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Use of Low Quality Pollen by Asteraceae-Specialist *Osmia* Mason Bees
(Hymenoptera: Megachilidae)

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**RESEARCH ARTICLE: Use of Low Quality Pollen by Asteraceae-Specialist *Osmia* Mason
Bees (Hymenoptera: Megachilidae)**

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Running head

Use of Low Quality Pollen

Keywords: Oligolecty, protein, Asteraceae, pollen, *Osmia*

Abstract:

The need for plants to protect their pollen from herbivory has contributed to pollen sources with wide variation in quality, including toxicity and low nutrient composition. With differing qualities of pollen, many bees have adapted to tolerate nutrient poor or toxic pollen. This contributes to pollen specialization, as bees develop physiological mechanisms to digest specific pollen. Among bees, oligolecty (use of pollen from only a few related species) has arisen multiple times. Within oligolectes, specialization on the low protein pollen from the plant family Asteraceae is common. This specialization has arisen multiple times, however, non-specialists appear to have a difficult time using the pollen. To compensate for the low quality of Asteraceae pollen, we expected that Asteraceae-specialist bees would make more foraging trips, brood cell provisions would be larger, and that consumption of this low quality food would produce more frass compared to bees using other pollens. Here we assessed the implications of specialization on Asteraceae pollen by comparing the number of foraging flights required to provision brood cells among Asteraceae-specialist *Osmia* and one Fabaceae-specialist *Osmia*. We compared size of brood provisions using mass of wet pollen and frass among six species of *Osmia*, including three Asteraceae specialists, one Fabaceae specialist, and two generalists. Mass of pollen was not significantly different among species. Mass of frass was significantly higher for Asteraceae specialist than bees using other pollens ($p < 0.001$). Asteraceae-specialist bees required significantly more foraging flights to provision brood cells than the Fabaceae specialist ($p < 0.001$). Although Asteraceae specialists made more foraging flights, there seemed to be no connection to brood cell pollen mass, so questions still remain regarding the benefits of the energetic output of significantly more foraging flights. Significant differences among species in

69 terms of mass of frass suggests that more waste is created by bees using low protein pollen
70 compared to bees using other pollens.

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Introduction:

Flowering plants and bees both rely on pollen for their reproductive success. Because plants need pollen to come into contact with pollinators, it must be accessible, but this also leads to pollen consumption by pollinators and non-pollinators alike. The amount of pollen collected by bees for food can be huge compared to the pollen that contributes to pollination (Schlindwein et al. 2005). This drives plant selection towards protection of pollen through wide variation in pollen quality. These variations include differing levels of toxicity or nutritional quality (Praz et al. 2008). With differing qualities of pollen, bees have adapted to tolerate nutrient poor or toxic pollen. This contributes to pollen specialization, as bees develop physiological mechanisms to digest specific pollen (Roulsten et al. 2000).

Among bees, oligolecty (use of pollen from only a few related species) has arisen multiple times (Müller 1996, Wcislo and Cane 1996). Within oligolectes, many specialize on pollen from the plant family Asteraceae. This pollen is known to be low in protein compared to other pollens and may lack essential amino acids (Levin and Haydak 1957, Roulston and Cane 2000, Müller and Kuhlmann 2008, Praz et al. 2008, Tasei and Aupinel 2008). Pollen is the only source of protein for bees and the protein content of pollen is important because it is the chemical constituent of pollen that most strongly influences the performance of consumers (Roulston and Cane 2000). Many studies have suggested that protein content of pollen may influence the number of foraging trips a bee must make in order to provision enough food for offspring dietary requirements, though this has not been quantitatively studied (Danforth 1990, Neff and Simpson 1997, Roulston and Cane 2000, Roulston et al 2000).

