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## Reproductive aggression and nestmate recognition in a subsocial bee

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In social contexts, the ability to recognize and discriminate among individuals is advantageous, because it allows individuals to adjust their behaviour so as to enhance both individual and group fitness. Eusocial insects have finely developed mechanisms of discrimination that promote many kinds of social interactions, but discrimination may also be adaptive in noneusocial species, including solitary ones. Physical traits such as reproductive status influence rates of aggression and discrimination, permitting individuals to share common resources and nesting sites and to cooperatively care for offspring, while excluding potential aggressors or social parasites. In this study, we examined reproductive aggression and nestmate recognition in a subsocial species of small carpenter bee, *Ceratina calcarata*, using circle tube behavioural assays. Not only does this subsocial bee show nestmate recognition, but there is seasonal variation in aggression that correlates with seasonal variation in reproductive status, illustrating that both aggressive behaviour and the consequences of nestmate recognition are context dependent. Females that were actively reproductive (ovaries fully developed) were more aggressive than pre-reproductive (ovaries undeveloped) or post-reproductive females (ovaries resorbed). Females altered their behaviour when interacting with nestmates versus non-nestmates. As in most social Hymenoptera, agonistic behaviour was observed to be greatest between unfamiliar, reproductively active individuals. However, post-reproductive females were tolerant towards unfamiliar females. During the natural adult cohabitation phase of the nesting cycle (the mature brood phase), mothers were aggressive towards daughters, whereas same generation pairs of nestmates or non-nestmates showed no signs of aggression. These results indicate that this subsocial bee species does possess the ability to recognize nestmates but the consequences of recognition vary seasonally, sometimes resulting in greater aggression towards nestmates than towards non-nestmates.

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The ability to discriminate among individuals with different social characteristics is a prerequisite to many kinds of social behaviour, including territoriality, pair bonding, care of young, maintenance of social hierarchies and colony defence (Fletcher & Michener 1987). Discrimination involves a minimum of two individuals, a cue bearer and a cue perceiver. Depending on the degree of similarity between the perceived cue and a learned template, the cue perceiver assesses the other individual and either treats it tolerantly or attacks it (Ribbands 1954; Bell 1974; Lacy & Sherman 1983; Gamboa et al. 1987b; Moritz & Neumann 2004; Kudo et al. 2007). Recognition of nestmates as well as other aspects of social status is indicated when perceivers react differently to different classes of individuals. In social bees and wasps, nestmate recognition is most easily inferred when familiar individuals are

more tolerant and unfamiliar individuals are more aggressive (Benest 1976; Greenberg 1981; Pabalan et al. 2000). However, the context of interactions between perceivers and cue bearers also matters, as recognition can result in different outcomes depending on the cues presented and upon the current situation. For instance, in the primitively eusocial sweat bee, *Lasioglossum zephyrum* (Hymenoptera: Halictidae), females distinguish not only familiar versus unfamiliar individuals, but also the degree of relatedness between females, and whether they are queens or workers (Breed et al. 1978). In two other sweat bees (*Lasioglossum figueresi* and *Halictus ligatus*), ovarian status influences reactions: females with larger ovaries are more aggressive whereas bees with smaller ovaries show more avoidance (Wcislo 1997; Pabalan et al. 2000). Therefore, in studies of behavioural interactions that might indicate nestmate recognition, it is important to also investigate context dependence.

An outstanding feature of the social insects is their frequent and elaborate cooperative behaviour, which depends on accurate discrimination between colony-mates and aliens (Hölldobler & Wilson

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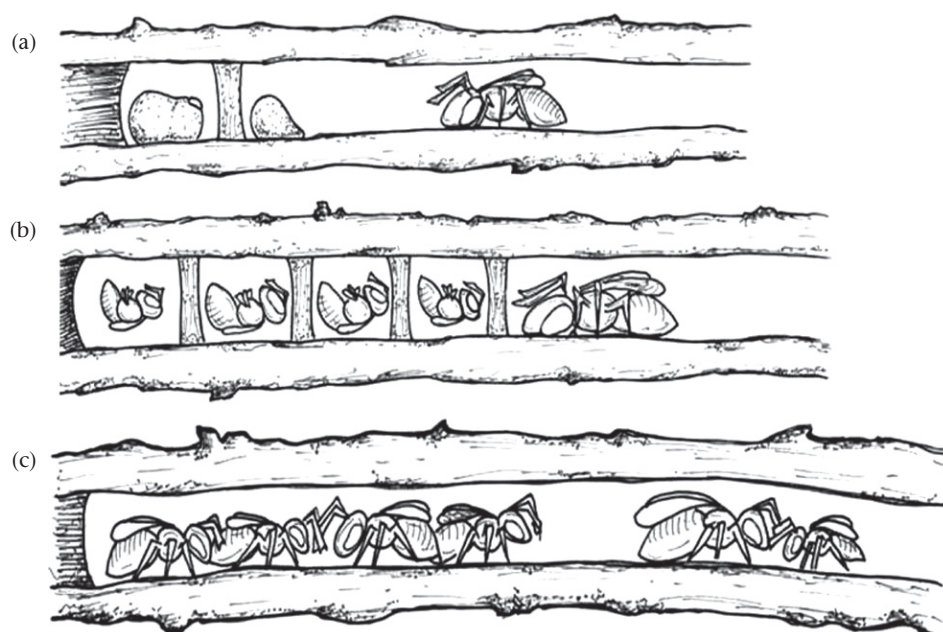
2008). Nestmate recognition has been posited as a precursor trait for the evolution of complex sociality (Breed et al. 1978; Flores Prado et al. 2008; Richards & Packer 2010). In social contexts, discrimination among individuals is clearly advantageous, because it allows individuals to adjust their behaviour so as to enhance both individual and group fitness (Hamilton 1972; Gamboa et al. 1987a). As a result, previous studies of recognition in bees and wasps have largely focused on obligately eusocial species, testing the complementary predictions that adult females should show tolerant or cooperative behaviour towards nestmates and intolerant or aggressive behaviour towards non-nestmates (Bell 1974; Shellman-Reeve & Gamboa 1984; Breed & Page 1991; Buchwald & Breed 2005; Kudo et al. 2007). However, the ability to discriminate between familiar versus unfamiliar individuals or between nestmates and non-nestmates may have adaptive functions in other contexts as well. Nestmate recognition is likely to occur in solitary, subsocial and facultatively social species in which there exist opportunities for repeated behavioural interactions among adults. For instance, discrimination of unfamiliar adults might be helpful when nesting females face conspecific nest parasitism (Field 1992; Hogendoorn & Leys 1993), which can occur in bees and wasps of any social type.

