

RESEARCH ARTICLE

Effects of nutritional deprivation on development and behavior in the subsocial bee *Ceratina calcarata* (Hymenoptera: Xylocopinae)

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ABSTRACT

By manipulating resources or dispersal opportunities, mothers can force offspring to remain at the nest to help raise siblings, creating a division of labor. In the subsocial bee *Ceratina calcarata*, mothers manipulate the quantity and quality of pollen provided to the first female offspring, producing a dwarf eldest daughter that is physically smaller and behaviorally subordinate. This daughter forages for her siblings and forgoes her own reproduction. To understand how the mother's manipulation of pollen affects the physiology and behavior of her offspring, we manipulated the amount of pollen provided to offspring and measured the effects of pollen quantity on offspring development, adult body size and behavior. We found that by experimentally manipulating pollen quantities we could recreate the dwarf eldest daughter phenotype, demonstrating how nutrient deficiency alone can lead to the development of a worker-like daughter. Specifically, by reducing the pollen and nutrition to offspring, we significantly reduced adult body size and lipid stores, creating significantly less aggressive, subordinate individuals. Worker behavior in an otherwise solitary bee begins to explain how maternal manipulation of resources could lead to the development of social organization and reproductive hierarchies, a major step in the transition to highly social behaviors.

KEY WORDS: Maternal manipulation, Division of labor, Small carpenter bee, Social evolution, Worker behavior, Phenotypic plasticity, Social insect

INTRODUCTION

Maternal 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). By limiting resources or nesting options, mothers can force offspring to remain at the nest and help raise siblings. This behavior has been observed across a wide variety of taxa from mammals (Briga et al., 2012) and birds (Clarke, 1984; Stacey and Koenig, 1990) to bees (Hogendoorn, 1996; Hogendoorn et al., 2001) and wasps (Gadagkar, 1991; Gadagkar et al., 1991). The imposed division of labor between a reproductive individual and her worker offspring is considered a key component in the evolution of highly social groups. Reproductive division of labor can arise through physical manipulation and differential maternal investment in each offspring (Mousseau and Fox, 1998). Investment in offspring, including 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, larval diet directly correlates with adult body size and influences caste determination, colony performance and social interaction (Andersson, 1984; Packer and Knerer, 1985; Hunt and Nalepa, 1994; Hunt and Amdam, 2005; Toth et al., 2009; Quezada-Euán et al., 2010; Brand and Chapuisat, 2012). Larval diet also allows for increased lipid stores, which are directly correlated to overwintering success (Tepedino and Torchio, 1982; Strassmann et al., 1984; Strohm and Linsenmair, 1999). In most bees, the mother forages for pollen and nectar to make a pollen ball to lay her egg upon. Each pollen ball contains all the nutrients each offspring will need to develop from larva to adult (Michener, 1974, 2007). Previous research in honey bees and bumble bees demonstrated that foraging workers can determine variations in the nutritional quality of pollen and will preferentially collect pollen containing higher essential amino acids or protein content (Cook et al., 2003; Behmer, 2009; Konzmann and Lunau, 2014; Somme et al., 2014; Vaudo et al., 2014; reviewed in Vaudo et al., 2015).

Poor larval nutrition significantly reduces body size and has been shown to have major impacts on adult physiology (Birkhead et al., 1999; Lummaa and Clutton-Brock, 2002; Ozanne et al., 2004) and behavior (Hunt et al., 2005). Specifically, mating and foraging success are negatively impacted (Muller et al., 2015; Xie et al., 2015). In eusocial honey bees, previous research has shown that larval developmental fate depends on diet and nutrients. Body and ovary size are the result of differences in larval nutrition provided by nurse workers (Linksvayer et al., 2011).

Less is known about how larval nutrition affects social interactions and the development of hierarchies in solitary or subsocial species. In primitively eusocial species, social dominance hierarchies are often determined by body size, where the smaller individual assumes a subordinate position (Smith et al., 2009); thus, poor larval nutrition would reduce competitive ability (Huntingford and Turner, 1987; Withee and Rehan, 2016, 2017). Comparisons of social complexity and life history between social species and their solitary relatives can offer insights into the evolution of social behavior (Michener, 1974; Rehan and Toth, 2015; Shell and Rehan, 2017). By understanding how maternal manipulation of larval nutrition affects growth, development and behavior of offspring in subsocial species, we can begin to understand the role maternal investment plays in the development of division of labor.