Despite many independent origins of Asteraceae specialization across clades, Asteraceae pollen appears to be difficult to use for bees not specialized on the family, including honeybees,

bumblebees, and polylectic (pollen-generalist) solitary bees (Levin and Haydak 1957, Müller 1996). Additionally, analysis of pollen use by bee species in the genus *Colletes* showed that most of the polylectic species examined do not collect any Asteraceae pollen (Müller and Kuhlmann 2008). Non-Asteraceae specialist bees experimentally reared on Asteraceae pollen have lower larval mass, longer development time, and decreased survivorship (Levin and Haydak 1957, Guirguis and Brindley 1974, Williams 2003).

Specialization in general is assumed to be efficient (Lovell 1913, MacArthur and Pianka 1966, Strickler 1979). In terms of Asteraceae pollen use, benefits of specialization include the family's ubiquity in most habitats, with flowers that provide large amounts of pollen and nectar. Many plants in Asteraceae bloom throughout the growing season providing a reliable source of food. Specialization on low quality foods can help release animals from competition (Linsley 1958, Michener 1979). Additionally, use of low quality or toxic food can serve as a protection against natural enemies. In the case of *Osmia* mason bees (Hymenoptera: Megachilidae), this specialization appears to protect against brood parasitism by sapygid wasps. The wasps both avoid nest sites of Asteraceae specialists, and their development is significantly reduced when experimentally reared on Asteraceae pollen (Spear et al. 2016). The exact mechanism for reduced development of sapygids on Asteraceae pollen is not known, but low nutrient composition of Asteraceae pollen is suggested. The costs of Asteraceae specialization, however, are largely unexplored.

Given all the evidence that Asteraceae pollen is low in protein and difficult to use, we investigate how so many bee lineages have evolved to specialize on it, and how they compensate for its nutritional deficiencies. Because protein is such an important driver of development, specialization on Asteraceae pollen is likely to require more time and effort due to the need to

compensate for the low quality pollen by making more foraging flights to provision a larger quantity of pollen.

We hypothesized that Asteraceae-specialists *Osmia* bees will compensate for low food quality by providing a high quantity of pollen. We predicted that 1) to offset the nutritional deficit in Asteraceae pollen, Asteraceae-specialist *Osmia* bees would provision brood cells with more pollen relative to their body mass compared to bees using other pollens, 2) that Asteraceae-specialist bee larvae would produce more frass as an effect of both a larger provisions, and their low quality food source which produces more waste, and 3) Asteraceae-specialist *Osmia* would make more foraging flights per brood cell provisioned than other specialists using pollen with higher protein content.

Methods:

Study System

Cavity-nesting bees, including many species of *Osmia*, use naturally occurring holes in trees and other woody material, but will voluntarily occupy trap nests (wooden blocks with drilled holes lined with paper straws). Trap nests were established at several sites around the Rocky Mountain Biological Laboratory, Crested Butte, Colorado in 2013 and 2014 (Figure 1). Among other species, trap nest occupants include *Osmia (Helicosmia) coloradensis*, *Osmia (Cephalosmia) montana*, and *Osmia (Cephalosmia) subaustralis*, all specialists on the pollen of plant family Asteraceae; *Osmia (Hapsidosmia) iridis*, a specialist on the pollen of the legume tribe Fabeae (Forrest & Chisholm, submitted); and *Osmia (Melanosmia) tristella* and *Osmia (Melanosmia) tersula*, both pollen generalists.

Mass of provisions

To compare mass of provisions among species, we weighed contents of brood cells relative to body mass for all six species. The wet mass of pollen provisions within brood cells was measured by excising individual cells from paper straw nests and weighing them (contained within their section of paper straw). Mass of an equal length of paper straw of the same diameter was subtracted. Mass of the mud or leaf wall separating individual brood cells was also subtracted from the brood cell mass after the bee had died or spun its cocoon and the wall could be extracted. As a relative measure of body mass, we used average dry mass of female adults of each species, obtained using specimens from the Rocky Mountain Biological Laboratory Natural History Collections.

Mass of frass

To compare mass of frass among species, we weighed frass from the previous year's nests. As a relative measure for body mass, we used cocoon mass from the same cell used for frass measurement. In some cases, bees had not consumed all of the pollen provisioned in their cells. We excluded these nest cells so that analysis included only measurements of frass and no pollen.