Although behavioural interactions among individuals in obligately solitary and obligately eusocial bees have been well studied (Breed et al. 1978, 2007; McConnell-Garner & Kukuk 1997; Buchwald & Breed 2005; Packer 2006; Flores Prado et al. 2008), subsocial and incipiently social species have received less attention (Wcislo 1997; Arneson & Wcislo 2003; Peso & Richards 2010). Subsocal bees are informative species for elucidating the intermediate evolutionary steps in transitions from solitary antecedents to eusociality (Linksvayer & Wade 2005; Wilson 2008). Subsocal bees are typified by nest loyalty, adult longevity and prolonged cohabitation, all necessary preconditions for solitary lineages to evolve more complex social traits (Tallamy & Wood 1986; Crespi 1994; Costa 2006; Wilson 2008). Since a likely evolutionary route between solitary and eusocial behaviour involves a subsocial intermediate, empirical data on intraspecific variation in the behavioural profiles of subsocial species can be used to test the hypothesis that natural variation in the behavioural propensities of solitary and subsocial species might

provide the phenotypic plasticity from which social behaviour can evolve (West-Eberhard 1967; Linksvayer & Wade 2005).

The small carpenter bee, *Ceratina calcarata* (Hymenoptera: Apidae), is a subsocial species endemic to eastern North America; its colony cycle and social structure are both well understood (Johnson 1988; Rehan & Richards 2010a, b). Unlike highly eusocial, caste-differentiated species such as honeybees (Hymenoptera: Apidae), *C. calcarata* is subsocial, having neither morphological castes nor reproductive division of labour. *Ceratina calcarata* females are long-lived and nest loyal, and provide care to their offspring throughout development and even into adulthood (Rehan & Richards 2010b). In spring, overwintered females emerge from their hibernacula, dispersing to construct new nests in twigs and stems. 'Active brood nests' are those in the first phase of brood production and contain the reproductively active mother bee and developing brood of various ages (Fig. 1a). The active brood phase generally lasts from May to June. When females complete oviposition in early to late July, the nest enters the 'full brood' phase. The mother becomes reproductively inactive but remains in the nest to guard against predators and parasites, periodically inspecting and interacting with her brood (Fig. 1b). From late July to early August, the brood begin to eclose as adults. During this 'mature brood' phase, mothers interact with young adult offspring of both sexes. Many of these remain within the natal nest until the following spring (Fig. 1c), but some adult brood disperse to hibernacula elsewhere. During the mature brood phase, all females are reproductively inactive but of different generations. Very few, if any, mothers survive a second winter to reproduce in a second season (Rehan & Richards 2010a).

The prolonged nesting cycle of *C. calcarata* provides an excellent opportunity to track how sociodemographic status of the nest influences behaviours considered to be important precursors to sociality, including nestmate recognition. The first objective of this study was to determine the role of seasonal variation and sociodemographic status in behavioural interactions between females of *C. calcarata*. We used circle tubes to assay the behaviour of females collected at different points in the cycle to compare reproductively active versus post-reproductive non-nestmate mothers, and post-reproductive mothers versus pre-reproductive non-nestmate



**Figure 1.** *Ceratina calcarata* nesting biology. (a) Active brood nest with reproductive mother actively foraging and ovipositing eggs. (b) Full brood nest with post-reproductive mother guarding the nest entrance as offspring develop in brood cells. (c) Mature brood nest with post-reproductive mother and callow offspring cohabiting.

daughters. Nest site competition and kleptoparasitism during the active brood phase may provide strong selective pressure to prevent nest usurpation and loss of brood earlier in the season. We predicted that mothers collected during the reproduction phases of the nesting cycle would be more aggressive and less tolerant towards non-nestmates than would mothers collected during the post-reproductive phase. The second objective of this study was to determine experimentally whether *C. calcarata* is capable of nestmate recognition. To do this, we assayed females collected during the mature brood phase of the nesting cycle, when mothers cohabit with adult daughters and sons for an extended period. If *C. calcarata* is capable of nestmate recognition, as we predicted, then nestmate pairs in circle tube assays of adult females should show more tolerance and cooperative behaviour whereas non-nestmates should show more aggressive behaviour.

## METHODS

### Bee Collection

Focal females for this study were collected from active brood (June 2009), full brood (July 2010) and mature brood (August 2011) nests. Nests were collected at dawn and dusk from dead, broken raspberry stems in St Catharines, Ontario, Canada. Nests were chilled on ice, then split longitudinally to obtain adult bees. Adult females were retained in microcentrifuge tubes and chilled on ice until behavioural assays on the same day. The duration between nest dissection and observation was kept to a maximum of 2 h to reduce the effect of captivity-induced changes in behaviour (Pabalan et al. 2000). Each bee was uniquely marked with a single dot of enamel paint on its mesonotum.

