The Influence of Maternal Quality on Brood Sex Allocation in the Small Carpenter Bee, *Ceratina calcarata*

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Abstract

In the twig-nesting carpenter bee, *Ceratina calcarata*, body size is an important component of maternal quality, smaller mothers producing significantly fewer and smaller offspring than larger mothers. As mothers precisely control the sex and size of each offspring, smaller mothers might compensate by preferentially allocating their investment towards sons. We investigated whether variation in maternal quality leads to variation in sex allocation patterns. At the population level, the numerical sex ratio was 57% male-biased (1.31 M/F), but the investment between the sexes was balanced (1.02 M/F), because females are 38% larger than males (1.28 F/M). Maternal body size explained both sex allocation pattern and size variation among offspring: larger mothers invested more in individual progeny and produced more female offspring than smaller mothers. Maternal investment in offspring of both sexes decreased throughout the season, probably as a result of increasing maternal wear and age. The exception to this pattern was the curious production of dwarf females in the first two brood cell positions. We suggest that the sex ratio distribution reflects the maternal body size distribution and a constraint on small mothers to produce small broods. This leads to male-biased allocation by small females, to which large mothers respond by biasing their allocation towards daughters.

Introduction

In dioecious organisms, natural selection usually favours equal parental investment in sons and daughters (Fisher 1958; Charnov 1982). In biparental, diploid organisms, this is ultimately because of the population sex ratio being balanced by the ratio of reproductive values of the two sexes; when one sex occurs in excess, per capita reproductive value declines until increased production of the rarer sex again balances the investment ratio. When the costs to rear male and female offspring are unequal, populations with even investment ratios should display biased numerical sex ratios that balance the production costs of sons and daughters. Moreover, in most diploid organisms, parents are equally related to male and female offspring, and so, on average, are expected to invest equally in sons and daughters

(Trivers & Hare 1976). However, even when population-level investment ratios are balanced, individual parents may preferentially allocate investment in daughters and sons in patterns that differ from the population optimum, but which maximize the fitness of particular parents in the below-mentioned variable ecological circumstances.

Three traits of many Hymenoptera (bees, ants and wasps) make them ideal candidates for studies of sex allocation patterns. First is the unique ability of hymenopteran mothers to precisely control the sex of individual offspring. Gender is determined by whether a mother allows an egg to be fertilized by sperm stored in her spermatheca, with fertilized eggs producing diploid daughters and unfertilized eggs producing haploid males. Second, many bees and wasps are mass provisioners, in which a mother provides each offspring with an individual food mass,

the size of which is the major proximate influence on the eventual adult body size of the offspring that will consume it (Danforth 1990; Bosch & Vicens 2002). Thus, mothers can precisely manipulate both the sex and the amount of investment in individual offspring. This level of maternal control creates the potential for optimizing brood sex ratios in response to a variety of selective pressures, including intrinsic factors such as maternal body size and condition, and extrinsic factors such as resource availability (Wrensch & Ebbert 1993). Third, females and males are often size dimorphic, so the production costs of daughters and sons are unequal for mothers; usually, but not always, females are bigger than males (Tepedino & Parker 1983, 1984; Johnson 1988; Visscher & Danforth 1993; Kim 1997; Martins et al. 1999; but see Paini & Bailey 2002 Alcock et al. 2005; Beveridge et al. 2006). Thus, in addition to manipulating the size and number of offspring, mother bees may also adjust the total investment in each sex. In general, high-quality mothers should allocate proportionately more of their investment to the more costly sex, whereas low-quality mothers should allocate proportionately more to the cheaper sex (Trivers & Willard 1973; Frank 1987).

What traits describe or predict maternal quality? Maternal quality refers to the set of maternal traits that influence the number and quality of offspring that a female can produce. In bees, maternal quality can be described in terms of a fixed component, body size as measured by exoskeletal measurements, and a variable component, maternal condition, which may change during a mother's lifetime. As in other insects, large females probably can produce more and larger eggs (Berrigan 1991; Honek 1993) and may also be more efficient foragers, because they can carry heavier loads and fly longer distances (Tomkins et al. 2001; Gathmann & Tscharntke 2002; Pereboom & Biesmeijer 2003). In short, larger bees should produce more and larger offspring than do small bees. Another potential influence on the maternal reproductive potential is wing wear; bees' wings tend to accumulate damage, starting with nicks and tears along the trailing edge, and then leading to progressive declines in wing surface area. Bees with worn wings cannot fly as efficiently and experience higher rates of physiological decline and higher mortality (Cartar 1992; Fox & Czesak 2000). More worn bees should provision fewer or smaller offspring than less worn bees, so over the course of a breeding season, progressively more worn mothers should produce progressively smaller and/or fewer brood.

