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ORIGINAL ARTICLE

Maternal manipulation of pollen provisions affects worker production in a small carpenter bee

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Abstract Mothers play a key role in determining the body size, behavior, and fitness of offspring. Mothers of the small carpenter bee, *Ceratina calcarata*, provide smaller pollen balls to their first female offspring resulting in the development of a smaller female. This smaller female, known as the dwarf eldest daughter, is coerced to stay at the nest to forage and feed siblings as a worker. In order to better understand how this maternal manipulation leads to the physiological and behavioral differences observed in dwarf eldest daughters, we characterized and compared the quality of the pollen balls fed to theses females vs. other offspring. Our results confirm earlier studies reporting that there is a female-biased sex allocation in the first brood cell position and these daughters received mass provisions significantly smaller than other daughters. In addition to the smaller quantities of pollen provisioned, we found evidence for maternal control of the quality of pollen invested in the dwarf eldest daughters. Late brood cells receive pollen balls with significantly less floral diversity than early brood cells. This difference in floral diversity affects the protein content of the pollen balls; in that, older offspring receive less protein than their younger siblings. These results reveal that *C. calcarata* mothers manipulate not only the quantity but also the quality of the provision provided to her first offspring to create a small worker she is able to coerce to remain at the nest to help raise her siblings. This overlapping of generations and division of labor between mother and dwarf eldest daughter may represent the first steps in the evolution of highly social groups. One of the major transitions to the formation of highly social groups is division of labor. By manipulating resource availability to offspring, parents can force offspring to remain at the nest to serve as a worker leading to a division of labor between parent and offspring. In the small carpenter bee, *C. calcarata*, mothers provide their eldest daughter with less food resulting in a smaller adult body size. This dwarf eldest daughter (DED) does not have the opportunity to reproduce and serves only as a worker for the colony. In addition to overall reduced investment, we found that mothers also provide a different variety of pollen to her DED. By exploring the factors and mechanisms that influence maternal manipulation in a non-eusocial bee, we can begin to understand one of the major transitions in social group formation.

Keywords Eusocial · Pollen · Foraging · Floral diversity · Protein content · Maternal manipulation · *Ceratina calcarata*

Introduction

Parental manipulation has been posited as a mechanism for the evolution of eusociality (Alexander 1974; Michener and Brothers 1974; Charnov 1978; Craig 1979; Queller 1996; Crespi and Ragsdale 2000; Kapheim et al. 2015). Parents force offspring to act as workers for the nest by manipulating resources and limiting offspring's opportunities to leave the nest. Some offspring thus remains at the nest and helps to raise siblings. This is common across diversity taxa including birds (Clarke 1984; Stacey and Koenig 1990), wasps (Gadagkar 1991; Gadagkar et al. 1991), and bees (Hogendoorn 1996; Mueller 1991; Hogendoorn et al. 2001). This behavioral mechanism leads to a syndrome known as sib-social care

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(Lin and Michener 1972; Alexander 1974; Michener and Brothers 1974).

In addition to physical manipulation, mothers can control investment in each offspring (Mousseau and Fox 1998). Maternal investment in offspring, including providing food and protection, can influence offspring development, survival, and behavior (Wade 2001; Reinhold 2002; Wilson et al. 2005; Wolf and Wade 2009; Kapheim et al. 2011). In *Hymenoptera*, body size is directly correlated to larval diet and often plays an important role in caste determination, colony performance, and sociality (Andersson 1984; Packer and Knerer 1985; Hunt and Nalepa 1994; Hunt and Amdam 2005; Toth et al. 2009; Quezada-Euan et al. 2010; Brand and Chapuisat 2012). Mothers provide each offspring with a pollen ball, which contains all the nutrients the offspring will need to develop from larva to adult (Michener 1974; Michener 2007). The mother forages for pollen and nectar to make a pollen ball to lay her egg upon. In generalist species, the mother is capable of selecting pollen collected for the mass provision. Previous research in honeybees and bumblebees demonstrate that worker bees can determine variations in nutritional quality of pollen and preferentially forage for pollen containing higher essential amino acids or protein (Cook et al. 2003; Behmer 2009; Konzmann and Lunau 2014; Somme et al. 2014; Vaudo et al. 2014; reviewed in Vaudo et al. 2015), but very little is known about how subsocial and solitary bees differentially forage for pollen provisions (Westrich 1996; Greenleaf et al. 2007).