Ceratina calcarata is a small carpenter bee widespread across eastern North America (Shell and Rehan, 2016). Females build nests in spring within branches of dead broken pithy stems. Over the next few weeks, females singly forage and lay eggs on nutrient-rich pollen balls (Rehan and Richards, 2010a). Like all hymenopteran species, *C. calcarata* mothers have complete control over the body size and sex of offspring (Rehan and Richards, 2010b). Mothers can adjust the quantity of food provided to offspring and in turn their

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resultant body size (Johnson, 1988; Rehan and Richards, 2010b). The pollen balls provisioned vary among brood cells and sexes, with larger females receiving larger mass provisions than males. Previous studies have shown that the first egg laid and first brood cell position are female biased (Johnson, 1988; Rehan and Richards, 2010b). This eldest daughter is smaller than her siblings because she receives a smaller mass provision (Lawson et al., 2016). Interestingly, this dwarf eldest daughter (DED) serves only as a worker for the colony and does not reproduce (Rehan and Richards, 2010b; Rehan et al., 2014). The DED is significantly smaller than other females in the nest and displays more subordinate behavior (Mikát et al., 2017). Manipulation of pollen quantity by the mother leads to one of the three hallmarks of eusocial behavior, reproductive division of labor, whereby the mother serves as the reproductive individual and the DED acts as the worker (Rehan and Richards, 2013; Rehan et al., 2014). Thus, understanding how pollen quantity provided to offspring affects body size, lipid stores and behavior may help us better understand how division of labor is established during the earliest stages of social group formation.

The goals of this study were: first, to measure how the amount of pollen provided to offspring affects body size and lipid stores, and second to determine whether and how the mass of the pollen ball affects offspring behavior. By experimentally manipulating the amount of pollen provided, we separated the effects of maternal manipulation of pollen quantity from the effects of maternal interactions. We could therefore determine whether pollen quantity alone is enough to create a worker-like daughter or whether maternal interaction is needed to develop the social hierarchy between mother and daughter.

MATERIALS AND METHODS

Collection and nest measurements

Nests of *C. calcarata* Robertson 1900 were collected from staghorn sumac (*Rhus typhina*) stands in Durham, NH, USA (43.1339°N, 70.9264°W) between 4 June and 1 August 2015. Nests were collected before 08:00 h 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. Measurements of adult bees included head width (which is a strong predictor of body size) and wing wear (a useful proxy for age and foraging; Rehan and Richards, 2010a). Adult bees were stored at -80°C for later lipid quantification. A total of 370 brood from 123 nests were used for pollen manipulation experiments and then stored in the incubator at 25°C with 50% humidity until eclosion.

Pollen manipulation

Early stage larvae and eggs were removed from nests, weighed using a Mettler analytical balance (accuracy 0.01 mg) and randomly assigned to a control group or one of two treatment groups. The pollen of the control group remained unmanipulated. For the treatment groups, approximately 1/3 of the pollen ball was removed from one group and added to the other group. Both treatment groups were reweighed and offspring and pollen ball were placed in PCR tubes in the incubator at 25°C with 50% humidity until reaching their final molt. Offspring from later developmental stages were raised in the incubator as additional controls. Every other day, the development of the offspring was assessed. The sex and mass were recorded for all brood that reached adulthood. Sex was determined by counting the number of metasomal terga; females have six segments, while males have seven (Rehan and Richards, 2010a).

Adults were used in circle tube assays followed by lipid quantification. To quantify the exact amount of pollen consumed, we calculated the amount of pollen provided minus the amount of pollen remaining when offspring began pupation.

Lipid quantification

To quantify body lipids from lab-reared adult bees raised on manipulated pollen quantities, we followed methods previously published for honey bees (Toth and Robinson, 2005) and solitary bees (Richards and Packer, 1994; O'Neill et al., 2015). Briefly, bees previously frozen at -80°C (see 'Behavioral assays', below) were placed in a homogenizer tube and ground with a glass rod. Extraction was carried out by addition of 5 ml of 2:1 chloroform:methanol solvent overnight. To purify the sample, we poured the solvent and sample through glass wool and rinsed with 2 ml of 2:1 chloroform:methanol. The sample was quantified using a Spectramax 250 spectrophotometer and compared with a standard curve of 0, 10, 50, 100 and 500 μl of cholesterol in petroleum ether.