### Behavioural Testing

Many studies of reproductive aggression and nestmate recognition have focused on observations of individuals within observation colonies. When observation colonies are impractical or unavailable, an alternative approach is to use circle tube arenas as a means of quantifying behaviours between two individuals (Breed et al. 1978; Packer 2006). Although circle tubes do not provide a particularly realistic environment for bees, there is evidence that interactions among individuals in circle tube arenas (e.g. passing, following and nudging behaviours) parallel those expressed in observation colonies (Brothers & Michener 1974). Moreover, behavioural profiles based on the frequencies of aggression, tolerance and avoidance in circle tube encounters can be used to evaluate the social status of sweat bees (i.e. whether they are solitary, communal or express caste-based sociality; Packer 2006; Richards & Packer 2010).

Two bees were introduced simultaneously into opposite ends of a clean plastic circle tube with an internal diameter of 4 mm (approximately double the average head width of *C. calcarata*) and a length of 30 cm (40 times the average *C. calcarata* body length). This tube diameter allows two individuals to turn around and pass one another, but is narrow enough that one bee can block an attempted pass by the other (Packer 2005). Simultaneous entry of individuals into the circle tube arena precludes ownership effects (Wcislo 1997). A new piece of tubing was used for each dyad.

Observations of each pair lasted for 20 min and took place outdoors between 1000 and 1500 hours, during the normal time at which bees are active. An encounter between bees was recorded when individuals came within one body length of each other (Kukuk 1992; Packer 2005). Encounters were classified into four categories: aggression, avoidance, tolerance and following (see Table 1 for definitions). 'Following' behaviour has been classified as cooperative in studies on communal species (McConnell-Garner &

Kukuk 1997; Boesi & Polidori 2011), as subordination by researchers on eusocial species (Breed et al. 1978; Michener 1990), and as dominance in other social contexts (West-Eberhard 1979). Due to the uncertainty of an appropriate designation for this behaviour we have retained 'following' as a separate behavioural category (Table 1) as per Packer (2006). Tolerance behaviours (Table 1) requiring two individuals to mutually engage in the same behaviour were recorded as a single event, but individual behaviours such as following, avoidance and aggressive acts were recorded as separate events. Behaviours were quantified in terms of their frequency during each 20 min trial, and latency, the time from the start of the trial until either bee first performed the behaviour. If a behaviour did not occur in a trial, the latency value was recorded as 1200 s (the total duration of the trial). To address our first objective to investigate seasonal variation in behaviour, we compared non-nestmate female dyads from spring (active brood), summer (full brood) and late summer (mature brood) colonies to determine whether sociodemographic status has marked effects on aggression and tolerance behaviour. To address our second objective of determining whether females are capable of nestmate recognition, we compared the behaviour of nestmates and non-nestmates from mature brood colonies (during this phase, mothers cohabit with adult offspring) to determine whether females alter their behaviour in the presence of familiar versus unfamiliar females.

After behavioural assay, all bees were killed by freezing at  $-20^{\circ}\text{C}$  and then transferred to 70% ethanol for storage until dissection. All bees were measured and assessed for relative age and reproductive condition. Head width is a strong predictor of body size in small carpenter bees and was measured from the dorsal view as the greatest distance across the compound eyes (Rehan & Richards 2010a, b). Wing

**Table 1**

Ethogram of behaviours of the carpenter bee *Ceratina calcarata*, and definitions of types of encounters observed in circle tubes

Behavioural category	Behaviour	Definition
Aggressive behaviours	Biting	The mandibles of one bee clamp around the legs, neck or antenna of the other bee
	C-posture	A female curls her abdomen under the thorax so her body forms a C-shape with mandibles and sting pointing at the other female
	Nudging	One bee applies force to another with its head (Steen 2000; Arneson & Wcislo 2003); this behaviour has also been termed 'pushing' (Peso & Richards 2010; Boesi & Polidori 2011), 'lunging' or 'head-butting' (Packer et al. 2003)
Avoidance behaviours	Back	A bee backs away from the other individual in the circle tube without turning
	Reverse	A bee makes a 180° turn and moves away from the other individual
Tolerance behaviours	Pass	Bees meet and manoeuvre to accommodate each other while they pass venter to venter in the circle tube
	Antennate	Both females in a frontal encounter stop when in contact with the other and slowly touch each other with antennae
	Head-Head touch	Both females in a frontal encounter stop when in contact with the other and pause touching each other with their faces; this behaviour has been reported only in the large carpenter bee, <i>Xylocopa virginica</i> (Peso & Richards 2010)
Following behaviours	Follow	A forward movement by a bee towards another bee that is walking or backing away from the first bee

An encounter between bees was recorded when individuals came within one body length of each other.

wear is a useful proxy for age and foraging activity in bees and was ranked by the number of nicks and tears in the apical margin of the forewing; pristine wing margins, with no nicks and tears receiving a score of zero, and wings with the apical margin completely obliterated receiving a score of five (Mueller & Wolf-Mueller 1993). Reproductive status was estimated based on dissection of the metasoma. The spermatheca was inspected for presence of sperm. Ovarian development was measured as the sum of the lengths of the three largest terminal oocytes. All active, full and mature brood mothers were mated, and all mature brood daughters were unmated, so insemination status was not considered further.

To examine the propensity of individuals to perform behaviours based on intrinsic differences, we compared the relative differences in physical traits to relative differences in behavioural frequencies. All differences between pair members were calculated as  $|(value\ for\ bee\ 1) - (value\ for\ bee\ 2)| / ((average\ value\ for\ both\ bees))$ , where the identities of bee 1 and bee 2 were randomly assigned for each dyad.

#### Statistical Analyses

Behavioural data were analysed in terms of frequency and latency to first instance. Data were not normally distributed and could not be transformed to produce normality, so nonparametric statistics based on ranks were employed. Statistical analyses were carried out in SAS v.9.1. Since the four behavioural categories (tolerance, avoidance, following and aggression) are tests of the same data for different variables, *P* values were adjusted for multiple comparisons using Bonferroni correction ( $\alpha = 0.05/N = 0.05/4 = 0.0125$ ).