Ceratina calcarata is a small carpenter bee endemic to eastern North America (Shell and Rehan 2016). In the spring, *C. calcarata* females build nests within the dead broken branches, commonly, staghorn sumac, *Rhus typhina* (*Sapindales*:*Anacardiaceae*). The mother hollows a broken stem to create a nest; she then lays eggs over the next few weeks and singly forages to provide each developing offspring with its own nutrient-rich pollen ball. Like all bee species, *C. calcarata* mothers have control over the sex and body size of offspring (Rehan and Richards 2010a). Mothers can manipulate offspring body size by providing differing amounts of pollen and nectar to each brood cell in the form of a pollen ball or mass provision, on which an egg is laid (Johnson 1988; Rehan and Richards 2010a). Overall, female offspring typically receive larger mass provisions than males, which impacts adult body size and fecundity. Past studies have found a female bias in the first brood cell position or first egg laid (Johnson 1988; Rehan and Richards 2010b). This eldest daughter receives a smaller mass provision compared to her female siblings and thus develops a smaller adult body size (Rehan and Richards 2010b). In addition to this nutritional manipulation, the mother uses physical force to nudge her eldest daughter out of the nest encouraging her to forage for provisions to feed her adult siblings (Rehan and Richards 2013; Rehan et al. 2014). This dwarf eldest daughter does not have the opportunity to reproduce and serves only as a worker for the colony (Rehan and Richards 2010a). This manipulation by the mother leads to one of the three hallmarks of eusocial behavior, reproductive division of labor, whereby the mother serves as the reproductive and the dwarf eldest daughter acts as the worker (Rehan and Richards 2013). Thus, understanding the mechanism of this maternal manipulation may help us better understand how division of labor is established during the earliest stages of social group formation.

The goals of this study are threefold, first, to assess the maternal investment patterns of *C. calcarata* in a new study population; second, to determine how both the quantity of pollen varies across brood cell positions and between offspring; and third, to identify how pollen diversity and quality vary between offspring. Additionally, we provide the first characterization of plant diversity utilized by a small carpenter bee that is also an abundant and widespread native pollinator.

Materials and methods

Collections

Nests of *C. calcarata* were collected from staghorn sumac (*R. typhina*) stands in Durham, NH, USA (43.1339°N, 70.9264°W) between 26 June and 17 July 2014. Nests were collected before 8 am to ensure the presence of mother and brood. Nests were dissected in the lab, and nest contents were recorded including brood developmental stages, number of brood cells, nest width and length, and the presence of an adult female who was assumed to be the mother. Each offspring was individually removed from the nest, along with their mass provision, and weighed using a Mettler analytical balance (accuracy 0.01 mg). Only complete pollen masses were used for analysis. The pollen of the mass provision was then sampled using pinpricks (see below), and the mass provision and offspring were placed in 200-μl microcentrifuge tubes in the incubator at 25 °C with 50 % humidity until reaching their final molt. The sex and weight were recorded for all brood that reached adulthood. The sex was determined by counting the number of metasomal terga; females have six segments, while males have seven (Rehan and Richards 2010b). The founding date for the nest was estimated based on the developmental stage of the innermost, or first laid, brood cell (Rehan and Richards 2010a). The date of nest collection minus the approximate number of days to reach that developmental stage gave an estimate for the nest founding date and subsequently the brood cell founding date (Rehan and Richards 2010b).

Pollen sampling from brood cell mass provisions

To identify the plant community of the pollen ball, an insect pin was moistened with water and pricked through the

provision as per Radmacher and Strohm (2010). Briefly, the pollen from the pin was rinsed with 100 % glacial acetic acid onto a well slide. Once all pollen was removed, the pin was wiped and rinsed in water. Each pollen mass was sampled five times in order to accurately sample the diversity of pollen in the provision mass. After the five pin pricks were completed, the provision mass was reweighed using a Mettler analytical balance and placed in an incubator.

To preserve the pollen samples, we used an acetolysis procedure modified from Delaplane et al. (2013). Briefly, a drop of glacial acetic acid was placed on the slide containing each pollen ball sample and allowed to dry. Then, one drop of 9:1 glacial acetic acid to sulfuric acid solution was added to each slide. The solution was stirred on the slide using an insect pin to expose all pollen grains to the solution. The slide was placed in a drying oven at 70 °C for 5 min. The slide was removed and a drop of water was added to keep the pollen moist. It was then returned to the drying oven for an additional 5 min. Once the slide was removed, another drop of water was added and the slide was stored in a closed container with drying beads for 48 h. The pollen sample was resuspended in water and the drying process was repeated. Finally, the sample was stained with 10 μl of safranin-O and allowed to dry for an additional 24 h in the enclosed container with drying beads and protected with a coverslip.