In general, studies of maternal investment patterns in solitary bees have focussed either on intrinsic factors, such as maternal quality, or on extrinsic factors, such as resource availability. In the former category, studies of several species of trap-nesters, bees that nest in pre-existing holes or crevices, support the predictions that higher quality mothers produce more and larger brood, as well as proportionately more brood of the larger sex (females in the following examples). In *Osmia cornuta* (Bosch & Vicens 2006) and in *Osmia cornifrons* (Sugiura & Maeta 1989), small mothers produce broods with more male-biased investment ratios. Moreover, in *O. cornifrons*, older mothers produce more male-biased sex ratios as their provisioning efficiency and ability to produce eggs declines (Sugiura & Maeta 1989). In *Osmia rufa*, declining maternal provisioning efficiency and increasing risk of parasitism may explain the observed seasonal shift from female-biased to male-biased offspring production (Seidelmann 2006). In *Megachile apicalis*, larger females lay more eggs in total and also produce larger daughters, which results in a more female-biased investment ratio (Kim 1997).

Studies of extrinsic factors influencing maternal brood allocation have focussed on pollen availability. Bee mothers faced with low or declining levels of pollen or nectar availability may respond by increasing their foraging time (Richards 2004), by producing smaller or fewer offspring (Minckley et al. 1994; Kim & Thorp 2001) or by producing proportionately more offspring of the cheaper sex. In an elegant set of experiments, Peterson & Roitberg (2006a,b) found that in *Megachile rotundata*, a species in which females are larger than males, mothers responded to lower pollen availability by adjusting total clutch size downward, and making smaller daughters but not smaller sons. As they did not adjust the brood numerical sex ratio, they proportionally decreased allocation to daughters and increased allocation to sons. When resource availability was similar but some mothers had to fly further than others, these produced fewer and smaller brood, and also proportionately fewer daughters. In an experimental study of *M. apicalis*, when more pollen was made available to nesting bees, they produced larger clutch sizes and more and larger daughters (Kim 1999). Seasonal patterns of brood production may reflect seasonal patterns of resource availability, more costly brood (of either sex) being produced at times when pollen availability is greater (Paini & Bailey 2002; Alcock et al. 2005).

As the above examples show, studies of factors influencing maternal brood investment decisions have focussed either on intrinsic or on extrinsic factors, but rarely on both simultaneously (Kim 1999 is an exception), and mostly on trap-nesters of a single family, Megachilidae. Here, we investigate how maternal quality, as measured by maternal body size, leads to variation in sex allocation patterns in *Ceratina calcarata*, a member of the family Apidae. In addition, we investigate evidence for seasonal patterns in investment potentially linked to seasonal changes in resource availability.

Ceratina calcarata is one of the most common bees in eastern North America. It is a small carpenter bee that nests in woody twigs, especially of raspberry and sumac (Daly 1973). Mothers produce one brood per year, and remain with their offspring until the brood emerge as adults in mid to late summer. *Ceratina calcarata* are known to be incredibly variable in body size and sexually dimorphic; females are larger (more costly) and males are the smaller (less costly) sex (Johnson 1988; Rehan & Richards 2010). In the population studied here, female body size is highly variable and small females experience greater overwintering mortality than larger females (Rehan & Richards 2010). This suggests that female body size is an important influence on reproductive success and that it could also be an important factor influencing maternal sex allocation decisions when producing brood. In particular, we test the hypothesis of Trivers & Willard (1973) that larger mothers will be of higher quality and therefore produce more and larger offspring, as well as proportionately more daughters, than smaller mothers in the population. Additionally, we test the hypothesis of Frank (1987), that because of increasing maternal wear, mothers should opt to produce the larger, more costly daughters sooner and switch to smaller, less costly sons later in the season.

Methods

Life Cycle of *Ceratina calcarata* in Southern Ontario

This study was carried out at field sites at Brock University and the adjacent Glenridge Quarry Naturalization Park in St. Catharines, Ontario (49°7′N, 79°14′W). Here, we provide a summary of the nesting biology of *C. calcarata* in southern Ontario; further details are provided in Rehan & Richards (2010). In mid-May, females emerge from their hibernacula in hollowed out twigs and independently found nests through early June. Nest founding requires locating a dead broken twig followed by chewing a long linear burrow into the pith; nests are not reused a second season. Following nest founding, a female forages for pollen and nectar provisions to form a loaf of food or provision mass on which she then lays an egg. After oviposition each brood cell is capped with a partition the female constructs out of pith scraped from the nest walls. This process is repeated in a serial manner until the female ceases brood production thus concluding her reproductive effort for the summer. Foraging and oviposition usually cease by mid-summer (mid to late July), and egg to adult development time is about 46 d. Many brood remain in their natal nests until the following spring.

Nest Collections

Between 26 June and 27 July 2006, 110 complete nests were collected from dead raspberry (*Rubus ideaus*) and staghorn sumac (*Rhus typhina*) twigs. Field sites consisted of forest edges and meadows along walking trails with frequent human activity, including moderate mowing and pruning. The constant human interference not only produced numerous dead broken shoots for bee occupancy, but also made nests visible and easy to collect. Nests were collected before eight am to ensure that all occupants would be present. Branches containing visible hollows were sealed at the entrance with masking tape and clipped at the base of the shoot with pruning shears.