Behavioral assays

To quantify the behavioral effects of nutritional addition or reduction, we used circle tube assays to observe behavioral interactions between age and size-matched treatment and control individuals. Circle tube assays provide an environment suitable for observation of interactions between individuals, which are similar to those in their natural nest (Brothers and Michener, 1974; Breed et al., 1978; Packer, 2006). To perform a trial, bees were simultaneously introduced to opposite ends of a clean plastic circle tube with an internal diameter of 4 mm (double the average head width of *C. calcarata*) and a length of 30 cm (40 times the average *C. calcarata* body length). A new tube was used for each trial. To decrease variation between trials, all circle tube assays were completed indoors in a standard arena. Tubes were placed under two UV lights to simulate UV rays and a single 120 V bulb for heat. Temperature was measured every minute to ensure that all trials were run between 32 and 38°C . Pairs were observed for 20 min and all interactions were documented. Interactions, when bees were within one body length of one another, were classified into four categories previously published by Rehan and Richards (2013): aggression, avoidance, tolerance and following. Briefly, aggressive behaviors include nudging, biting or C-posturing, when the bee curls its abdomen under the thorax, displaying both mandibles and sting. Avoidance behaviors include backing away from or reversing 180 deg to move away from the other bee. Passing, and antenna-to-antenna or head-to-head contact were considered tolerant behaviors. Following has been classified as a cooperative behavior in some communal species (McConnell-Garner and Kukuk, 1997; Boesi and Polidori, 2011), a subordinate behavior in some eusocial species (Breed et al., 1978; Michener, 1990) and as a dominant behavior in other species (West-Eberhard, 1979). Because of the uncertainty of the meaning of this interaction, following behaviors were classified separately. After behavioral trials, all bees were frozen at -80°C for later lipid quantification. To account for differences in paired circle tube assays, we compared the relative differences in behavioral frequencies. All differences between pair members were calculated as (value for treatment bee, pollen added or removed)–(value for control bee).

Statistical analysis

Pollen consumed, head width and lipid content of the adult bees were all separately tested with the Shapiro–Wilk test for normality and Levene's test for equality of variance. All comparisons were

analyzed using a two-way ANOVA followed by *post hoc* Tukey–Kramer test. Where necessary, data were normalized via log transformation (Sokal and Rohlf, 1995). If the data were not normal, a Kruskal–Wallis test was used followed by a *post hoc* Dunn’s test. Differences in behavioral frequencies between control and treatment groups were compared using a two-way ANOVA for normal data or a Kruskal–Wallis test for non-normal data. All statistical analyses were performed in JMP v.7.01 (SAS, Cary, NC, USA). All reported *P*-values are two-tailed.

RESULTS

Pollen manipulation

To confirm the effectiveness of the pollen manipulation treatment, we first compared the amount of pollen consumed by the control group and each treatment group. We calculated the amount of pollen consumed as the amount of pollen provided minus the amount of pollen remaining at the onset of pupation. There was a significant difference in the amount of pollen consumed between the three groups (Kruskal–Wallis test $\chi^2=57.17$, d.f.=2, $P<0.0001$; Dunn’s test, control versus pollen added $P=0.30$, control versus pollen reduced $P<0.0001$, pollen added versus pollen reduced $P<0.0001$; Fig. 1). *Ceratina calcarata* raised with additional pollen consumed significantly more pollen ($N=56$, mean \pm s.e.m., 25.04 \pm 1.06 mg) than *C. calcarata* raised on a pollen ball in which pollen had been reduced ($N=67$, 15.05 \pm 0.54 mg). However, bees raised with additional pollen did not consume significantly more pollen than controls ($N=51$, 22.08 \pm 0.79 mg).

The difference in pollen consumption between the groups was directly associated with adult head width. *Ceratina calcarata* raised with additional pollen were significantly larger ($N=56$, head width mean \pm s.e.m., 1.63 \pm 0.02 mm) than those raised with less pollen ($N=67$, 1.51 \pm 0.02 mm), but not significantly different from controls ($N=51$, 1.66 \pm 0.02 mm; ANOVA with log transformation F -ratio=14.71, d.f.=2, $P<0.0001$; Tukey–Kramer test, control versus pollen reduced, $P<0.0001$; control versus pollen added, $P=0.59$; pollen added versus pollen reduced, $P=0.0002$; Fig. 2).