Pollen reference collection

To create a reference collection of the pollen common in the area, blooming plants were collected from a variety of locations near sumac stands in and around Durham, NH, between 29 May and 27 August 2014, which overlaps the nest collection dates of the pollen balls used in the study. A total of 63 plant specimens were photographed and identified using Gleason and Cronquist (1991). After identification of the plant specimen, pollen was collected and treated using the acetolysis method adapted from Delaplane et al. (2013). Briefly, pollen was added to a glass test tube containing 3 ml of 100 % glacial acetic acid and centrifuged at approximately 5000 rpm for 5 min. The glacial acetic acid was decanted, and 2 ml of 9:1 glacial acetic acid:sulfuric acid solution was added to each tube. The mixture was heated in a water bath at 100 °C and stirred continuously for 10 min. The mixture was allowed to cool and centrifuged at approximately 5000 rpm for another 5 min. The sulfuric acid solution was decanted and resuspended in 5 ml of distilled deionized water. The mixture was again centrifuged as above. The treated pollen was then stained using 10 μl of safranin-O stain, mounted in glycerin jelly, and protected with a glass coverslip. Images were taken using a Nikon SMZ800 light microscope at ×40 magnification for each pollen sample. Reference images and slide mounts were deposited in the University of New Hampshire Herbarium.

Pollen identification

The pollen samples were identified using a Nikon SMZ800 light microscope at ×40 magnification and compared to our reference collection. The reference collection was supplemented with images from PalDat, a palynological database (paldat.org), and Atlas of pollen and spores of the Florida Everglades (Willard et al. 2003). Additionally, we compiled a list of plant families blooming in southern New Hampshire between May and July from the USDA (plants.usda.gov). To minimize observer bias, blinded methods were use when all pollen identification data were recorded.

Because protein content is highly conserved within plant family, for families with multiple unidentifiable species, the average protein content for each plant family was used to calculate proportion protein in each mass provision. For families, such as *Anacardicaceae*, where only one genus in the family is present in the area, *R. typhina* and *Rhus glabra*, and *Rosaceae*, where all species could be identified, *Rubus fruticosus*, *Rubus idaeus*, and *Rosa multiflora*, an average of protein concentrations from those species was used. Specifically, the total protein in each mass provision was calculated by multiplying the total number of pollen grains for each plant family times the protein content for that plant family (Rayner and Langridge 1985; Day 1990; Roulston et al. 2000). For each sample of the 71 brood cells across 9 nests, we summed the protein content for all of the plant families identified in the sample. To calculate the percent protein in the sample, the total protein content was divided by the total number of pollen grains in the sample. Then, the percent protein was multiplied by the total mass of the provision to calculate the total protein amount in the sample in milligrams. To estimate percent protein in the sample, the top eight most abundant families (representing 96 % of the total pollen diversity) were used and the remaining families were grouped into an "other" category (each family representing less than 1 % pollen diversity; Table 1).

Statistical analysis

All statistical analyzes were performed in JMP v.7.01 (SAS, Cary, NC, USA). Sex ratio was calculated using a chi-squared goodness-of-fit test. Comparisons of offspring weight and relative abundance of pollen type (by family) were analyzed using two-way ANOVAs or non-parametric equivalents (Sokal and Rohlf 1995). All reported p values are two tailed.

Pollen diversity indices, abundance, and similarity measurements were calculated using EstimateS v.9.1.0 (Colwell 2013). To measure species diversity, we compared Shannon diversity index (Shannon 1948) and Simpson diversity index (Simpson 1949). All diversity indices showed similar patterns and only Shannon diversity index is reported. Diversity indices were compared across groups using two-way ANOVAs or

Table 1 Plant families, relative abundance, and protein concentration from pollen identified in mass provisions

Plant family	Relative abundance (%)	Protein content (%)
Anacardiaceae	52.88	28.93
Rosaceae	12.36	18.90
Fabaceae	11.31	30.85
Caprifoliaceae	9.43	15.00
Rhamnaceae	2.66	40.40
Unknown	2.41	30.00
Campanulaceae	1.85	51.45
Geraniaceae	1.76	14.20
Cluseaceae	1.33	30.30
Other	**4.01**	**36.17**
Cornaceae	0.64	
Vitaceae	0.64	
Berberaceae	0.48	
Rubiaceae	0.47	
Solanaceae	0.33	
Ranunculaceae	0.30	
Violaceae	0.28	
Boranginaceae	0.26	
Primulaceae	0.13	
Gentianaceae	0.08	
Asteraceae	0.06	
Fumaraceae	0.04	
Ulmaceae	0.04	
Caryophyllaceae	0.03	
Ericaceae	0.03	
Eleagnaceae	0.03	
Aceraceae	0.02	
Polygonaceae	0.02	
Onagraceae	0.01	

The most abundant families (representing >96 % of the total plant diversity) were used to categorize pollen types, and the remaining families were grouped into an "other" category (bold). Protein content is highly conserved within plant families (Roulston et al. 2000). The average protein content for each plant family was used to calculate proportion protein in each mass provision

non-parametric equivalents. To compare the composition of the pollen balls, we used the Bray-Curtis index, which uses count data to quantify the compositional dissimilarity between the groups (Bray and Curtis 1957).