Nests were dissected in the laboratory the same day they were collected in the field. Mothers and pollen masses were weighed using a Mettler analytical balance (accuracy 0.001 mg). Maternal wing wear was scored from zero to five; mothers with no nicks or tears on the apical margin of their forewing receive a score or zero and mothers with the apical margin of their forewing completely shredded away receive a score of five. Females were dissected under light microscopy and insemination status was determined by the presence or absence of sperm in the spermatheca. The spermatheca of a mated female is opaque whereas an unmated female has a transparent spermatheca. Nest contents were recorded when the nests were opened, including brood developmental stages, number of brood cells, and the presence of an adult female who was assumed to be the mother of the brood. Only full-brood nests in which the youngest juvenile was at least a small larva (eggs take 5 d to hatch) were deemed to contain complete broods (see Johnson 1988; Rehan & Richards 2010).

All immatures along with any remaining mass provisions were removed from the nest and reared in clear 200 μl microcentrifuge tubes in an incubator at 25–27°C until they reached their final moult or died. The sex was recorded for all brood that reached the pupal stages. Sex was determined by counting the number of metasomal terga; females and males with six vs. seven segments, respectively. Following final moult, revealing their wings, immatures were removed from their rearing tube and weighed live using a Mettler analytical balance. Offspring live weight gives a reasonably precise estimate of maternal investment (Bosch & Vicens 2002). We use the term 'clutch size' to refer to the total number of brood in a nest.

Nest-founding date was approximated using the innermost basal brood cell. The date of nest dissection minus the number of days approximated for brood to reach that developmental stage (see Rehan & Richards 2010) gives an estimate of the nest-founding date.

Sex Ratio, Cost Ratio and Sex Investment Calculations

For each nest, we calculated the numerical sex ratio as the proportion of males among brood that reached the pupal stages. Cost ratios were calculated based on the mean live weights of newly emerged (callow) male divided by female brood. The live weight of newly emerged adults is directly correlated with the mass of pollen and nectar provisions that mothers provide to each offspring (Klostermeyer et al. 1973; Johnson 1988; Danforth 1990; Bosch & Vicens 2002), so the total mass of brood in a clutch is directly proportional to the mother's total energetic investment in reproduction. For each nest, we calculated maternal investment in each sex as the sum of the live weights of all sons or daughters in the nest. The population investment ratio was calculated as the total live weight of callow sons divided by the total live weight of callow daughters in all nests.

Statistical Analyses

Relationships between clutch size and sex ratios are complicated by the fact that same-sex clutches are more likely in smaller broods. We used the binomial sampling probability $p^n + q^n$, where p = probability of laying a male egg, q = probability of a female egg, and n = clutch size, to calculate the expected number of same-sex clutches for each observed clutch size. We then compared this to the observed number of same-sex clutches to determine whether the observed frequency was different from that expected based on the observed sex ratio.

Statistical analyses were carried out using SAS 9.1 (SAS Institute Inc., Cary, NC, USA). We used multiple regression to examine the influence of maternal live weight, maternal wing wear and nest-founding date on clutch size, brood sex ratio, total brood mass and investment ratio. All variables except sex ratio were normally distributed. Clutch size was calculated as the total number of brood per nest. Following the usual practice in studies of bee sex ratios, brood sex ratio is presented as the proportion of male brood in each clutch, but as this variable was not normally distributed, the following transformation was used for regression analyses: $\ln [(m + 1)/(f + 1)]$, where m is the number of male brood per nest and f is the number of female brood per nest (this circumvents difficulties inherent in using ratios with denominators of 0). For each sex of brood, brood mass was calculated as the number of brood multiplied by the average live weight of newly emerged individuals. This calculation allowed us to assess total brood mass even if not all brood survived to adulthood. The investment ratio for each nest was calculated as male brood mass/total brood mass.

Only complete data were used for the regression analyses, so sample sizes were different for each analysis. In total, there were 93 clutches for which maternal live weight and wing wear were known, 40 clutches in which all brood survived to pupation so that they could be sexed, and 26 clutches in which total broodmass could also be measured.

Chi-square and G-tests of goodness-of-fit were used to test for deviation from equality in population and brood cell sex ratios, and offspring live-weight ratios. Statistical significance was usually assessed at $\alpha = 0.05$, but in a few multi-way linear models, partial effects with p < 0.1 are reported when the overall model was significant at p < 0.05.

Results

Maternal Size and Condition

There was considerable variation in maternal body size, both in terms of live weight (mean ± SD, 14.16 ± 3.35 mg, range 5.05–22.15 mg, n = 93) and head width (1.92 ± 0.17 mm, range 1.33–2.23 mm, n = 108). These two measures were highly correlated ($r = 0.88$, n = 104, p < 0.0001; Fig. 1). Neither size measure was associated with nest-founding date
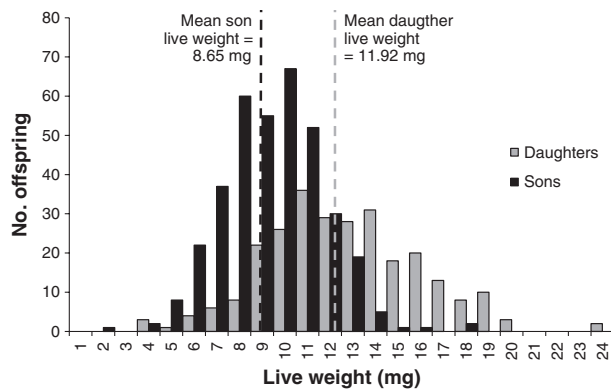
Fig. 1: Offspring live-weight distributions. Male live weights ranged from 1.98 to 17.66 mg, while females ranged from 3.06 to 23.53 mg live weight. Female offspring were significantly larger than male offspring.