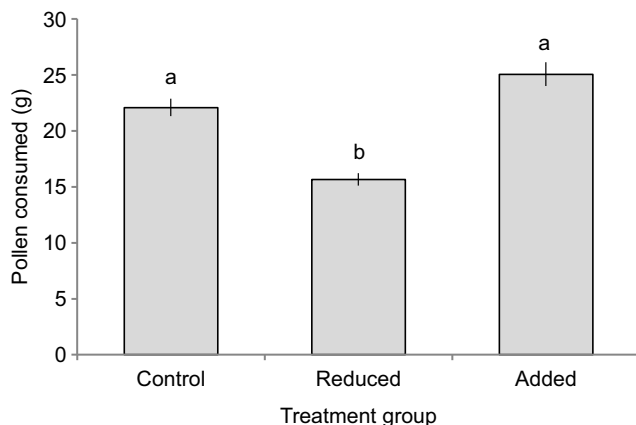


Fig. 1. Pollen manipulation. To confirm the effectiveness of the treatment, we calculated the amount of pollen consumed by each group (data are means \pm s.e.m.). *Ceratina calcarata* raised with additional pollen consumed significantly more pollen ($N=56$) than *C. calcarata* raised on a pollen ball in which pollen had been reduced ($N=67$). However, bees raised with additional pollen did not consume significantly more pollen than controls ($N=51$). There was a significant difference in the amount of pollen consumed between the three groups (Kruskal–Wallis test $\chi^2=57.17$, d.f.=2, $P<0.0001$; Dunn’s test, control versus pollen added $P=0.30$, control versus pollen reduced $P<0.0001$, pollen added versus pollen reduced $P<0.0001$). Letters indicate significant differences.

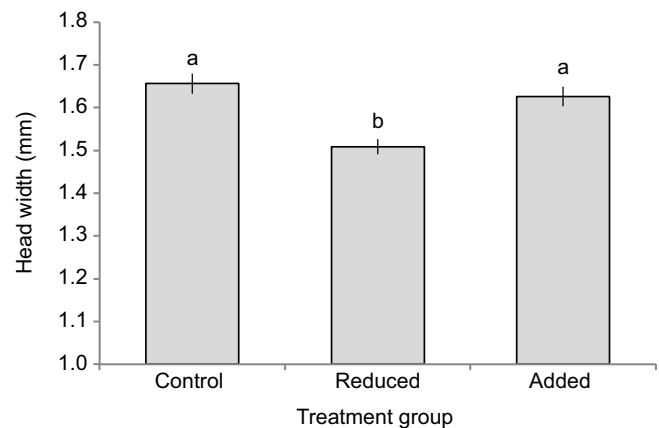


Fig. 2. The amount of pollen consumed during development is directly associated with adult body size as measured by head width. *Ceratina calcarata* raised with additional pollen were significantly larger ($N=56$) than those raised with less pollen ($N=67$), but not significantly different from controls ($N=51$; ANOVA with log transformation F -ratio=14.71, d.f.=2, $P<0.0001$; Tukey–Kramer test, control versus pollen reduced, $P<0.0001$; control versus pollen added, $P=0.59$; pollen added versus pollen reduced, $P=0.0002$). Data are means \pm s.e.m.

Stored fats, as measured by lipid quantity in the abdomen, were significantly affected by pollen consumption. *Ceratina calcarata* raised with additional pollen had significantly greater fat stores ($N=50$, mean \pm s.e.m., 3.34 \pm 0.17 g) than those raised with reduced pollen ($N=47$, 2.85 \pm 0.19 g). Similar to head size, lipid quantity was not significantly different between *C. calcarata* raised with additional pollen and controls ($N=39$, mean \pm s.e.m., 3.65 \pm 0.24 g), but bees raised with less pollen had significantly less fat than controls (Kruskal–Wallis test $\chi^2=9.90$, d.f.=2, $P=0.007$; Dunn’s test, control versus pollen added $P=1.00$, control versus pollen reduced $P=0.01$, pollen added versus pollen reduced $P=0.05$; Fig. 3).