Results

Sex allocation and body size across brood cell position

Our sample of 627 brood cells from 105 full brood nests (range 1–18 brood cells, average brood size = 7) confirmed a female sex bias in the first brood cell position in this population of *C. calcarata* (female = 69.23 %, male = 30.77 %, chi-squared goodness of fit $\chi^2 = 4.26$, $df = 1$, $p = 0.04$; Table 2). The overall population sex ratio is 43.54 % male ($\chi^2 = 5.00$, $df = 1$, $p = 0.03$; Table 2). Overall, female offspring were significantly larger ($N = 741$, mean ± SD, 12.41 ± 3.79 mg) than male offspring ($N = 603$, 9.35 ± 2.73 mg; Wilcoxon-Mann-Whitney test $\chi^2 = 205.53$, $df = 1$, $p < 0.0001$; Fig. 1a). There was no significant difference in the size of male offspring by brood cell position ($r^2 = 0.003$, $F = 1.87$, $p = 0.17$; Fig. 1a). Daughters in the first brood cell position (mean ± SD, 11.27 ± 3.80) were significantly smaller than daughters in other brood cell position (mean ± SD, 12.65 ± 3.75; Wilcoxon-Mann-Whitney test $\chi^2 = 15.70$, $df = 1$, $p < 0.0001$). Additionally, there was a negative correlation for both daughters and sons between brood cell founding date and offspring live weight. This means that daughters and son laid later in the season (based on brood cell founding date) were significantly larger than those laid earlier in the season (linear regression sons $r^2 = 0.06$, $F = 33.83$, $p < 0.0001$; daughters $r^2 = 0.10$, $F = 75.23$, $p < 0.0001$).

Quantity of pollen ball

Sons receive significantly smaller pollen balls ($N = 265$, mean ± SD, 20.40 ± 0.67 mg) than daughters ($N = 302$, 24.37 ± 0.07 mg; Wilcoxon-Mann-Whitney test $\chi^2 = 55.23$, $df = 1$, $p < 0.0001$; Fig. 1b), and daughters in the first brood cell position received provisions more similar in size to that of male offspring ($N = 65$, 20.26 ± 0.20 mg). Overall, for both sons and daughters, pollen ball mass increased significantly with brood cell position (linear regression sons $r^2 = 0.03$, $F = 8.28$, $p = 0.0043$; daughters $r^2 = 0.09$, $F = 29.77$, $p < 0.0001$; Fig. 1b). Additionally, pollen ball mass also increased with brood cell founding date (linear regression $r^2 = 0.02$, $F = 22.25$, $p < 0.0001$).

Pollen composition

Pollen samples were collected from 71 mass provisions across 9 nests. A total of 27 plant families were identified across all mass provisions (Table 1). The top eight most abundant families (representing 96 % of the total pollen diversity) along with relative protein content are reported in Table 1. The diversity of pollen based on plant family in the mass provisions varied significantly across brood cell positions. The pollen diversity was negatively correlated with brood cell position (linear regression $r^2 = 0.23$, $F = 20.99$, $p < 0.0001$; Figs. 2 and 3). The highest Shannon diversity index was in brood cell position one (1.37), while the lowest diversity was in brood cell position 9+ (0.35; Fig. 3). The most common pollen type across all brood cell positions was the family *Anacardiaceae* (52.88 %; Fig. 2 and Table 1). The diversity of pollen in the

Table 2 Sex allocation across brood cell positions

Brood cell position	No. of males	No. of females	Total no.	Proportion male (%)	Chi-squared	*p* value
1	20	45	65	30.77	4.26	*0.04*
2	30	44	74	40.54	0.78	0.38
3	40	26	66	60.61	1.10	0.29
4	22	36	58	37.93	1.26	0.26
5	21	33	54	38.89	0.94	0.33
6	30	23	53	56.60	0.23	0.63
7	22	25	47	46.81	0.01	0.92
8	22	21	43	51.16	0.01	0.91
9+	66	101	167	39.52	3.31	0.07
Overall	273	354	627	43.54	5.00	*0.03*

The numbers of males and females and total number for all complete full brood nests are reported. A chi-squared goodness of fit was used to compare sex ratio to expected equal sex ratio. Sex ratios significantly different from the expectation of equality are italicized.