(live weight: $r = -0.06$, n = 93, n.s.; head width: $r = 0.05$, n = 108, n.s.). In short, there was no tendency for large mothers to nest earlier than small mothers.

Wing wear scores for mothers of complete broods ranged from 0 to 5, but the average score was only 1.5, because only 28 of 108 (26%) had very worn wings (scores of 3 or higher). Maternal wing wear was not related to body size (live weight: $r = 0.14$, n = 93, n.s.; head width: $r = 0.13$, n = 108, n.s.), but tended to be higher among mothers that founded nests later in the season ($r = 0.18$, n = 108, p = 0.0545). In a sample of founding nests (collected prior to the onset of brood provisioning), only four of 118 mothers collected in May and June had any sign of wing wear, whereas four of five mothers

collected from very late founded nests in July had worn wings. In other words, increases in maternal wing wear over the season were at least partly because of flight activities prior to brood provisioning.

Variation in Number and Sex of Offspring

Our sample of 110 full-brood nests contained a total of 679 brood that survived at least to pupation. Of these, 385 (56.7%) were male and 294 (43.3%) were female, which differs significantly from an even sex ratio (log likelihood chi-square, $G_1 = 12.23$, p = 0.00047). In a smaller sample of 45 nests with 100% brood survival to pupation, the proportion of males was 58.1% (180 males and 123 females) which was not significantly different from that calculated for all complete nests ($G_1 = 0.00134$, n.s.).

Three variables, maternal wing wear, nest initiation date and maternal live weight, were significant predictors of variation in both clutch size (the number of brood per nest) and total brood mass (Table 1). In general, larger clutches were produced by larger mothers that exhibited less wear and which started brood production earlier in the season, whereas smaller clutches were produced by smaller mothers that exhibited more wear and tended to start brood production later in the season.

Variation in the sex ratios of individual nests was dramatic, ranging from all female, to even, to all male (Fig. 2). The most important predictor of brood sex ratio was maternal live weight, whereas maternal wing wear and nest-founding date were not significant (Table 1). Separate multiple regression

Table 1: Results of multiple regression analyses for three predictors of maternal brood productivity. The complete model refers to the following regression equation: brood productivity = β_1(maternal live weight) + β_2(maternal wing wear) + β_3(nest-founding date) + ε. The sex ratio was analysed as the log-transformed ratio of males to females, $\ln[(m + 1)/(f + 1)]$

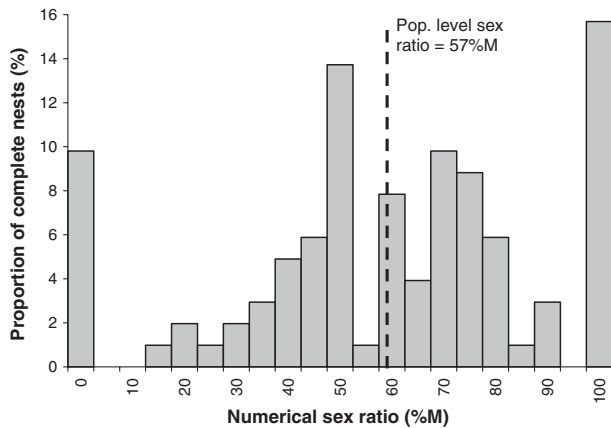| Brood productivity measure	Complete model	Parameter estimates		
		Maternal live weight	Maternal wing wear	Nest-founding date
Clutch size	$R^2 = 0.392$	$\beta_1 = 233.4 \pm 75.5$	$\beta_2 = -0.99 \pm 0.18$	$\beta_3 = -0.11 \pm 0.03$
	$F_{3,92} = 19.11$	$t = 3.05$	$t = -5.38$	$t = -3.58$
	$p < 0.0001$	$p = 0.0030$	$p < 0.0001$	$p = 0.0006$
Number of daughters	$R^2 = 0.295$	$\beta_1 = 223.88 \pm 53.69$	$\beta_2 = -0.29 \pm 0.13$	$\beta_3 = -0.07 \pm 0.02$
	$F_{3,89} = 12.45$	$t = 4.17$	$t = -2.25$	$t = -3.41$
	$p < 0.0001$	$p < 0.0001$	$p = 0.0266$	$p = 0.0010$
Number of sons	$R^2 = 0.357$	$\beta_1 = -14.09 \pm 58.12$	$\beta_2 = -0.52 \pm 0.14$	$\beta_3 = -0.12 \pm 0.02$
	$F_{3,89} = 16.48$	$t = -0.24$	$t = -3.70$	$t = -5.15$
	$p < 0.0001$	$p > 0.1$	$p = 0.0004$	$p < 0.0001$
Sex ratio	$R^2 = 0.210$	$\beta_1 = -97.77 \pm 36.80$	$\beta_2 = -0.12 \pm 0.09$	$\beta_3 = -0.01 \pm 0.02$
	$F_{3,36} = 3.20$	$t = -2.66$	$t = -1.40$	$t = -0.36$
	$p = 0.0348$	$p = 0.0117$	$p > 0.1$	$p > 0.1$

Fig. 2: Population-level sex ratio calculated from the 385 male and 294 female offspring reared in the laboratory. This 57% male-biased population-level sex ratio was significantly different from unity. Individual nests ranged from all-female to all-male broods.

analyses of the number of female or male brood per nest (Table 1) suggest that larger mothers produced larger clutches as a result of adjusting the number of daughters but not sons (Fig. 3). The numbers of both daughters and sons were negatively correlated with maternal wing wear and nest-founding date.