In addition to final adult size, total pollen consumption had a significant effect on developmental rate. The total development time

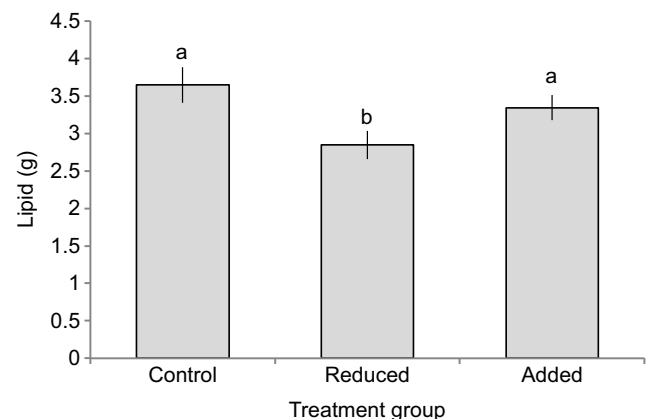


Fig. 3. The amount of pollen consumed during development has a significant effect on lipid stores. Shown is the lipid quantity for the control group and the two treatment groups (data are means \pm s.e.m.). *Ceratina calcarata* raised with additional pollen had significantly greater fat stores ($N=50$) than those raised with reduced pollen ($N=47$). Lipid quantity was not significantly different between *C. calcarata* raised with additional pollen and controls ($N=39$), but bees raised with less pollen had significantly less fat than controls (Kruskal–Wallis test $\chi^2=9.90$, d.f.=2, $P=0.007$; Dunn’s test, control versus pollen added $P=1.00$, control versus pollen reduced $P=0.01$, pollen added versus pollen reduced $P=0.05$).

of bees in the control group was 36.96 ± 0.98 days (mean \pm s.e.m.). Although final body size was significantly different between control offspring and those raised with less pollen, developmental rate did not differ significantly between these groups (36.33 ± 0.95 days; Figs 2 and 3). *Ceratina calcarata* provided with more pollen, took significantly longer to develop than either of the other groups (39.21 ± 1.11 days; Kruskal–Wallis test $\chi^2=16.56$, d.f.=2, $P=0.0009$; Table 1). Specifically, development was significantly longer during three developmental stages: early in the larval stage (when the larva is 1/3 to 1/2 the length of the pollen ball), the prepupal stage and the early pigmented pupal stage (Table 1).

Behavioral assays

To assess the behavioral effects of food deprivation and abundance, we used circle tube assays to compare the number of interactions of each type between control and treatment groups: a positive value indicated the treatment group had more interactions than the control group, while a negative value indicated the treatment group had fewer interactions. The offspring provided with less pollen were significantly smaller than those provided with additional pollen (pollen added $N=20$, 0.52 ± 0.22 ; pollen removed $N=12$, -1.45 ± 0.23 ; ANOVA F -ratio=8.31, d.f.=1, $P=0.01$). When compared with controls, *C. calcarata* raised with additional pollen ($N=20$, mean \pm s.e.m. interactions, 0.68 ± 1.37) were significantly more aggressive than those raised with less pollen ($N=12$, -6.00 ± 1.27 ; Kruskal–Wallis test $\chi^2=5.40$, d.f.=1, $P=0.02$; Fig. 4A). There was no significant difference between treatment groups in any other behavioral type tested: following (pollen added $N=20$, 0.25 ± 1.29 ; pollen reduced $N=13$, -2.23 ± 1.26 ; Kruskal–Wallis test $\chi^2=0.36$, d.f.=1, $P=0.55$; Fig. 4B), avoidance (pollen added $N=20$, 0.30 ± 1.13 ; pollen reduced $N=13$, 0.38 ± 1.25 ; Kruskal–Wallis test $\chi^2=0.00$, d.f.=1, $P=1.00$; Fig. 4C), or tolerance (pollen added $N=20$, 4.95 ± 3.03 ; pollen reduced $N=13$, -0.62 ± 3.93 ; Kruskal–Wallis test $\chi^2=1.18$, d.f.=1, $P=0.28$; Fig. 4D). Additionally, there

was no significant difference between the total number of interactions (pollen added $N=20$, 2.65 ± 4.45 ; pollen reduced $N=13$, -6.31 ± 6.02 ; Kruskal–Wallis test $\chi^2=1.71$, d.f.=1, $P=0.19$).