#### Ethical Note

All work was conducted in accordance with regulations and guidelines established by the Canadian Council for Animal Care and

the Brock University Animal Care Committee. No licenses or permits were required for this research.

## RESULTS

### Seasonal Variation

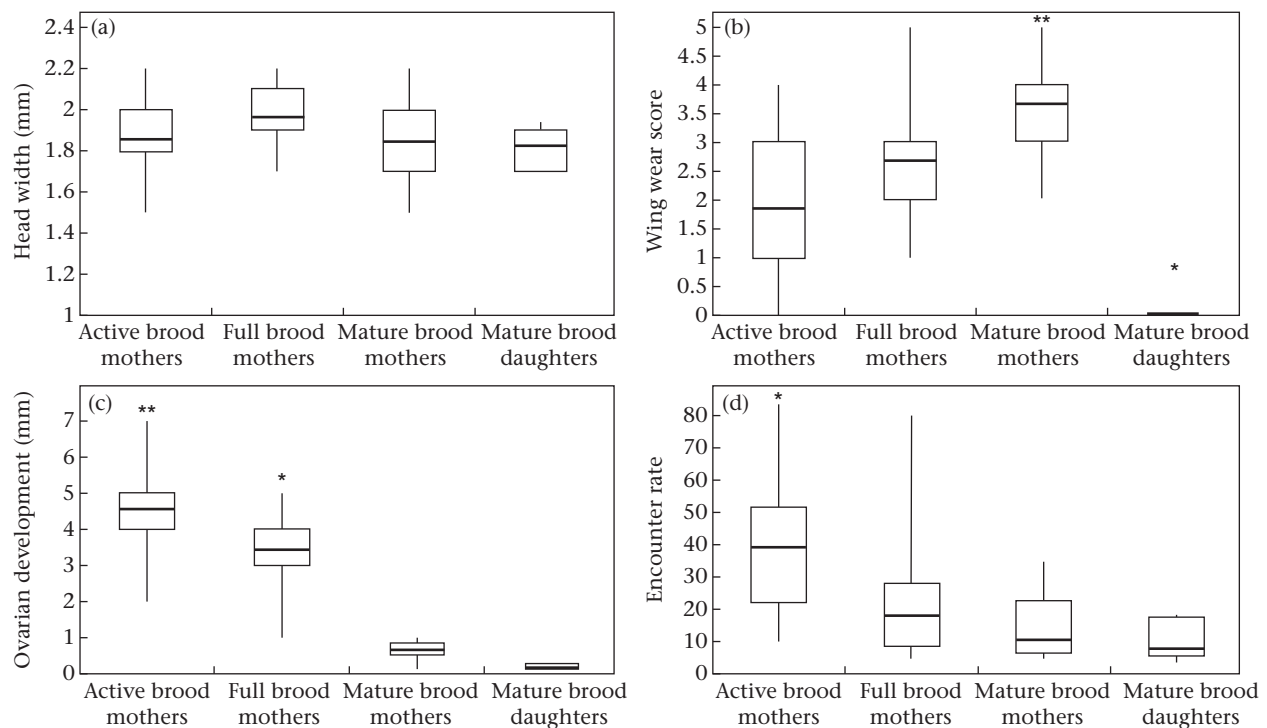
#### Female traits

There were no significant differences in head width among females collected in different seasons (Kruskal–Wallis test:  $H_3 = 1.06$ ,  $P = 0.79$ ; Fig. 2a). There was a significant seasonal increase in maternal wing wear, with mature brood mothers showing significantly more wear than active and full brood mothers, while mature brood daughters showed little to no wing wear ( $H_3 = 9.03$ ,  $P = 0.02$ ; Fig. 2b). There was a significant seasonal decline in ovarian development, as indicated by comparison of active brood mothers, full brood mothers, mature brood mothers and mature brood daughters ( $H_3 = 8.15$ ,  $P = 0.04$ ; Fig. 2c). There was no significant influence of any physical trait (ovarian development, head width, wing wear) on the latency or frequency of encounters, tolerance, aggression, avoidance or following in circle tube arenas (Supplementary Table S1).