Same-sex clutches (all-male or all-female) were smaller (mean $\pm$ SD 3.8 $\pm$ 2.5) than mixed-sex clutches (7.8 $\pm$ 3.2; ANOVA, $F_{1,41}$ = 12.17, p = 0.0012). There was one all-female clutch of 4 (probability q^4 = 0.028), but the majority of same-sex clutches were all-male, including four relatively large nests with clutch sizes of 6, 6, 8 and 8. Dissection of three preserved mothers that produced all-male broods, indicated that one was unmated (clutch size 5) and two (clutch sizes 6 and 8, respectively) possessed sperm in their spermathecae. Overall, the observed number of same-sex clutches in the sample of completely known nests was 11, significantly higher than
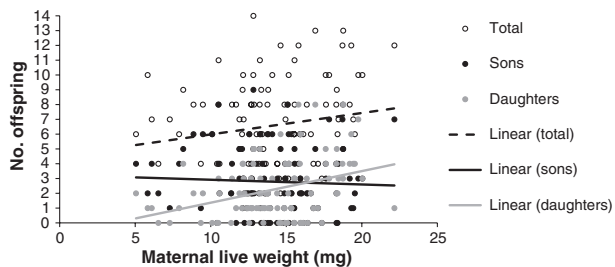
expectation based on the observed p = 0.59 and q = 0.41 (χ^2 = 47.07, df = 13, p < 0.001). As small clutches included both all-female and all-male broods, there was no correlation between clutch size and brood sex ratio (r = 0.06, n = 45, n.s.).

To determine whether the sex of offspring varied with brood cell order, the sex ratio was examined for each brood cell position, position one being the innermost brood cell (Table 2). The two innermost brood cell positions usually contained females, the bias being especially apparent in position one, whereas the remaining brood cells usually contained males.

Variation in Brood Body Size and Maternal Investment

Ceratina calcarata is moderately sexually dimorphic, with newly emerged female brood weighing 11.92 $\pm$ 3.45 mg (mean $\pm$ SD) and males weighing 8.65 $\pm$ 2.31 mg on average (Fig. 1). Mean female weight was 38% larger than mean male weight ($F_{1,535}$ = 192.61, p < 0.0001), so the female/male cost ratio (the estimated energetic cost to a mother of producing a daughter vs. a son) was 1.38. The male-biased numerical sex ratio (1.31:1) was almost exactly balanced by the female-biased cost ratio (1.38:1), resulting in a calculated investment ratio (M/F) for all nests of 1.02 (30.2 g of male brood/ 29.5 g of female brood), which was not significantly

Fig. 3: Maternal live weight was compared to the number of sons and daughters she produced. Larger mothers produced significantly more daughters than smaller mothers, yet son production remained consistent across maternal live weights.

Table 2: Sex allocation sequence among brood cell positions. Each brood cell position was examined across all complete nests analysed to determine whether there is a pattern of sex allocation within *Ceratina calcarata* nests. The first two positions were female-biased (brood cell position 2 is significant at p < 0.1), and the remaining positions were male-biased. No further predictable sex allocation pattern exists across all nests

| Brood cell position	No. of males	No. of females	Total no.	Proportion male (%)	G	p value
1	28	78	106	26.4	24.548	**<0.0001**
2	45	62	107	42.1	2.712	0.0996
3	63	40	103	61.2	5.179	**0.0229**
4	64	38	102	62.7	6.701	**0.0096**
5	53	35	88	60.2	3.708	**0.0271**
6	46	32	78	58.9	2.526	0.1119
7	45	16	61	73.8	14.360	**0.0001**
8	27	20	47	57.4	1.046	0.3063
9	14	12	26	53.8	0.1540	0.6947
10	10	7	17	58.8	0.5322	0.4657
11	10	2	12	83.3	5.8221	**0.0158**
12–14	8	7	15	53.3	0.0667	0.7962
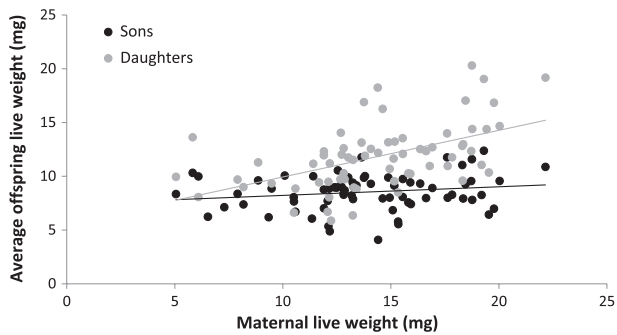
Fig. 4: Maternal live weight when compared to the body size of sons and daughters she produced. Larger mothers produce significantly larger daughters than smaller mothers, yet son's body size did not vary across maternal live weights.