DISCUSSION

Maternal manipulation of resources by *C. calcarata* mothers results in the development of a DED (Rehan and Richards, 2010b). The DED receives significantly less pollen and protein than other daughters, resulting in a smaller adult body size (Rehan and Richards, 2010b; Lawson et al., 2016). The DED serves as the worker for the nest, sacrificing her own direct fitness to help raise siblings (Rehan and Richards, 2010b; Rehan et al., 2014; Mikát et al., 2017). By manipulating the quantity of pollen provided to developing offspring, we were able to successfully manipulate adult body size, lipid stores and behavior.

Confirming our methods, we found that offspring provided with additional pollen consumed significantly more pollen than offspring provided with reduced amounts of pollen. Surprisingly, offspring provided with more pollen did not consume significantly more than controls and had pollen remaining following larval developmental stages (Fig. 1). This implies there is an upper limit to the amount of pollen that can be consumed. This is one of the first reports of an upper limit to the amount of pollen consumed by immature bees. Past research in the sweat bee, *Lasioglossum zephyrum*, has shown there is a maximum level of protein that can be consumed, but not total pollen (Roulston and Cane, 2002). Research in bumble bees has shown that workers forage specifically for essential proteins and macronutrients, implying that specific nutrients might be more important than pollen quantity or protein amount (Vaudo et al., 2016). Further research is needed to explore the effects of macronutrients in larval development and pollen consumption.

Next, we measured how the quantity of pollen provided to offspring affects body size and lipid stores. As with most

Table 1. Development rate of *Ceratina calcarata* from the pollen manipulation treatment

Stage	Control		Pollen reduced		Pollen added	
	Days	<i>N</i>	Days	<i>N</i>	Days	<i>N</i>
Egg	3.20 ± 1.64	44	3.59 ± 1.56	58	3.54 ± 1.48	50
Larva						
1/3 pollen ball	1.33 ± 0.68	51	1.44 ± 0.84	64	1.89 ± 0.98	57
1/2 pollen ball	1.22 ± 0.67	51	1.22 ± 0.64	69	1.67 ± 0.96	58
2/3 pollen ball	1.55 ± 0.99	51	1.46 ± 0.87	70	1.94 ± 1.38	60
1× pollen ball	2.09 ± 1.65	50	1.72 ± 1.15	68	2.69 ± 2.13	59
1.5× pollen ball	1.34 ± 0.66	51	1.61 ± 1.16	69	1.53 ± 1.06	59
2× pollen ball	1.37 ± 0.79	51	1.40 ± 0.82	66	1.28 ± 0.70	59
Trace bit pollen ball	1.48 ± 0.77	51	1.40 ± 0.79	66	1.26 ± 0.76	59
Full grown	2.05 ± 1.34	51	2.13 ± 1.40	66	1.89 ± 1.28	59
Prepupae	5.06 ± 1.88	51	5.06 ± 2.19	62	6.19 ± 3.49	56
Pupa						
White	1.99 ± 0.83	51	2.18 ± 0.81	59	2.08 ± 0.81	54
Pink	1.57 ± 0.62	51	1.58 ± 0.52	59	1.70 ± 0.72	53
Red	1.63 ± 0.83	51	1.49 ± 0.56	59	1.69 ± 0.84	53
Brown	2.47 ± 1.23	51	1.97 ± 0.95	59	2.27 ± 1.22	53
Black	1.64 ± 0.73	51	1.58 ± 0.89	59	1.40 ± 0.58	53
1/4 pigment	1.24 ± 0.65	50	1.18 ± 0.59	59	0.95 ± 0.44	53
1/2 pigment	1.19 ± 0.80	49	1.06 ± 0.51	59	0.98 ± 0.49	53
3/4 pigment	1.36 ± 0.76	46	1.24 ± 0.62	58	1.13 ± 0.49	52
Fully pigment	3.18 ± 1.11	38	3.02 ± 1.09	51	3.13 ± 1.25	48
Total	36.96 ± 0.98	940	36.33 ± 0.95	1180	39.21 ± 1.11	174

The duration of each developmental stage (grouped under the main developmental stages egg, larva and pupa) is given as the mean \pm s.d. number of days. *Ceratina calcarata* raised with more pollen took significantly longer to develop than those in the control and pollen reduced groups ($P=0.0009$). Most of the additional development time was in the larval stage, where all feeding occurs. Bold indicates a significant difference.