Foraging flights

To assess energetic cost of provisioning brood cells, we observed number of foraging flights per brood cell completed for four *Osmia* species occupying trap nests. We observed three Asteraceae-specialist species and one Fabaeae-specialist species. We chose to observe only the specialist *Osmia* that occupy trap nests to control for the fact that specialist may be more efficient than generalists in pollen collection. Observations of foraging flights were made at several established field sites where nesting was most active in June and July of 2016. We attempted to begin observations with the first foraging flight for each cell and continue

observations until the cell was complete. Where this wasn't possible, we recorded foraging flights for the portion of the brood cell that was observed being constructed. 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 by excavation of the pollen provision in nests of *O. montana* and *O. subaustralis*, which bury their eggs within the pollen mass (Torchio 1989).

Data Analysis

Analyses were performed in R, version 3.2.2 (R Core Team 2013). Brood cells from the same nest, and nests constructed by the same individual bee, could not be considered independent data points. To address the lack of independence in analyses of provision mass and frass mass, we constructed linear mixed effect models (LME) using the lme4 package in R (Bates et al. 2014) with nest identity and mother bee identity as nested random effects. We selected the appropriate random-effects structure based on Akaike Information Criterion (AIC). Fixed effects in our model were mass of frass or pollen provision controlled for bee size and *Osmia* species. For wet mass of pollen provision, the dependent variable (wet pollen mass as a proportion of mean dry species mass) was square root transformed to meet assumptions for normality. For frass mass, the dependent variable (frass mass as a proportion of total cell mass) was square root transformed to meet assumptions of normality. Replicates were the multiple measurements of different pollen provisions for each species. For both wet pollen and frass models, significance of fixed factors was determined using the "anova" function in the package lmerTest (Kuznetsova). Differences among species were assessed subsequently by individual pairwise comparisons using the "summary" function.

Because brood cell completion was not always observed in the field, differences among species in terms of numbers of foraging flights per brood cell were tested using a Kaplan Meier

estimator (Kaplan and Meier 1958) calculated by the R package survival (Therneau 2014). For this analysis, cells not completed in the observation period were considered surviving. Differences between the four observed species were assessed with individual pairwise comparisons. Replicates were multiple observations of brood cell completion by multiple individual bees; for this analysis, we did not account for this non-independence.

Results:

We measured pollen provisions from 67 nests constructed in 2016. A linear mixed effects model (LME) comparing mean adult bee mass by species divided by provision mass among species showed that there were no differences among species ($F = 2.1019$, $df = 5$, 17.313 , $p = 0.11$).

We measured frass and cocoon masses from 96 nests constructed in 2015. A linear mixed effects model (LME) comparing mass of frass as a proportion of total cell mass among species revealed that there were differences among species ($F = 17.3$, $df = 5$, 13.154 , $p < .001$). Pairwise comparisons showed that the three Asteraceae specialists all had significantly larger relative frass masses than the generalists and Fabeae specialist (Figure 1). There was no significant difference in mass of frass among Asteraceae specialists or among the non-Asteraceae bees (Table 1).

We observed construction and provisioning of 16 *Osmia* brood cells. A Kaplan Meier survival analysis showed that number of foraging flights per completed brood cell differed among species ($\chi^2 = 16.8$, $df = 3$, $p < 0.001$, Figure 2). Individual pairwise comparisons revealed that *Osmia iridis* makes significantly fewer foraging flights per brood cell than all three Asteraceae specialists. Because we had no complete observations of cell provisioning for two Asteraceae specialists *O. montana* and *O. subaustralis* pairwise comparisons between the two species were not applicable. Aside from the interaction between those species, there were no

230 significant differences among the Asteraceae specialists in terms of number of foraging flights
231 per brood cell. This may be an effect of incomplete observation of cell provisioning for some
232 species, and our small sample size.