#### Behaviour

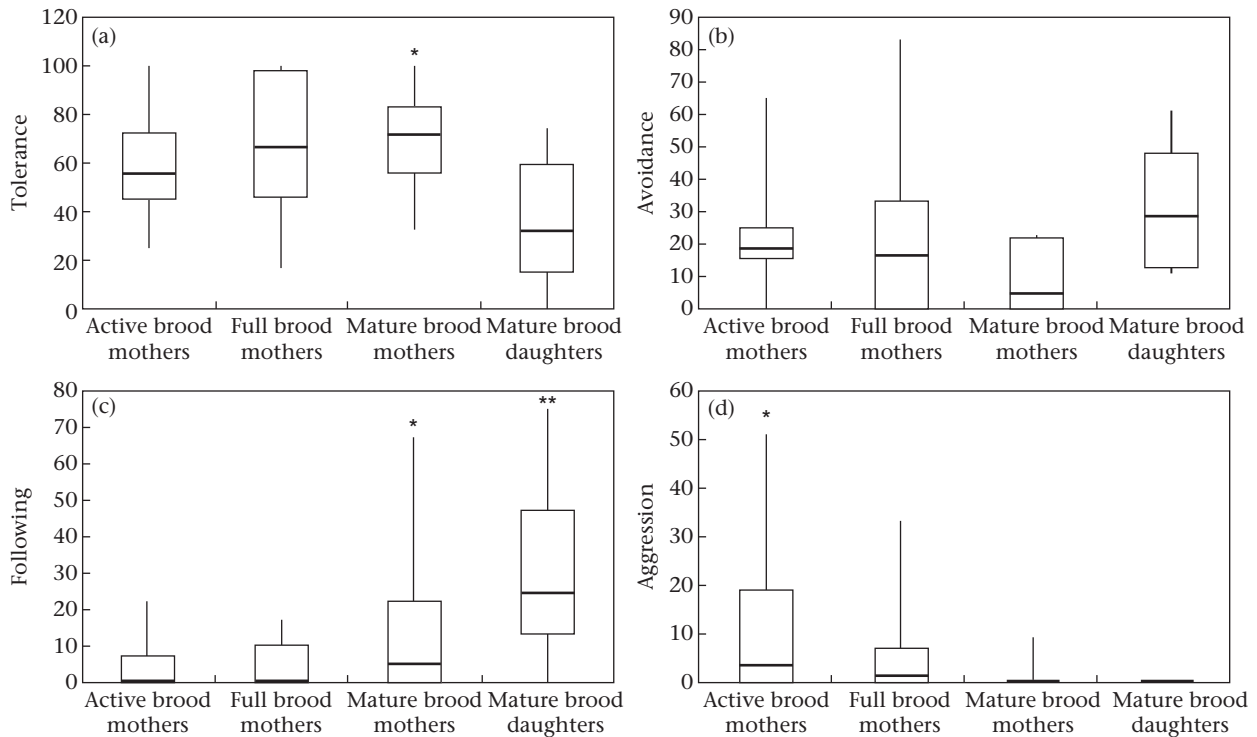
Active brood mothers encountered each other significantly more frequently ( $40 \pm 4$ ; mean  $\pm$  SD) than full brood mothers ( $19 \pm 3$ ), mature brood mothers ( $10 \pm 1$ ) or mature brood daughters ( $15 \pm 2$ ) ( $H_3 = 35.5$ ,  $P < 0.001$ ; Fig. 2d).

There was significant seasonal variation in the behavioural profiles of non-nestmate females among the four stages assayed, as indicated by differences in the relative proportions of encounters classified as tolerance, avoidance, following and aggression (Fig. 3). Active brood mothers spent 58% of encounters tolerating, 23% of



**Figure 2.** Seasonal variation in physical traits and encounter rates of the carpenter bee *Ceratina calcarata*. Boxes signify the medians and lower and upper quartiles, and whiskers signify the maximum and minimum observations. Asterisks signify statistically significant differences ( $P < 0.05$ ): \*Significantly different from treatments with no asterisks. \*\*Significantly different from treatments with one or no asterisks. Average (a) head width, (b) wing wear accumulation and (c) ovarian development, and by season. (d) Encounter rates in two female circle tube trials by season.





**Figure 3.** Box plots of sociodemographic variation in the relative frequencies (as a percentage) of behavioural categories for non-nestmate female trials from different times in the colony cycle. Boxes signify the medians and lower and upper quartiles, and whiskers signify the maximum and minimum observations. Asterisks signify statistically significant difference ( $P < 0.01$ ): \*Significantly different from treatments with no asterisks. \*\*Significantly different from treatments with one or no asterisks.

encounters avoiding, 5% of encounters following and 14% of encounters aggressing each other. Active brood mothers were significantly more aggressive than all other classes ( $H_3 = 28.4$ ,  $P < 0.001$ ; Fig. 3d), and avoided each other sooner than females from other classes ( $H_3 = 25.1$ ,  $P < 0.001$ ; Fig. 4b). Full brood mothers spent 68% of encounters tolerating, 21% avoiding, 5% following and 6% aggressing each other. Full brood mothers had the greatest latency to follow each other ( $H_3 = 20.9$ ,  $P < 0.001$ ; Fig. 4c). Mature brood mothers spent 71% of encounters tolerating, 11% avoiding, 18% following and 0% aggressing each other. Mothers from mature brood nests were more tolerant ( $H_3 = 21.2$ ,  $P < 0.001$ ; Fig. 3a) and were observed following more frequently than other classes of adult female pairings ( $H_3 = 28.8$ ,  $P < 0.001$ ; Fig. 3c). Aggression was not observed in dyads of mature brood mothers (Fig. 3d).

Newly eclosed daughters from mature brood nests had behavioural profiles that particularly differed from those of mothers in mature brood nests. Daughters had the lowest average activity rates, and they spent 37% of encounters tolerating, 31% avoiding, 31% following and 1% aggressing each other. Daughters from mature brood nests were slightly but not significantly less avoidant ( $H_3 = 8.4$ ,  $P = 0.04$ ; Fig. 3b) than other classes of female pairings. Daughters followed significantly more frequently than mothers from mature brood nests, and far more frequently than active and full brood mothers (Fig. 3c). There was no difference in latency to tolerance, avoidance, following or aggressive interactions compared to other mature brood mother dyads (Fig. 4).

