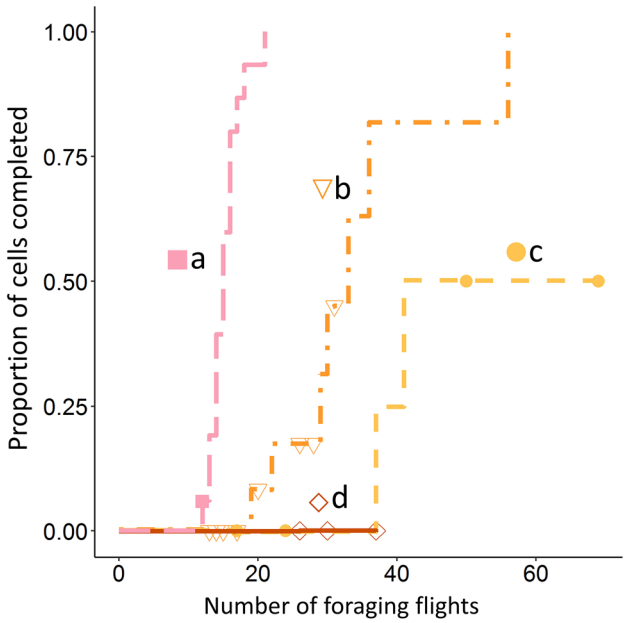

Fig. 5 Inverted survival curves for the number of foraging flights to brood cell completion in the 2016 and 2017 sampling years. Four bee species were observed including the Aster bees *Osmia coloradensis* (16; "open triangle"), *O. montana* (6, "filled circle"); and *O. subaustralis* (3, "open diamond"); and the Fabaceae specialist *O. iridis* (17; "filled square"). The symbols illustrate brood cells that were not observed to completion. Letters indicate significant differences between bee species. Colour figure available in the online version

amino acid requirements for honeybees (pairwise comparisons: honeybees and Aster bee provisions $P = 0.69$; honeybees and other bee species provisions $P = 1.00$). These marginal differences between the Aster bees 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, $R^2 = 0.28$, $F_{2, 10} = 1.97$, $P = 0.10$).

Provision mass

Provision mass (divided by estimated adult body mass) differed significantly among species ($F_{4, 44.4} = 7.83$, $P < 0.001$; Fig. 2b). Pairwise comparisons among species showed that the Aster bees *O. coloradensis* and *O. montana* collected significantly larger provisions relative to body size than *O. iridis* (Fabaceae specialist) and the generalist *O. tristella* (Fig. 2b). *Osmia tersula* was omitted from the analysis due to low sample size, but its provision mass was similar to that of *O. tristella*, the other generalist species (Fig. 2b).

Foraging flights

We observed construction of 42 brood cells during our observations of pollen foraging by the Aster bees *O. coloradensis* (16 brood cells constructed by 11 individual bees), *O. montana* (6, 6) and *O. subaustralis* (3, 3), and the Fabaceae specialist *O. iridis* (17, 13).

The number of pollen foraging flights per brood cell differed significantly among species (Fig. 5, Online Resource Table I1). The Fabaceae specialist *O. iridis* made significantly fewer flights per brood cell (max. 21) than the Aster bees *O. coloradensis*, *O. montana*, and *O. subaustralis.* This result actually underestimates the difference between *O. iridis* and the Aster bees, because the latter often spent more than 1 day provisioning a single cell and we were therefore rarely able to observe brood cell completion for these bees. Maximum observation time for an Aster bee without cell completion was 15 h, during which 69 foraging flights were completed. Among the Aster bees, *O. coloradensis* made significantly fewer flights per cell than *O. montana* and *O. subaustralis*. In addition, *O. montana* made fewer foraging flights than *O. subaustralis*, and none of the three *O. subaustralis* bees was observed until cell completion.