233 **Discussion:**

234 Contrary to our initial predictions, Asteraceae specialists did not appear to compensate
235 for poor quality pollen by provisioning a larger quantity of it, despite the fact that they made
236 more foraging flights per brood cell. It still remains unclear why they made more foraging flights
237 if pollen provisions of Asteraceae specialists do not reflect a larger quantity of pollen, as extra
238 flights are energetically costly. The differences in number of foraging flights may even be more
239 pronounced than reported because our data set includes few completed Asteraceae cell
240 observations. We were not able to observe cell completion for many Asteraceae specialist bees
241 because they often spent more than one day provisioning a single cell. Maximum observation
242 time for an Asteraceae specialist without cell completion was 15 hours.

243 Mass of frass from Asteraceae specialist nests was proportionally greater than the mass of
244 frass from other species indicating that they produce more waste from lower quality pollen than
245 bees using other pollens. This may be attributed to the low protein content of Asteraceae pollen
246 in general, and that in bees using other higher quality pollens, more of the original nutrient
247 profile of the pollen is assimilated, compared to Asteraceae specialists. The lack of differences in
248 the masses of frass from generalists and *O. iridis*, a Fabaeae tribe specialist, indicates that pollen
249 hosts of those species may be nutritionally similar.

250 Given the low quality of Asteraceae pollen and the costs associated with specialization,
251 Asteraceae specialists must receive some benefits as well. Protection against natural enemies is
252 one possible explanation, exemplified by the protection against brood parasitism in Asteraceae

specialists by a sapygid wasp (Spear et al. 2016). Specialization on low-quality foods is common among animals, especially where the low quality or toxicity of the food is conveyed to the specialist's predator (Freeland and Janzen 1974, Smiley 1978, Bernays and Graham 1988).

Aside from the benefits that may maintain specialization on Asteraceae pollen, there may be physiological or neurological constraints that maintain it as well (Sedivy et al 2008). Several studies showed that Asteraceae specialists developed on non-Asteraceae pollen, and in some cases, better than they did on their host pollens (Williams 2003, Praz et al. 2008). This suggests that Asteraceae bees might not be physiologically constrained, as bees were able to digest and develop on other pollens. The specialization might have more to do with neurological constraints related to flower handling or host plant recognition (Sedivy et al 2008).

This study may help to explain the mechanisms responsible for reduced sapygid development on Asteraceae pollen. If Asteraceae specialist *Osmia* are provisioning proportionally more pollen to make up for protein deficits, the absolute protein content of Asteraceae provisions alone does not explain reduced development of sapygids, as additional protein via larger provisions is presumably available to them as well. This suggests the toxicity hypothesis might be a more likely explanation. It is also possible that sapygids may not physiologically tolerate pollen with low concentrations of protein, and that their inability to process the pollen the same way an Asteraceae specialist can explains their failure to develop on it.

Future work should include measuring the actual nutrient content of the pollens collected by *Osmia* bees in our system. Assumptions of low protein content of Asteraceae pollen compared to other pollens come from analysis of many genera not collected by bees in our system (Roulston et al. 2000). These data are probably similar to protein content of pollens from

other genera but more information about specific protein content of pollen collected by bees in our system may help elucidate the relationship between pollen provisions and frass content in cells as it relates to digestive efficiency. Additionally, we report on two independent origins of Asteraceae specialization, generalists from a single clade, and only one legume specialist. Replication including broader taxonomic sampling is necessary to further examine the patterns revealed in this study. Studies including specialists using other protein-poor pollens would increase the generality of these results as well.

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386 Table 1. Means of mass of frass and cocoon with standard error.

Species	Frass Mass		Cocoon Mass		N
	Mean (g)	SE	Mean (g)	SE	
<i>O. coloradensis</i>	0.0206	0.0017	0.0554	0.0032	8
<i>O. iridis</i>	0.0064	0.0005	0.0757	0.0051	39
<i>O. montana</i>	0.0277	0.0031	0.1068	0.0074	23
<i>O. subaustralis</i>	0.0371	0.0026	0.1051	0.0049	17
<i>O. tersula</i>	0.0058	0.0011	0.0729	0.0039	6
<i>O. tristella</i>	0.0030	0.0007	0.0239	0.0014	2

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Table 2. P-values for differences in number of foraging flights among species. Bolded text indicates significant p-values. Relationship between *O. montana* and *O. subaustralis* are not applicable because the data does not include complete cell observations for either of those species.