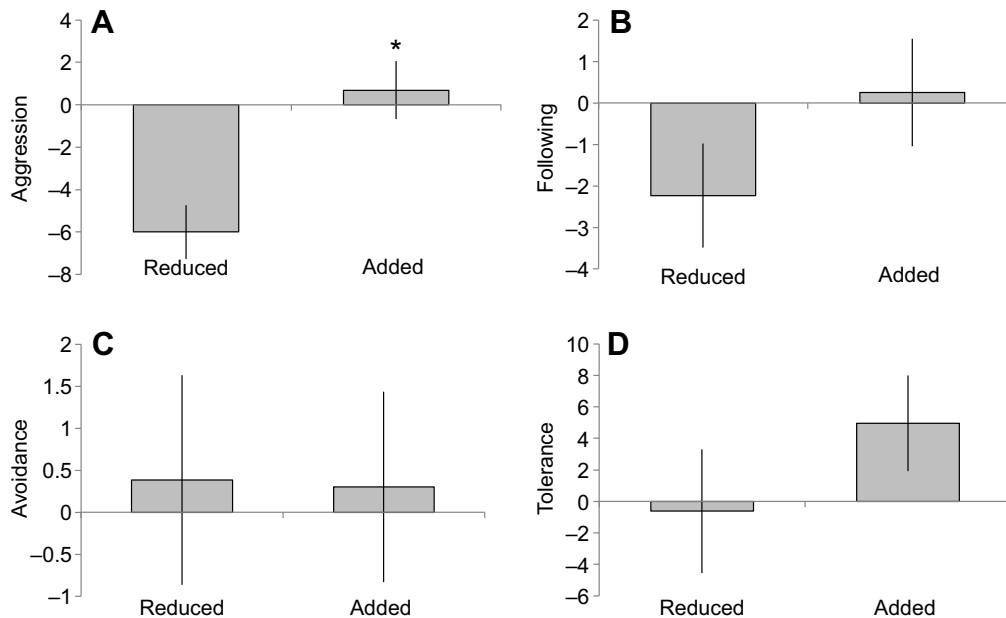


Fig. 4. Differences in the number of behavioral interactions between bees raised with additional or reduced pollen quantities relative to controls.

Positive numbers indicate more interactions by the treatment group relative to the control group, while negative numbers indicate fewer interactions relative to the control group (data are means \pm s.e.m.). (A) When compared with controls, *C. calcarata* raised with additional pollen ($N=20$) were significantly more aggressive than those raised with less pollen ($N=12$; Kruskal–Wallis test $\chi^2=5.40$, d.f.=1, $*P=0.02$). There was no significant difference between treatment groups in any other behavioral type tested: (B) following (pollen added $N=20$; pollen reduced $N=13$; Kruskal–Wallis test $\chi^2=0.36$, d.f.=1, $P=0.55$), (C) avoidance (pollen added $N=20$; pollen reduced $N=13$; Kruskal–Wallis test $\chi^2=0.00$, d.f.=1, $P=1.00$), or (D) tolerance (pollen added $N=20$; pollen reduced $N=13$; Kruskal–Wallis test $\chi^2=1.18$, d.f.=1, $P=0.28$).

Hymenoptera, pollen consumption in *C. calcarata* was directly associated with adult body size (Fig. 2) (Andersson, 1984; Packer and Knerer, 1985; Hunt and Nalepa, 1994; Hunt and Amdam, 2005; Toth et al., 2009; Quezada-Euán et al., 2010; Brand and Chapuisat, 2012). Offspring provided with less pollen were significantly smaller than controls or those provided with more pollen (Fig. 2). Smaller adult body size in Hymenoptera has been associated with a number of negative fitness correlates. Larger individuals maintain higher body temperatures allowing for foraging earlier in the season, with more frequent trips (Stone, 1993). Larger females have larger brood (Tengo and Baur, 1993), more offspring surviving to adulthood (Sugiura and Maeta, 1989; Larsson, 1990; Kim, 1997) and more female-biased nests (Rehan and Richards, 2010b). In some species, male mating success is tightly correlated with body size. For example, in the anthophorine bee *Centris pallida*, larger males are better at defending mating territories (Alcock, 1995). Larger male Dawson’s burrowing bees, *Amegilla dawsoni*, displace smaller males, forcing them to adopt alternative mating strategies (Alcock, 1997).