#### Social Variation

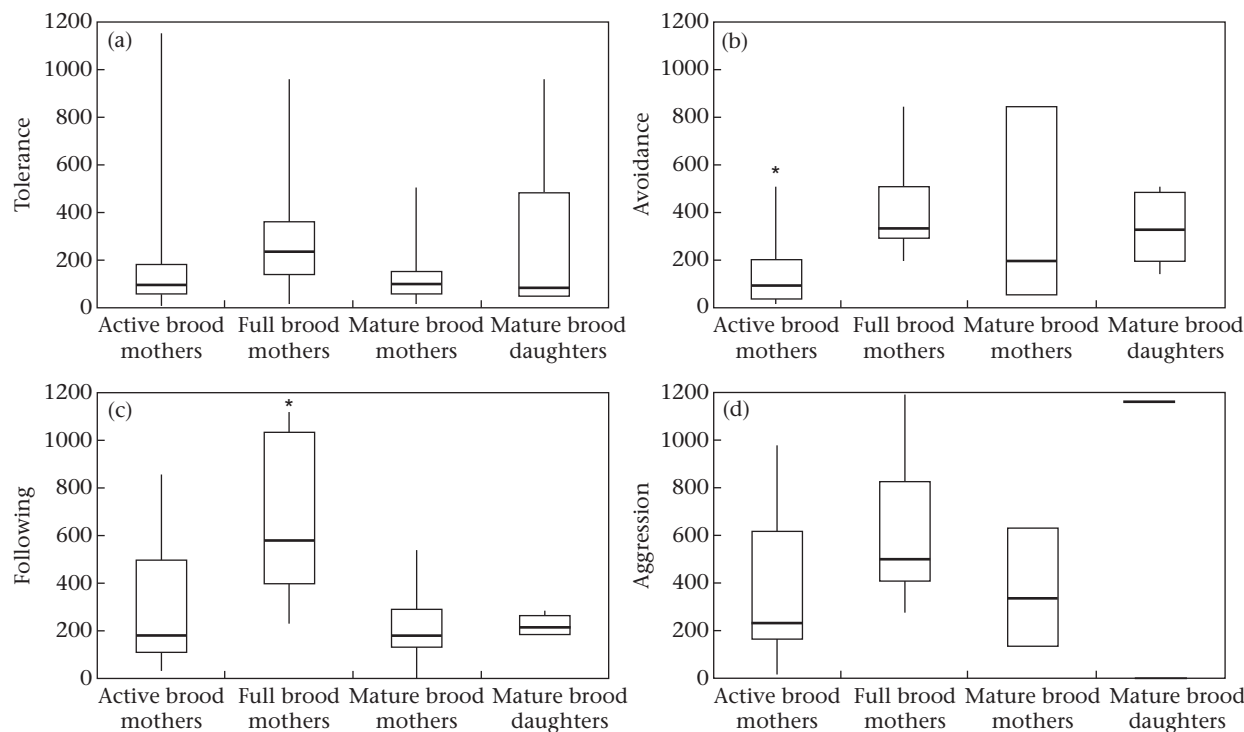
##### Female traits

Ovarian development was not assessed for mature brood colonies since females were not reproductive at this time. Mothers were significantly more worn than daughters (Fig. 2c, see Seasonal

Variation above). The influence of proportional size differences between females, based on head widths, was considered because relative size might influence behavioural interactions (Supplementary Table S2). There was a significant negative correlation between head width difference and the relative difference in avoidance in mother–daughter pairings (Spearman rank correlation:  $r_s = -0.80$ ,  $N = 13$ ,  $P = 0.001$ ). Conversely, there was a significant positive correlation between head width difference and relative difference in avoidance between unfamiliar daughter pairings ( $r_s = 0.84$ ,  $N = 14$ ,  $P = 0.0001$ ). There was a positive correlation between head width difference and latency to follow in unfamiliar mother–daughter pairings. Mature brood mothers with larger head widths took longer to follow smaller, unrelated daughter generation females ( $r_s = 0.76$ ,  $N = 20$ ,  $P = 0.0001$ ).

#### Behaviour

We investigated socially induced variation in females' behaviour by pairing familiar and unfamiliar females collected during the mature brood phase of natural cohabitation. There was no significant difference in encounter frequencies among the four categories of mature brood female pairings: mothers versus daughters, sisters (daughters from the same nest), unfamiliar females from the maternal and daughter generations, and unfamiliar females from the daughter generation ( $H_3 = 2.23$ ,  $P = 0.5$ ). Mature brood females were generally inactive, with unfamiliar maternal and daughter generations encountering each other  $10 \pm 1$  times per trial, familiar mothers and daughters encountering each other  $8 \pm 1$  times per trial, familiar sisters encountering each other  $8 \pm 1$  times per trial, and unfamiliar females from the daughter generation encountering each other  $10 \pm 1$  times per trial. There were no differences in frequencies of tolerance ( $H_3 = 7.2$ ,  $P = 0.07$ ) or avoidance ( $H_3 = 6.8$ ,  $P = 0.08$ ) during encounters among these dyads (Fig. 5). However, pairs of mothers



**Figure 4.** Latency to encounters of each behavioural category by season. Boxes signify the medians and lower and upper quartiles, and whiskers signify the maximum and minimum observations. Significant differences in behavioural tendency are noted with an asterisk.

with their own daughters showed significantly more aggression than did other pairs ( $H_3 = 34.8$ ,  $P < 0.001$ ; Fig. 5d); the aggression was mostly displayed by mothers towards daughters. Furthermore, following behaviour was much less frequent in pairs of mothers with their daughters than in other pairings ( $H_3 = 15.2$ ,  $P = 0.002$ ; Fig. 5c). There were no significant differences in latency to show tolerance ( $H_3 = 4.2$ ,  $P = 0.2$ ), avoidance ( $H_3 = 7.0$ ,  $P = 0.7$ ) or aggression ( $H_3 = 5.3$ ,  $P = 0.6$ ) in bigenerational pairings (Fig. 6). Daughters from the same nest followed each other significantly later than did females in other types of pairings ( $H_3 = 8.7$ ,  $P = 0.01$ ; Fig. 6c).

## DISCUSSION

The findings of this study were two-fold. Firstly, we showed that a subsocial apid bee, *Ceratina calcarata*, changes its level of agonism with ovarian development. Females that were actively reproductive (ovaries fully developed) were more aggressive than pre-reproductive (ovaries undeveloped) or post-reproductive females (ovaries resorbed). These results indicate that females of this species vary their aggression levels according to their level of ovarian development. Secondly, we found that females alter their behaviour when interacting with nestmates and non-nestmates. In late summer, solitary mothers naturally cohabitate with newly eclosed daughters, and comparison of nestmate versus non-nestmate females assayed at this time indicated nestmate recognition.