	<i>O. iridis</i>	<i>O. coloradensis</i>	<i>O. montana</i>	<i>O. subaustralis</i>
<i>O. coloradensis</i>	0.0191	-	0.116	0.48
<i>O. iridis</i>	-	0.0191	0.0125	0.0125
<i>O. montana</i>	0.0125	0.116	-	NA
<i>O. subaustralis</i>	0.0125	0.48	NA	-



Figure 1. Map of study area. Red points indicate study sites.

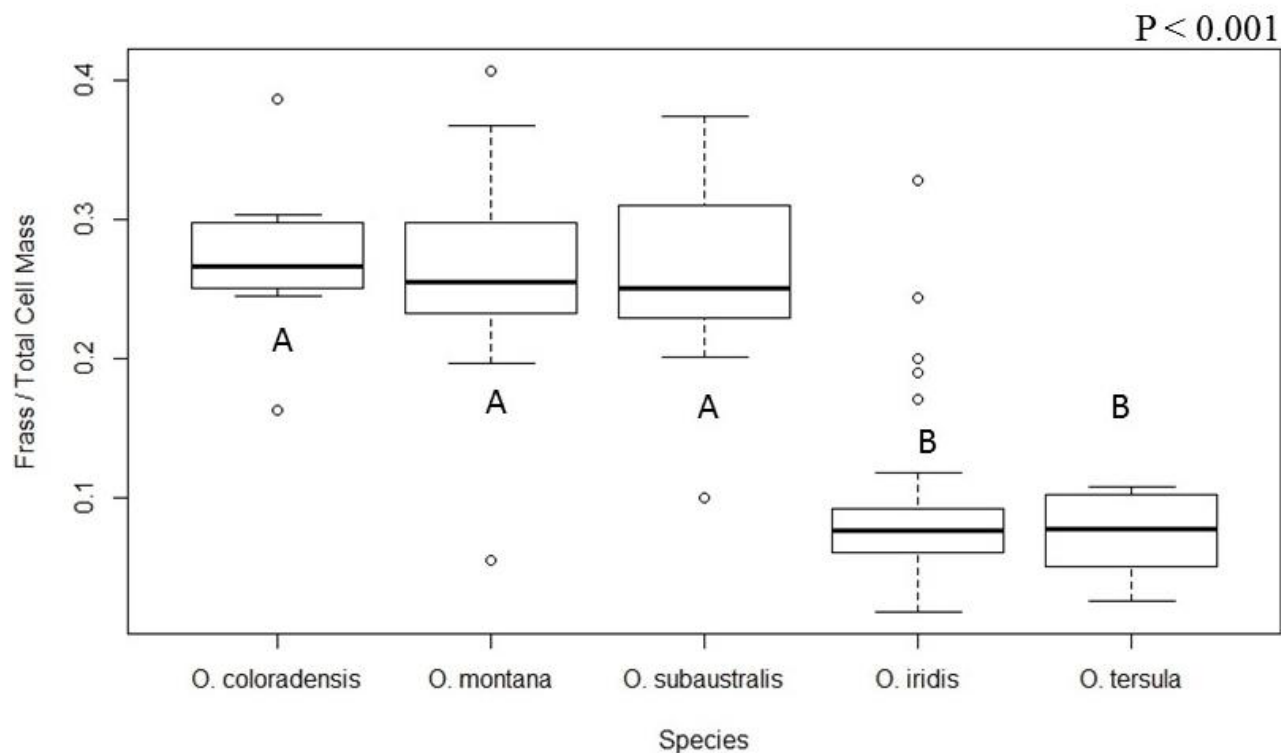


Figure 2. Differences in mass of frass as a proportion of total cell mass among species. Error bars are one standard error of the mean.