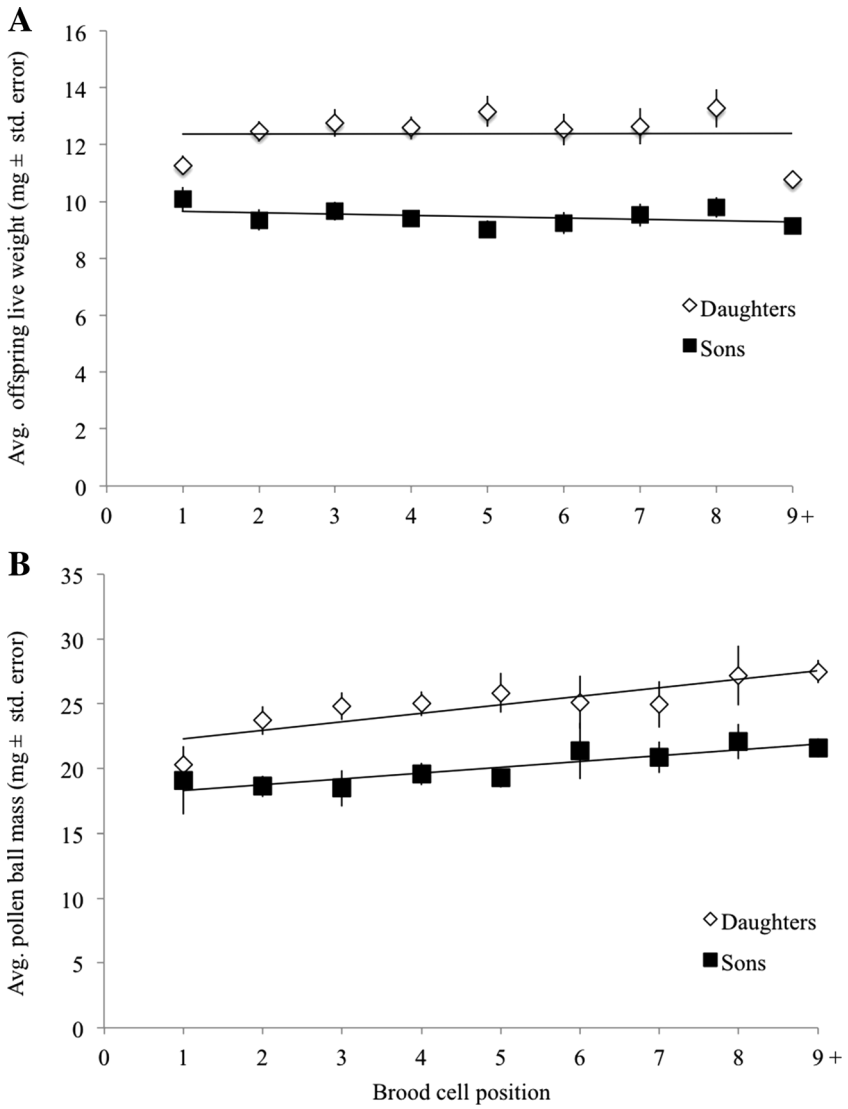

Fig. 1 The average mass (±standard error) of male (*black*) and female (*white*) offspring. **a** Live weight of offspring across brood cell position. **b** Pollen ball mass provisions across brood cell position. Overall, sons receive less pollen than daughters. The live weight of sons did not vary based on brood cell position. Brood cell position 1 is laid first and often contains a daughter that receives significantly less pollen and is significantly smaller than other daughters. Brood cell positions 9 and later were grouped for a larger sample size

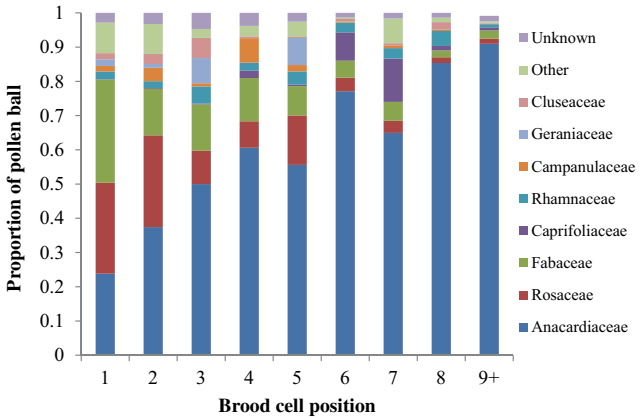

Fig. 2 The average pollen diversity of pollen balls provided to offspring across brood cell position. Mothers collect significantly more pollen from their nesting substrate (*Anacardiaceae*) in later brood cells

pollen balls was also negatively correlated to the brood cell founding date (linear regression $r^2 = 0.08$, $F = 5.48$, $p = 0.02$).