Discussion

Pollen provisions from *Osmia* species that are Asteraceae specialists were inadequate larval diets for *Osmia* species that do not normally use this pollen type. However, the three Aster bee species we studied could survive and develop on nonhost pollens. We found that the Aster bee provisions were not amino acid deficient, although two of these species (*O. coloradensis* and *O. montana*) collected significantly larger pollen provisions than the Fabaceae specialist (*O. iridis*) and generalist species (*O. tristella*), and all Aster bees made more foraging flights than *O. iridis* to complete brood cells. The numbers of foraging flights per brood cell we recorded among Asteraceae specialists are some of the highest reported for any bees (reviewed by Franzén and Larsson 2007; Neff 2008). The differences we observed in provision mass and number of foraging flights per brood cell seem unrelated to the floral densities of host plants, which varied by orders of magnitude through time and across sites (unpublished data). In addition, the larvae of the Aster bees produced significantly more frass than the other bee species, but they had a similar ability to digest their host provisions compared to the other bee species (at least as reflected by removal of pollen grain contents). *Osmia iridis* and *O. tristella* may have been unable to survive on Aster provisions due to difficulties with digestion and nutrient assimilation, but, unfortunately, their digestive efficiency on Aster provisions could not be examined since both species died before reaching the defecating larval stage. Overall, these results suggest that (a) Aster bees do not specialize on Asteraceae pollen because their larvae require it for development; (b) the poor quality of Asteraceae pollen provisions for nonspecialists is due to something other than low protein content; and (c) Aster bees compensate for low pollen quality by providing relatively larger provisions that require more foraging flights and generate more waste than other pollen types. We discuss each of these conclusions in more depth below.

Larval performance on host and nonhost provisions

All three Aster bees 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 findings of two previous studies. First, larval survival of the Asteroideae (Asteraceae) 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). Second, the Heliantheae (Asteraceae) specialist *O. californica* grew larger when fed a diet containing some nonhost pollen (*Phacelia tanacetifolia*, Boraginaceae) than when reared on a 100% host pollen diet—yet adult *O. californica* ceased nesting rather than collect nonhost pollen when their host plants were unavailable (Williams 2003). Similarly, *H. truncorum* bees refused to collect either *Echium* or *Campanula* pollen even in the absence of host plants, suggesting that host selection could also be neurologically constrained in this species (Praz et al. 2008b). Together, these results suggest that the larval diet breadth of Asteraceae specialists often exceeds the host range of the adult bees, which is inconsistent with the "mother-knows-best" hypothesis and suggests that other hypotheses (e.g. involving enemy-free space) must be invoked instead.

Aster bees also seemed to develop more slowly on their host pollen than the other bee species reared on their host provisions. For example, the Aster bees *O. montana* and *O. subaustralis* reared on host pollen required on average 27 and 36 days, respectively, after hatching or transfer (for bees transferred as larvae) just to reach the defecating larval stage (Online Resource Fig. F1). Conversely, *O. iridis* (Fabaceae specialist) and *O. tristella* (generalist) bees fed host pollen had reached the defecating stage after an average of only 19 and 12 days, respectively.

For unknown reasons, larvae of *O. coloradensis*, despite this species being an Asteraceae specialist, did not develop on Aster provisions, and the average lifespan of *O. coloradensis* bees fed Aster provisions was less than 16 days. It is unclear why *O. coloradensis* larvae failed to develop on their host pollen during the experiment, since they can evidently develop successfully in the field; we can only speculate that the eggs or larvae of this species were particularly sensitive to the experimental conditions when placed on this (dry and crumbly) provision type. Interestingly, data from unmanipulated nests collected from a single field site in 2017 suggest that *O. coloradensis* bees also develop more slowly than the Fabaceae specialist *O. iridis*. For example, none of 33 surviving *O. coloradensis* bees from five nests initiated within a 2-week period in June 2017 had progressed beyond the defecating stage by 6 Aug 2017, while, of 48 surviving *O. iridis* bees from 18 nests initiated during the same 2-week window, 19 (40%) had reached the cocoon-spinning stage or even completed cocoon construction by the same date. 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.

The other bee species in our study (i.e., *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 Asteraceae 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 generalist blue orchard bee, *O. lignaria*, resulted in longer development and reduced larval mass. Heliantheae pollen was also rejected by nesting *O. lignaria* (Williams 2003). Together, these results suggest that physiological adaptation of some sort or, perhaps, possession of a specialized gut microbiota (see next section), is necessary to tolerate the unfavourable qualities of Asteraceae pollen.