Reduced body size is correlated with fewer energy reserves in many insect species (Briegel et al., 2001; Barrett et al., 2009; Lease and Wolf, 2011; Sisterson et al., 2015). Our experiments revealed that offspring provided with less pollen had significantly smaller lipid stores than offspring provided with additional pollen (Fig. 3). Increased lipid stores allow larger females to better survive diapause (Tepedino and Torchio, 1982; Strassmann et al., 1984; Strohm and Linsenmair, 1999). Past research on *C. calcarata* found that lipid metabolism is upregulated in overwintering bees (Durant et al., 2016). Additionally, increased lipid stores have been linked to an increase in reproductive potential in *Polistes metricus* (Toth et al., 2009) and *Apis mellifera* (Toth and Robinson, 2005).

Finally, our results show that larger offspring provided with more pollen are significantly more aggressive than offspring provided

with less pollen. No other behavioral class significantly differed between treatment groups (Fig. 4). In social insects, dominance hierarchies are thought to be a precursor to reproductive hierarchies of caste systems (West-Eberhard, 1967; Gadagkar, 1980). In forced association studies, largely solitary species will form social dominance hierarchies, indicating these species possess behavioral precursors to higher levels of social organization (Sakagami and Maeta, 1977; Michener, 1985; Arneson and Wcislo, 2003). Dominance hierarchies are often determined by body size, where the smaller individual assumes a subordinate role (Smith et al., 2009; Tribble and Kronauer, 2017); thus, poor larval nutrition would reduce competitive ability (Huntingford and Turner, 1987; Withee and Rehan, 2016). For example, hierarchies in subsocial and primitively eusocial Hymenoptera, such as *C. calcarata*, *Lasioglossum zephyrum* and *Mischocyttarus mastigophorus*, are determined by dominance interactions, where larger females often become the reproductive individual and smaller females serve as workers (Kumar, 1975; Buckle, 1982; Molina and O’Donnell, 2008; Withee and Rehan, 2016). Specifically, in *C. calcarata*, mothers produce a DED by providing her with less pollen and nutrients than other daughters, resulting in a significantly smaller adult body size (Rehan and Richards, 2010b; Lawson et al., 2016). Because the DED is significantly smaller and typically less aggressive, the mother is able to coerce the DED to forage, thus forgoing her opportunity to reproduce and instead serving as the worker for the nest (Rehan and Richards, 2010b; Rehan et al., 2014). By manipulating the quantity of pollen, we were able to recreate the DED phenotype. By limiting pollen to offspring, we created significantly smaller, less aggressive individuals. Future work is needed to tease apart the effects of nutritional quantity versus quality on the development of social hierarchies in a subsocial species.

Conclusions

Ceratina calcarata mothers provide the first daughter with significantly less pollen than other offspring, resulting in a smaller daughter that will serve as the worker for the nest. By manipulating pollen quantity during development, we recreated the conditions of maternal manipulation and found that larval nutritional deprivation has significant effects on development, adult body size, lipid stores and behavior. We demonstrated how maternal manipulation of nutrition allows the mother to control adult body size. By creating a smaller daughter, the mother is able to coerce the daughter into serving as the worker for the nest. This maternal manipulation of resources leads to the development of the social organization and reproductive hierarchies, a major step in the transition to highly social behavior.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: S.P.L., S.M.R.; Methodology: S.L.H., S.M.R.; Formal analysis: S.P.L., S.M.R.; Investigation: S.P.L., S.L.H., S.M.R.; Resources: S.M.R.; Data curation: S.L.H.; Writing - original draft: S.P.L., S.M.R.; Writing - review & editing: S.P.L., S.L.H., S.M.R.; Supervision: S.M.R.; Project administration: S.M.R.; Funding acquisition: S.M.R.

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Data availability

Data are available from figshare (Lawson et al., 2017): <https://doi.org/10.6084/m9.figshare.5596621.v1>

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