The overall pollen diversity for sons and regular daughters was more similar than regular daughters to dwarf eldest daughters (Bray-Curtis index sons vs. regular daughters = 0.70, sons vs. dwarf eldest daughters = 0.63, regular daughters vs. dwarf eldest daughters = 0.52; Fig. 4). However, there was no significant difference in pollen diversity between sons, regular daughters, and dwarf eldest daughters (mean ± SE sons = 0.89 ± 0.16, daughters = 0.84 ± 0.18, dwarf eldest daughters = 1.31 ± 0.17; Wilcoxon-Mann-Whitney test $\chi^2 = 2.10$, $df = 2$, $p = 0.35$; Fig. 4).

Quality of pollen ball

Protein content for each pollen ball was calculated based on protein content for each pollen type in the pollen ball. Protein content is based on research by Roulston et al. (2000). Protein content was positively correlated with brood cell position (linear regression $r^2 = 0.09$, $F = 5.50$, $p = 0.02$; Fig. 5). Although pollen diversity was negatively correlated with brood cell founding date, the overall protein content of the pollen ball was not significantly correlated with brood cell founding date (linear regression $r^2 = 0.01$, $F = 0.30$, $p = 0.58$).

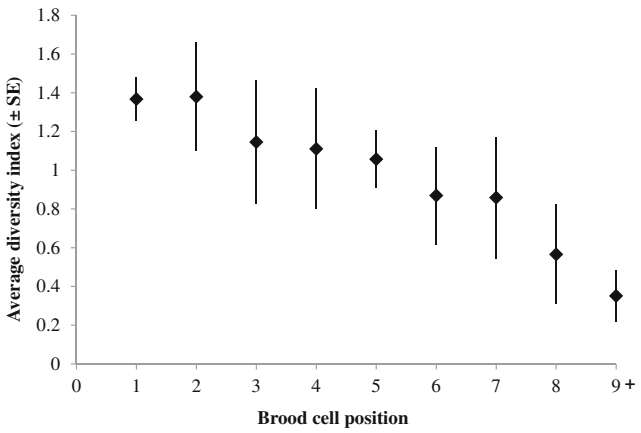

Fig. 3 Average Shannon diversity index of pollen balls (±standard error) across brood cell position. Brood cell positions 9 and later were grouped for a larger sample size. Pollen diversity significantly decreases with brood cell position

Discussion

Sex allocation and body size vary across brood cell position

Our results verify the presence of the dwarf eldest daughter in the New Hampshire population of *C. calcarata* (Rehan and Richards 2010b). The maternal manipulation of pollen balls results in the production of smaller females in the first brood cell position, known as dwarf eldest daughters (Table 2 and Fig. 1). Overall, the population sex ratio was 43.54 % male, which is mostly attributable to the female bias in the first brood cell position. Interestingly, these results differ from former reports of a male-biased sex ratio in other populations of *C. calcarata* (Johnson 1988; Rehan and Richards 2010b). Bees are among few organisms that are capable of precisely adjusting brood sex allocation (reviewed in West 2009). In species where females are the larger sex, investment in female offspring is costlier and past research has shown that resource availability is positively correlated with female-biased investment in solitary bees (Kim 1999). More data are needed to determine if the distribution of brood sex ratio tracks annual variation in resources as hypothesized by Tepedino and Torchio (1982) or if brood sex ratio consistently differs across populations.

Dwarf eldest daughters have been reported in *C. calcarata* populations in Ontario (Rehan and Richards 2010b) and Indiana (Johnson 1988), as well as in Japanese congeners, *Ceratina flavipes* (Sakagami and Maeta 1977), *Ceratina japonica* (Sakagami and Maeta 1984), and *Ceratina okinawana* (Sakagami and Maeta 1995). Behavioral observations in *C. japonica* and *C. okinawana* have shown that dwarf eldest daughters emerge earlier than their siblings. Mothers of these species will use physical force in addition to their larger size to manipulate the eldest daughter to forage and provide food for their siblings before overwintering just as in *C. calcarata* (Rehan et al. 2014). The dwarf eldest daughter effectively occupies the role of a worker, and thus, a reproductive division of labor—considered a necessary precondition for the evolution of more complex social groups—emerges in this system (Rehan et al. 2014). This maternal manipulation restricts the reproductive options of the dwarf eldest daughter, which forces her to invest in the fitness of her siblings, instead of her own direct fitness (Alexander 1974; Andersson 1984).