Digestibility and nutrient content of pollen provisions

Pollen digestibility

Our results suggest that Aster provisions are not difficult for the Aster bees (*O. montana* and *O. subaustralis*) to digest. However, we could not compare their ability to digest Aster provisions to that of other bee species, since none of the latter species lived long enough on this provision type to defecate. It is therefore possible the non-Aster bee species (*O. iridis* and *O. tristella*) could not survive on Aster provisions due to difficulties with digestion and nutrient assimilation. The Fabaceae specialist, *O. iridis*, may also possess adaptations that increase its ability to digest its host pollen (Fabeae), since this species digested Fabeae provisions more completely than the Aster bees (though not *O. tristella*). Interestingly, the *Cephalosmia* species (*O. montana* and *O. subaustralis*) seem to have more difficulty digesting Fabeae provisions than their host Aster provisions, possibly due to adaptations to physiologically tolerate their host pollen.

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 (*Helianthus annuus*, Asteroideae) pollen could impede digestion. The thickness of the intine, the inner pollen wall that covers the germinal pores, can also influence digestion of pollen grains by bees. 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, intine disruption at the germinal pores prior to consumption could facilitate digestion. Alexander (1980) noted that several Asteraceae species produced pollen grains with thicker, more impervious pollen walls than those of Fabaceae species; so, thicker pollen walls may play a role in the relative indigestibility of Asteraceae pollen. In addition, differences in the amount of nectar (or sugar) added to pollen provisions might influence the degree of protoplasmic extrusion and, consequently, subsequent larval digestion (e.g. Turner 1984; mentioned by Dobson and Peng 1997). There could be differences in the volume of nectar added to pollen provisions among bee species, since the provisions of the Aster bees tend to be dry and crumbly and the provisions of the other bee species (*O. iridis*, *O. tersula* and *O. tristella*) are wetter and stickier. In the future, it would be interesting to examine provision samples from each bee species prior to consumption for signs of intine disruption and protoplasmic extrusion.

Finally, it is possible that bee gut microbiota play a role in pollen grain digestion, and that the gut microbial community of Aster bees facilitates nutrient extraction from Asteraceae pollen specifically. Solitary bees lack the specialized, vertically transmitted core microbiota of social bees, but they acquire a gut microbiota from their environment, particularly from the pollen provision (Voulgari-Kokota et al. 2019b). Gut bacteria contribute to pollen digestion in honeybees (Engel et al. 2012; Lee et al. 2015), and they may play a similar role in *Osmia* (Voulgari-Kokota et al. 2019b). In addition, pollen microbes were recently found to be a significant protein source for bees (Steffan et al. 2019) and to play an important role in larval bee development (Dharampal et al. 2019). In our study, bees reared on nonhost pollen would also have been exposed to any pollen-associated microbes, so the failure of non-Aster bees to develop on Asteraceae pollen provisions cannot be due to lack of access to Asteraceae-associated microbes. However, guts of Aster bee larvae may be uniquely able to support Asteraceae-associated microbes (see Voulgari-Kokota et al. 2019a) that might aid in Asteraceae pollen digestion. At present, we know too little about the solitary bee gut microbiota to judge whether this is likely.

Pollen nutrient content

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

Previous studies have reported amino acid deficiencies in the pollen of several 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). However, in this study, the concentrations of total amino acids

and essential amino acids were comparable between the provisions of Aster bees and the other bee species (Fig. 4, Online Resource Table H1). We suspect that this apparent contrast is driven by the Aster bees adding less nectar to their provisions than do the other bee species. Thus, the pollen and protein content in the provisions of the other bee species may be more diluted—resulting in similar amino acid levels in provisions across all six species. This contrast could also be driven by the microbial community on the pollen provisions, which can be an important protein source for bees (Steffan et al. 2019). Regardless, given the similar amino acid contents of the larval provisions, it is unlikely that amino acid deficiencies are the reason the Fabaceae specialist *O. iridis* and the generalist *O. tristella* could not survive and develop on Aster provisions.