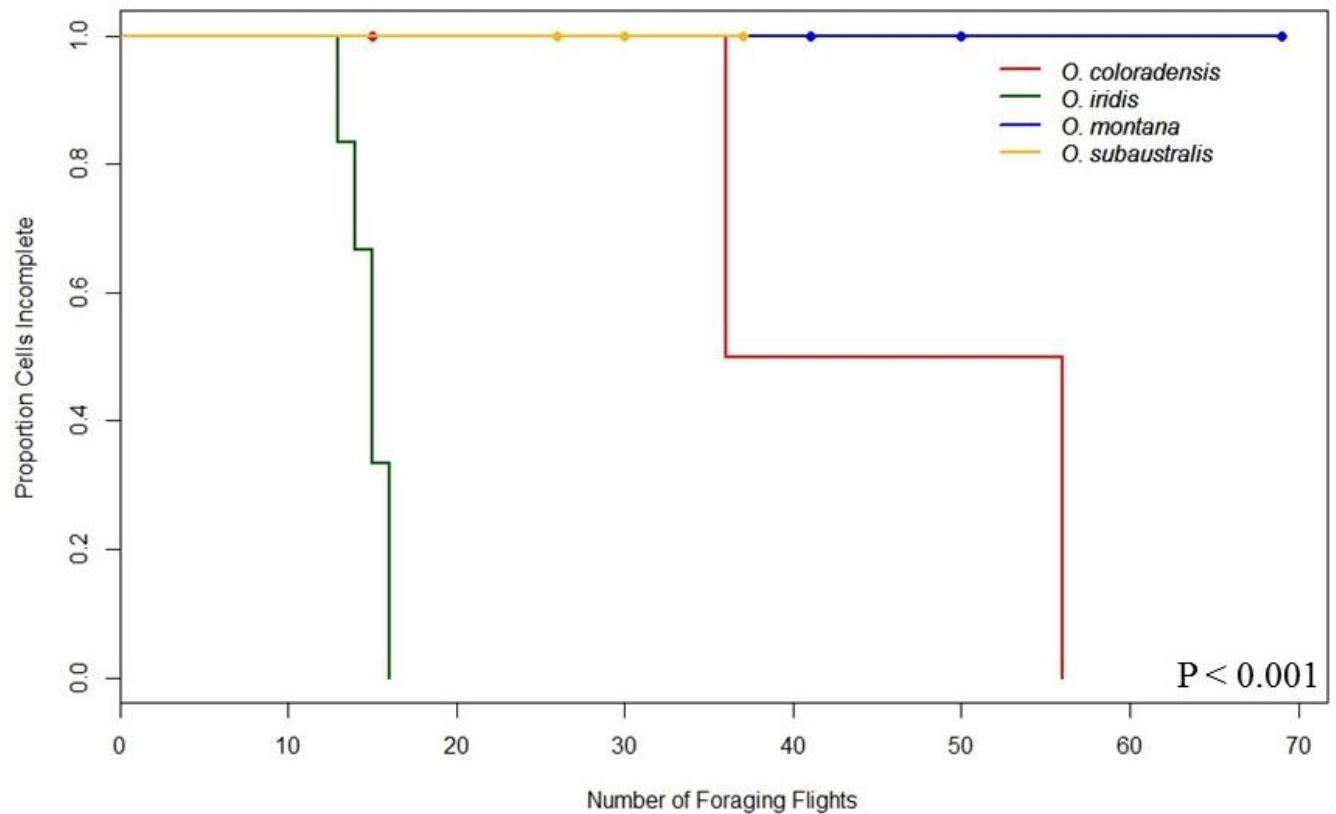


Figure 3. Kaplan Meier survival curves for number of foraging flights per brood cell completed among species. Points along the top correspond to the x-axis and indicate observations of brood cell construction that were not completed during the observation time. Differences among species $p < 0.001$.