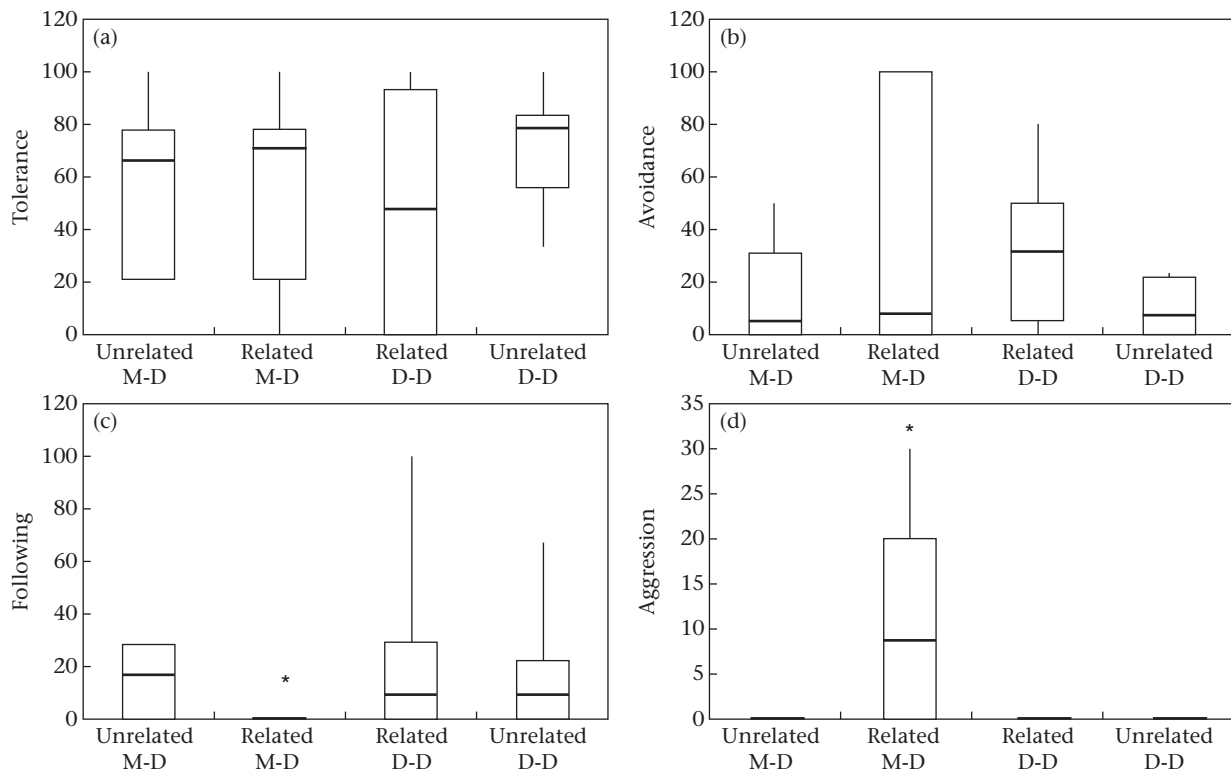
### Seasonal Variation in Aggressive Behaviour due to Changes in Reproductive Status

Physical traits and reproductive status influence rates of aggression in primitively eusocial bees and wasps (Breed et al. 1978; Pabalan et al. 2000; Cant et al. 2006; Field & Cant 2009). Sweat bee and paper wasp studies suggest that correlations between ovarian development and aggressive behaviour in unfamiliar

pairs might be explained through fitness arguments if conspecifics pose a threat of egg replacement. Consistent with circle tube studies on other bees (Wcislo 1997; Pabalan et al. 2000), in *C. calcarata*, ovarian development was correlated with levels of agonism. Active brood females were more aggressive than the post-reproductive full and mature brood females, whereas in the latter phase of the colony cycle, females showed no signs of aggression towards each other. High rates of aggression during the active brood phase accord with field observations of marked females, which indicate high rates of attempted nest usurpation that result in violent encounters between resident females and usurpers (S. M. Rehan, unpublished data).

Body size is not a predictor of reproductive dominance in reproductive nests of this and other *Ceratina* species (reviewed in Sakagami & Maeta 1995; Hogendoorn & Velthuis 1999; Rehan et al. 2009, 2010; Rehan & Richards 2010b). It remains unknown how reproductive division of labour is decided in facultatively eusocial and semisocial *Ceratina* (*C. japonica* and *C. australensis*, respectively), but in at least one allodapine bee, reproductive hierarchies are determined by order of adult eclosion (Schwarz & Woods 1994). Same-generation, age-based reproductive hierarchies have been reported across the apid bees (Hymenoptera: Apidae), including the large carpenter bees (Hogendoorn & Leys 1993) and euglossine bees (Augusto & Garófalo 2010).

When discussing the relative importance of physical traits on aggressive behaviour, maternal manipulation and physical coercion are important considerations. Nestmates in mature brood *C. calcarata* colonies do not show reproductive hierarchies as such, as mothers are post-reproductive during this phase of the nesting cycle, and daughters, having not yet mated, are pre-reproductive (Fig. 2c; Rehan & Richards 2010a). However, division of labour is observed between cohabiting females in late-summer nests, and is associated with maternal aggression towards adult daughters from mature brood nests (Fig. 5d). *Ceratina calcarata* mothers usually lay



**Figure 5.** Box plots of the relative frequency of encounters by behavioural category in mature brood trials. Boxes signify the lower and upper quartiles, and whiskers signify the maximum and minimum observations. Unrelated M-D: non-nestmate dyad of females from different generations; related M-D: nestmate dyad of females from different generations; related D-D: nestmate dyad from the same newly eclosed age cohort; unrelated D-D: non-nestmate dyad of newly eclosed females. Significant differences in behavioural tendency are noted with an asterisk.

female eggs in the first brood cell position, and they also underprovision this brood cell to produce a dwarf eldest daughter. Dwarf eldest daughters are smaller than their younger sisters and sometimes even smaller than their younger brothers (males are generally smaller than females; Johnson 1988; Rehan & Richards 2010b). Consistent with observations of nestmate aggression by mothers towards their daughters late in the mature brood phase, bigenerational females of similar body size were more avoidant in nestmate conditions (Supplementary Table S2). Dwarf eldest daughters are recurrent in *Ceratina* species (Sakagami & Maeta 1977, 1984, 1995), and mothers expel these daughters to forage and feed 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 coercion (Michener 1990; Maeta et al. 1992).