However, the higher foraging investment per brood cell by the Aster bees and the larger pollen provisions of two Aster bee species (*O. coloradensis* and *O. montana*) could be a means to compensate for other nutritional deficiencies, or, perhaps, for less effective nutrient extraction or assimilation from Asteraceae pollen. Consequently, comparative studies that examine other pollen constituents are required (e.g. secondary metabolites: Detzel and Wink 1993; sterols: Vanderplanck et al. 2014; fatty acids: Ruedenauer et al. 2020). For example, the lipid or sterol content of Asteraceae pollen might help explain why it is an inadequate diet for bees that are not Asteraceae specialists (see e.g. Vanderplanck et al. 2018). Secondary metabolites occurring in pollen of other plant taxa can affect survival of bee larvae (Arnold et al. 2014; Trunz 2017) and foraging preferences of adults (Wang et al. 2019), although such compounds have not been shown to be responsible for the low dietary quality of Asteraceae pollen (Vanderplanck et al. 2018). Recent studies have also found that nutrient ratios—specifically protein:lipid ratios (Vaudo et al. 2016, 2020)—play a role in pollen preferences and development of generalist bees. Future research is needed to improve our understanding of the nutritional requirements and tolerances of different bee species across the pollen specialization spectrum (Vaudo et al. 2015).

Why specialize on Asteraceae pollen?

Our results show that specializing on Asteraceae pollen has not reduced the ability of *Osmia* bees to tolerate other pollens and that the dietary breadth of Aster bee larvae may be larger than the host range of the adults. However, since the foraging decisions of mother bees ultimately determine the pollen composition and nutritional quality of offspring provisions, future 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).

Neurological constraints, such as sensory limitations (reviewed by Bernays 2001 in the context of phytophagous insects) could restrict the host range of specialist bees (Praz et al. 2008b). Indeed, research on phytophagous insects has shown that behavioural traits (e.g. host acceptance), rather than morphological or physiological traits, are most often the mechanisms maintaining specialization (Futuyma and Moreno 1988; Forister et al. 2012). Selection may have favoured specialization on Asteraceae pollen for a variety of reasons including the accessibility and availability of floral resources (i.e., the 'predictable plethora' hypothesis, Wcislo and Cane 1996), or to escape natural enemies (e.g. heterospecific competitors: Thorp 1969). In general, the combined effects of interactions with multiple species (e.g. hosts, competitors, and predators) are likely to shape the evolution of ecological specialization (reviewed by Forister et al. 2012). Although a complete escape from competitors is unlikely since generalists also tend to visit plants associated with specialist bees (Minckley and Roulston 2006; Wcislo and Cane 1996), 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 bees (same species as in this study) were not attacked as frequently by kleptoparasitic wasps (*Sapyga* sp., Sapygidae) as other bee species (*O. iridis*, *O. tersula*, and *O. tristella*), and that the wasps could not develop on Aster provisions. Since pollen consumption by adult bees is necessary for egg maturation (Cane 2016), another possible explanation is that Asteraceae pollen improves the mother's brood production, similar to what was observed by Scheirs et al. (2000) in a dipteran leaf-miner. However, this suggestion is purely speculative. Further research will be needed to improve our understanding of the drivers of Asteraceae pollen specialization in bees.

Conclusion

In this study, we found that Aster bees could develop on nonhost provisions, suggesting that these species do not specialize on Aster pollen because it is required for larval development and that the larvae can tolerate a wider range of pollens than the host range of the adults. Aster provisions were inadequate larval diets for both *O. iridis* and *O. tristella*, but their poor performance on this pollen diet was likely not due to amino acid deficiencies. We also found that Aster bees collect relatively larger provisions that require more foraging flights and produce more larval waste, possibly to compensate for low pollen quality. Future studies focused on other nutritional constituents or nutrient ratios could help shed light on the observed differences in performance across the provision types.

Understanding the nutritional requirements and 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; Vanderplanck et al. 2017), 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). Loss of floral diversity is of special concern for specialized bee species, notably those species that diapause and cease nesting in the absence of their host plants (e.g. Bohart and Youssef 1976; Minckley et al. 1994), since their narrow host ranges could put them at greater risk than generalists of local extinction during host shortages (Gathmann and Tscharntke 2002).

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Author contribution statement MKM, JRKF and SZK conceived, designed and conducted the study. MKM and SZK analyzed the data with guidance from JRKF. MKM prepared the manuscript. JRKF and SZK provided editorial advice.

Data availability Data related to this manuscript are available here: https://doi.org/10.17605/OSF.IO/R4EQ3.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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