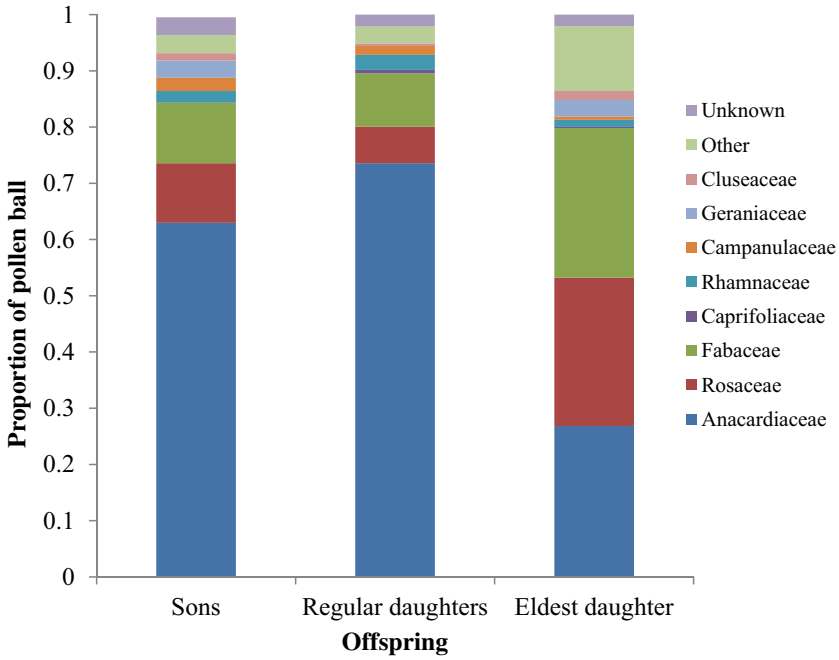

Fig. 4 The average pollen diversity of pollen balls for sons, regular daughter (in brood cells 2 and beyond), and eldest daughters (smallest daughter in the first brood cell position). The average pollen diversities of sons and regular daughters are more similar to one another than either is to the pollen diversity of dwarf eldest daughters

Variations in pollen quantity and quality

Our result demonstrated that not only is the dwarf eldest daughter provided with less pollen (Fig. 1) but also the mother provides the dwarf eldest daughter with a different variety of pollen from her siblings (Figs. 2, 3, and 4). Based on the Bray-Curtis similarity index, the pollen diversity of mass provisions of sons and regular daughters are more similar than either is to mass provisions of dwarf eldest daughters (Fig. 4). Additionally, our results showed a significant negative correlation between pollen diversity and brood cell position, indicating that the first brood cell position, which houses the dwarf eldest daughter, receives a different and more diverse pollen ball than later brood cells (Figs. 2 and 3). Interestingly, although the pollen ball provided to the dwarf eldest daughter is more diverse (Figs. 2 and 3), these pollen balls contain less protein than pollen balls in the later brood cell positions (Fig. 5).

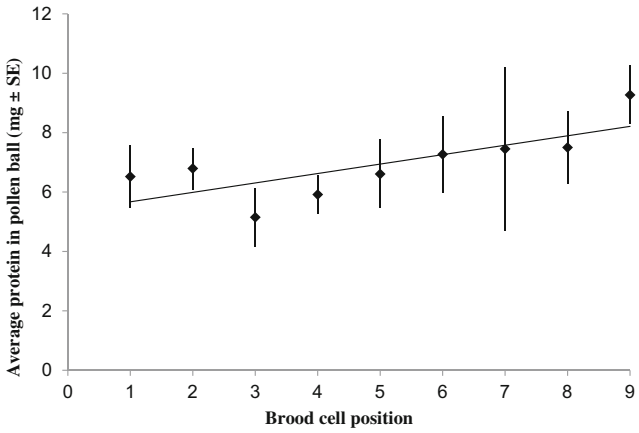

Fig. 5 The average protein content of pollen balls (±standard error) across brood cell positions. Protein content is based on the pollen diversity of each pollen ball. Protein content is positively correlated with brood cell position