Fig. 5: Maternal investment was examined for each brood cell position to examine investment allocation within a nest. Investment steadily declines from inner to outermost brood cell positions for sons. Innermost daughters are smaller than later sisters and often smaller than some of their brothers within the same nest.

different from equal investment in male and female brood ($G_1 = 0.00019$, n.s.).

Larger mothers produced larger offspring of both sexes ($F_{3,448} = 106.21$, p < 0.0001; Fig. 4), but the influence of maternal size was much more pronounced in daughters ($R^2 = 0.249$, $F_{1,186} = 61.88$, p < 0.0001) than in sons ($R^2 = 0.023$, $F_{1,262} = 6.08$, p = 0.0143). The influence of four variables, maternal live weight, maternal wing wear, nest-founding date and brood cell position, on the live weight of individual brood was investigated using multiple regression. For male brood, only maternal weight and brood cell position were significant predictors (model $R^2 = 0.144$, df = 4, 259, p < 0.0001; maternal weight partial $F_1 = 6.86$, p = 0.0093; brood cell position partial $F_1 = 32.94$, p < 0.0001). For female brood, only maternal weight and nest-founding date were significant predictors (model $R^2 = 0.310$, df = 4, 183, p < 0.0001; maternal weight partial $F_1 = 66.25$, p < 0.0001; founding date partial $F_1 = 13.89$, p = 0.0003). However, there was a categorical (as opposed to linear) positional effect on female brood body size; those in the innermost cells (positions 1 and 2) were conspicuously smaller (average live weight 11.4 mg) than those produced in later positions (average live weight 12.6 mg) (inner two cells vs. others, after accounting for variation because of maternal size and date, partial $F_1 = 13.26$, df = 1, p < 0.0004; Fig. 5). New provision masses also decreased in size as time progressed ($F_{1,122} = 41.93$, p < 0.0001); however, we did not know the sex of brood that would be produced on individual provision masses.

Maternal allocation of investment to sons vs. daughters was assessed by calculating male mass as a proportion of total brood mass. Multiple regression mode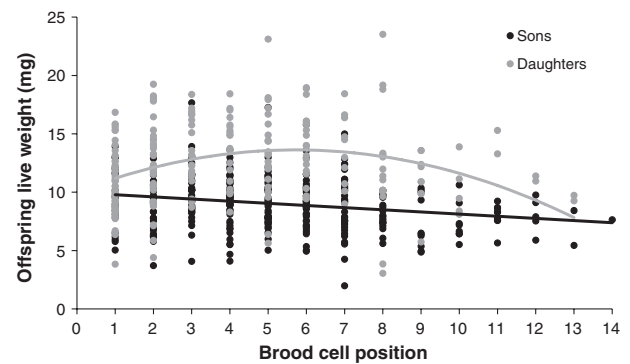ls (Table 3) indicated that total investment in brood (measured as total brood mass) was positively associated with maternal live weight and negatively with wing wear and founding date. Larger mothers produced more daughter biomass than smaller mothers (Table 3; Figure 6). Maternal wing wear was not associated with total daughter biomass but was negatively associated with total son biomass. The total biomass of both sons and daughters was less in nests founded later in the season. In terms of allocation of resources to sons vs. daughters (the investment ratio), larger mothers allocated a significantly greater proportion of their investment to daughters, as a result of producing both more and larger female offspring than did smaller mothers. Mothers that founded nests later in the season allocated proportionately more of their investment to sons (Table 3).

Discussion

The Influence of Maternal Quality on Sex Allocation in *Ceratina calcarata*

Many organisms can manipulate sex allocation ratios among their brood by adjusting offspring body size, but the ability to precisely control the sex of each offspring produced has been demonstrated in far fewer organisms (reviewed in West 2009). In bees, the combination of these two abilities allows mothers to precisely adjust brood sex allocation to their individual circumstances, including their own phenotypic limitations. In the current study of *C. calcarata*, there was dramatic variation among nests in sex and maternal investment ratios, and maternal body size was a strong predictor of both, as

Table 3: Results of multiple regression analyses for predictors of female and male brood production. The complete model refers to the following regression equation: brood investment = β_1(maternal live weight) + β_2(maternal wing wear) + β_3(nest-founding date) + ε. The investment ratio was analysed as the proportional weight of male brood per nest

Brood investment measure	Complete model	Parameter estimates		
		Maternal live weight	Maternal wing wear	Nest-founding date
Total brood mass	$R^2 = 0.523$	$\beta_1 = 3714.68 \pm 1601.20$	$\beta_2 = -11.87 \pm 3.95$	$\beta_3 = -2.98 \pm 0.98$
	$F_{3,22} = 8.04$	$t = 2.32$	$t = -3.01$	$t = -3.03$
	$p = 0.0008$	$p = 0.0300$	$p = 0.0065$	$p = 0.0062$
Total mass of daughters	$R^2 = 0.500$	$\beta_1 = 5395.11 \pm 1276.82$	$\beta_2 = -4.42 \pm 3.12$	$\beta_3 = -1.80 \pm 0.73$
	$F_{3,26} = 8.65$	$t = 4.23$	$t = -1.42$	$t = -2.47$
	$p = 0.0004$	$p = 0.0003$	$p > 0.1$	$p = 0.0250$
Total mass of sons	$R^2 = 0.370$	$\beta_1 = -1014.86 \pm 810.80$	$\beta_2 = -5.49 \pm 2.01$	$\beta_3 = -1.09 \pm 0.47$
	$F_{3,29} = 5.68$	$t = -1.25$	$t = -2.73$	$t = -2.33$
	$p = 0.0035$	$p > 0.1$	$p = 0.0106$	$p = 0.0270$
Investment ratio	$R^2 = 0.321$	$\beta_1 = -41.13 \pm 13.40$	$\beta_2 = -0.04 \pm 0.03$	$\beta_3 = -0.01 \pm 0.01$
	$F_{3,36} = 3.47$	$t = -3.07$	$t = -1.19$	$t = -1.19$
	$p = 0.0336$	$p = 0.0056$	$p > 0.1$	$p > 0.1$

larger mothers produced more offspring of both sexes, and their broods contained more and larger daughters. Johnson's (1990) study of *C. calcarata* in Indiana failed to find a relationship between maternal body size and sex ratio variation, but sample sizes were small (n = 20 nests), and all-female broods were excluded because of taxonomic confusion with a morphologically similar species. In fact, the patterns we observed in *C. calcarata* may be typical of this genus. In an Asian species, *Ceratina flavipes*, large mothers provisioned larger daughters and produced female-biased broods, while smaller mothers produced less costly sons resulting in slightly male-biased brood (Maeta et al. 1992).

Virgin oviposition may account for some but not all of the sex ratio variation that we observed among
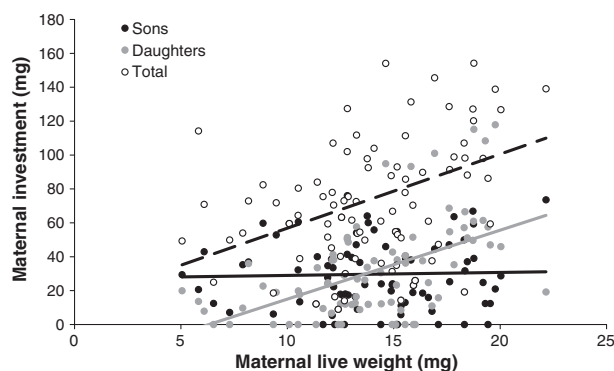

Fig. 6: Maternal live weight compared to maternal investment in offspring biomass. Investment in offspring biomass increases with maternal body size. Investment in sons remains constant across maternal body sizes but larger mothers invest more in daughters than smaller mothers.

nests of *C. calcarata*. Unfertilized females can found nests but are constrained to oviposit all-male broods (Tepedino & Torchio 1982; Frohlich & Tepedino 1986). In *C. calcarata*, the period of nest establishment can be quite protracted, and it is possible that some females fail to mate, because they emerge from their hibernacula late in spring when males have mostly disappeared. Dissection of three preserved mothers that produced all-male broods, confirmed that one female was unmated, but the other two females possessed sperm in their spermathecae. The unmated female produced a clutch size of five, and the two that had mated produced clutch sizes of six and eight. Hymenopteran females can precisely control sex of their offspring by releasing of sperm from the spermatheca to produce daughters or withholding sperm to produce sons (Gerber & Klostermeyer 1970). In addition, prolonged sperm viability within the spermatheca is well known for bees (Verma 1973; Collins et al. 2004). When mated females produce only sons, this could suggest barriers to fertilization such as morphological deformities of sperm or of the spermatheca It also is possible that females may choose to lay only unfertilized eggs.

The existence of females constrained to produce males may be an important contributing factor to the more or less split sex ratio distribution that we observed (Godfray 1990). Although chance deviations from the binomial distribution might explain all-female production in small broods, there may also be females constrained to produce all-female broods. In Hymenoptera, endosymbionts that cause sex ratio distortions and production of same-sex broods of either sex are well known (Werren 1997),

although there appear to be no examples in which both types of distortion are found in the same species. Two recent studies of female production by unfertilized *Apis mellifera* workers (Hoy et al. 2003) and of male-biased sex ratios in *Osmia* (Jeong et al. 2009) failed to support the hypothesis that these patterns were caused by endosymbiont infection. Indeed, there is so far little evidence to support the role of endosymbionts as proximate causes of highly biased sex ratios produced in many social or presocial Hymenoptera (Wenseleers & Billen 2000, Zchori-Fein and Perlman 2004). However, endosymbiont sex ratio distortion certainly does occur in Hymenoptera, and this could potentially have contributed to production of either all-male or all-female broods in *C. calcarata*.