Pollen is the main food source for many bee species and is the primary source of dietary protein, amino acids, starch, vitamins, sterols, and lipids (Roulston and Cane 2000; Michener 2007; Brodschneider and Crailsheim 2010). Pollen quality has been shown to play a significant role in larval development and adult reproduction in honeybees, multiple bumblebee species, and the sweat bee *Lasioglossum zephyrum* (Sutcliffe and Plowright 1990; Genissel et al. 2002; Roulston and Cane 2002; Human et al. 2007; Tasei and Aupinel 2008; Li et al. 2014; Vanderplanck et al. 2014). Changes in foraging strategies of multiple species have been observed in relation to the caloric value of floral resource, demonstrating that bees are capable of selecting resources in relation to nutritional content (Amaya-Marquez 2009). Specifically, research has shown that bees choose plant hosts on the basis of the protein content (Roulston et al. 2000; Hanley et al. 2008; Vanderplanck et al. 2014), concentrations of essential amino acids (Cook et al. 2003), or sugar content (Pernal and Currie 2001). Given that other species of bees are capable of selecting pollen based on nutritional content, *C. calcarata* may similarly be able to affect the nutritional content of the food provided to each offspring.

Characterization of common pollen diversity

Although most studies of *Ceratina* species have found that they are generalists, floral constancy has been observed in

C. flavipes (Kobayashi-Kidokoro and Higashi 2010). A single brood cell provision was found to contain 80–100 % of one species of pollen and on average, two to three species (Kobayashi-Kidokoro and Higashi 2010). Similarly, in twig-nesting halictid bees, *Megalopta genalis* and *Megalopta centralis*, although individuals used pollen from a wide variety of plants that varied by year, pollen balls were dominated by five main species (Smith et al. 2012). Although our data focused on plant family diversity, instead of plant species, our data are consistent with the results of these studies; we found that the abundance of the three main pollen groups remained fairly constant and comprise approximately 75 % of each pollen ball (Fig. 2 and Table 1). *Anacardicaceae* (including sumac) remained the most abundant pollen type across all nests and the majority of male (63 %) and regular female (74 %) offspring but was far less provisioned to dwarf eldest daughters (27 %). This is perhaps unsurprising, as sumac is the primary nesting substrate of this population of *C. calcarata*. Utilization of this resource as both a nesting and food material could allow a mother to invest in offspring that are more numerous, larger, and female biased, thus increasing her own fitness. Future studies comparing maternal investment across different nest substrates types will help resolve whether sumac is a preferred pollen type or simply readily available to mothers nesting in this substrate. The abundance of *Anacardicaceae* pollen increased in later brood positions, suggesting one possible option that as the season progressed, the mother began to forage closer to home (Fig. 2).

We found a significant negative correlation between brood cell founding date and pollen diversity and richness. This variation in pollen diversity is probably due to mothers' selectivity and learning rather than total availability of resources, as foraging takes place during only a few weeks, while most local flora are in bloom (Gyan and Woodell 1987; Rehan personal observation). Additionally, the pollen balls samples were sampled over 3 weeks in June and July. During this entire time, sumac (*Anacardicaceae*) is in full bloom (Gilbert 1961; Rehan personal observation). Further evidence of this maternal selectivity is seen in the protein content of the pollen balls. Protein content was not correlated with brood cell founding date, but protein content was significantly correlated with brood cell position (Fig. 5). Further research is needed to explore how resource availability, foraging distance, and diversity affect foraging habits in *C. calcarata* and how these differences in foraging behavior affect division of labor and social hierarchies within the nest. Furthermore, new research has demonstrated that rising levels of atmospheric carbon dioxide can reduce the protein concentration of floral protein (Ziska et al. 2016). In order to begin to understand the effects of global climate change on our native pollinators, we must begin with a baseline understanding of protein availability and how that effects foraging preferences.

Conclusions

One of the major transitions to the formation of highly social groups is overlapping generations and parental manipulation that reduce the options of some offspring to leave the nest to begin their own nest (Michener and Brothers 1974; Rehan and Toth 2015). By exploring the factors and mechanisms that influence maternal manipulation in a non-eusocial bee, we can begin to understand one of the major transitions in social group formation (Rehan et al. 2012). While maternal investment is correlated to offspring size via the mass of the provision, we found that floral preference also plays an important role in the differentiation between offspring. Additionally, the variation in the pollen composition and protein content of the pollen provided to the dwarf eldest daughter could be a function of maternal control of the quality of provision mass. Further studies into the nutritional value of pollen resources, along with the relationship between foraging range and floral preference in the investment of provision mass and how they affect offspring size and behavior, will offer important insights into the factors determining offspring development and the formation of social hierarchies in non-eusocial species.

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

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