Overall, we found that the population sex ratio was male-biased, whereas the cost per offspring (cost ratio) was female-biased, and that these two balanced to produce a population sex investment ratio that was almost exactly even. This in turn suggests a population-level balance between higher quality mothers that produce more and larger daughters vs. lower quality mothers producing fewer and smaller sons. However, it is unlikely that mothers can detect and respond to the population sex ratio among brood at the time of egg-laying, as brood production occurs over an extended period of time and individual nests are scattered throughout the landscape (Rehan & Richards 2010). On the other hand, it does seem likely that the sex allocation strategy exhibited by each individual mother is a flexible strategy that depends on her assessment of her own body size and condition. In *C. calcarata* as in other bees, small mothers probably forage less efficiently (Tomkins et al. 2001; Gathmann & Tscharntke 2002; Pereboom & Biesmeijer 2003) and do not lay as many eggs (Berrigan 1991; Honek 1993). In contrast, large mothers that forage efficiently and lay more eggs probably face much lower constraints; they could produce brood of any size or sex, but tend to produce proportionately more daughters. Therefore, the tendency for high-quality females to produce female-biased broods and more and larger daughters is likely an evolutionary response of large mothers to a numerical male bias created by constraints on low-quality mothers (Werren & Charnov 1978), as predicted by Trivers & Willard (1973). This suggests that overall the population is Fisherian, but that over time the distribution of brood sex ratios might track maternal body size variance. This hypothesis could eventually be tested by comparisons over time (Tepedino & Torchio 1982), among populations in different environments, or even among related species.

Temporal Patterns in Maternal Investment

In this study, we observed seasonal declines in the average size of both provision masses and offspring. These declines could reflect either declines in pollen and nectar availability or a decline in the ability of mothers to access those resources. As *C. calcarata* are small and polylectic (they forage on many different types of flowers), pollen availability is unlikely to decline in areas such as our study sites that experience a continuous sequence of bloom. On the other hand, maternal wing wear was a significant predictor of declines in clutch size, brood mass and brood body size. As wing wear is incremental and cumulative and has negative effects on a bee's overall physiological condition (Cartar 1992), it was the most likely cause of overall declines in maternal investment from the beginning to the end of the brood provisioning period.

Mothers with declining resource provisioning abilities should produce the more costly sex of offspring while they are in good condition, and switch to production of the cheaper sex as their condition declines (Frank 1987). In the population as a whole, expensive brood should be produced before cheaper brood, as observed in several species of bees (Kim 1999; Paini & Bailey 2002; Alcock et al. 2005; Peterson & Roitberg 2006b) and wasps (Strohm & Linsenmair 1997). In *C. calcarata*, we predicted that being larger, daughters would tend to be produced earlier than sons. In fact, we did find that daughters were produced in the first two brood cells, whereas sons were more frequent in later brood cells. However, we also found that the first two daughters were distinctly smaller than subsequent daughters, contradicting the hypothesis that daughters are produced first because they are larger and more costly. We suggest that this pattern is more likely related to the potential social roles of these daughters rather than to seasonal changes in maternal resource-gathering ability.

Potential Social Significance of Maternal Allocation Patterns in *Ceratina calcarata*

Female numerical bias in the first two brood cells, combined with small body size of such daughters, is recurrent throughout *Ceratina*. Johnson (1988) found strongly female-biased first brood cell positions in populations of *C. calcarata* in Indiana, noting

their reduced body size in comparison with younger sisters and even younger brothers. Dwarf eldest daughters have also been reported in *Ceratina dupla* (Vickruck 2010), *C. flavipes* (Sakagami & Maeta 1977), *Ceratina japonica* (Sakagami & Maeta 1984) and *Ceratina okinawana* (Sakagami & Maeta 1995). In the latter two species, there are observations that the small eldest daughter emerges earlier than her siblings by breaking down and rebuilding brood cell walls to pass over her younger siblings and that she often takes over feeding nestmates prior to overwintering (Sakagami & Maeta 1989; Sakagami et al. 1993). Carpenter bees (including both *Ceratina* and *Xylocopa*) are somewhat unusual among bees in that newly eclosed, callow brood must be fed before overwintering (Michener 1990). As mothers can precisely control the size of each offspring, the initial production of very small daughters, suggests that the dwarf elder daughters are intended to become nest helpers, their small size rendering them susceptible to maternal manipulation (Michener 1990; Maeta et al. 1992).

The production of dwarf elder daughters would help to explain the persistence in *C. calcarata* populations, of very small females which have low overwintering survival (Rehan & Richards 2010), low reproductive success and which are constrained to produce male-biased broods. Ironically, it appears that only relatively large mothers have sufficiently high reproductive success that they can afford to make one or two dwarf elder daughters (instead of large daughters) that would eventually become helpers and that might also eventually produce small, mostly male brood. The smallest mothers, which might benefit most from being helped, are more likely to make all-male or highly male-biased broods. When small females do make daughters, the daughters are about the same size as their mothers, even in the first brood cell position, and so would probably not be susceptible to the physical aggression that mothers would likely use to exact helping behaviour from worker-like daughters (Crespi & Ragsdale 2000). Above, we proposed that production of female-biased broods by high-quality mothers represents an evolutionary response to male-biased production by low-quality mothers, and this is a novel route to production of the female-biased sex ratios so commonly associated with incipient stages of social evolution (Seger 1983; Schwarz 1994). The scenario we propose here suggests that high-quality mothers themselves produce a few low-quality daughters as potential helpers, and if these survive

hibernation, they join the pool of low-quality mothers nesting the following spring.

Acknowledgements

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