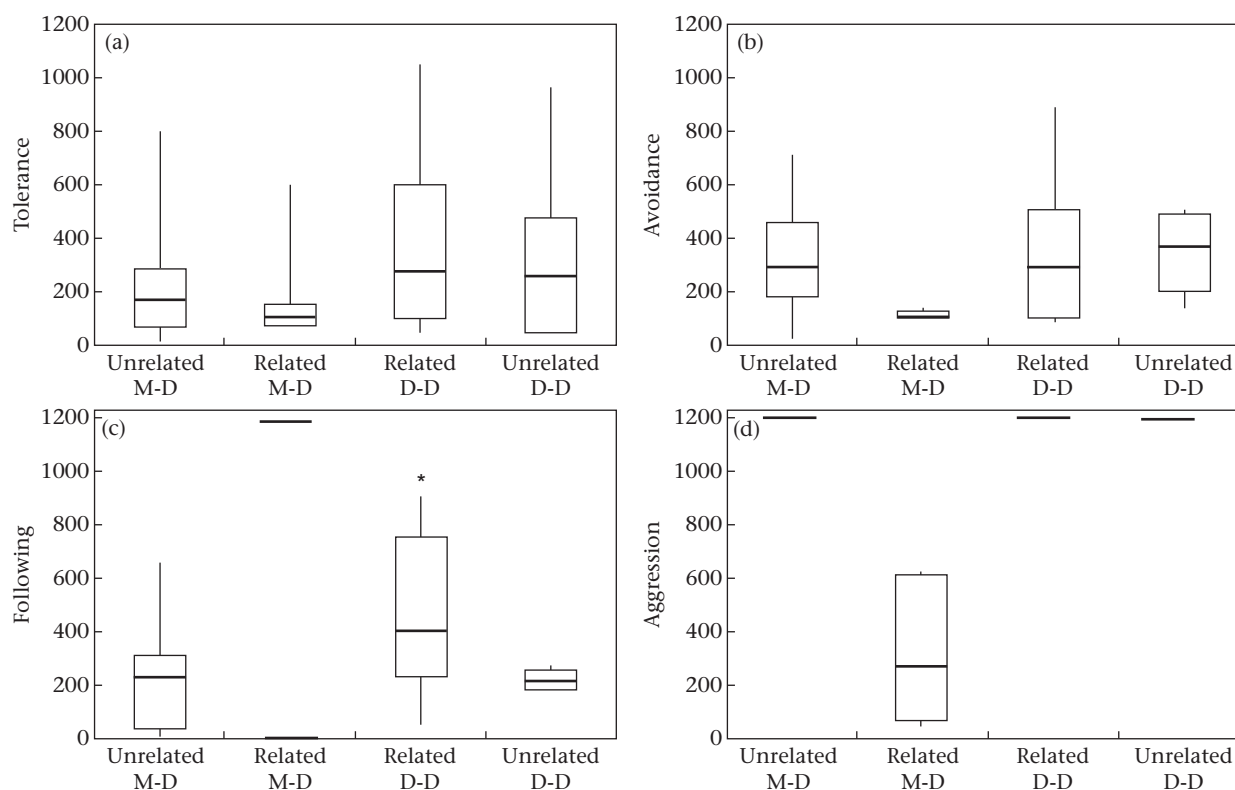
#### Nestmate Recognition in *C. calcarata*

This study shows that females of the small carpenter bee, *Ceratina calcarata*, alter their behaviour when interacting with nestmates and non-nestmates, showing significantly more aggression in nestmate than non-nestmate pairs. This difference in behaviour towards familiar versus unfamiliar individuals logically implies that this subsocial bee species is capable of nestmate recognition. However, the observed form of discrimination is surprising: usually, nestmate recognition in carpenter and other bees

is suggested by greater aggression towards non-nestmates and greater tolerance towards nestmates (e.g. Schwarz & Blows 1991; Flores Prado et al. 2008; Peso & Richards 2010), whereas we found the opposite. That recognition can result in either heightened tolerance or heightened aggression towards familiar individuals indicates that its outcome is strongly influenced by current behavioural context. We assessed the effects of familiarity during the mature brood phase of the colony cycle when mothers resided with adult daughters, and thus, at a time requiring mutual tolerance. However, the mature brood phase is also the season in which some adult brood emerge from the natal nest to forage and feed siblings prior to overwintering. During this phase, paint-marked and wing-worn mothers are occasionally observed to aggressively push unworn adult daughters out of the nest (S. M. Rehan, personal observation). Increased aggression towards daughters at the end of summer would be consistent with these observations.

#### Social Plasticity in Forced Association Experiments

Forced association experiments on ceratinines have received much attention and have the potential to uncover traits in solitary species that could be used to build eusocial systems (Sakagami & Maeta 1984, 1987, 1989; Michener 1985; Hogendoorn & Velthuis 1999). Social behaviour such as mutual tolerance or reproductive division of labour arising from forced cohabitation of solitary individuals is often posited as an emergent property (Fewell et al. 2009). There is also the possibility that reproductive dominance and division of labour are not emergent traits, but instead expression of traits that evolved in social ancestors and can be provoked in descendant species that have since reverted to solitary nesting (Rehan et al. 2012). Social behaviour in the ceratinines has been



**Figure 6.** Latency to encounter by behavioural category among nestmate and non-nestmate mature brood trials. Boxes signify the lower and upper quartiles, and whiskers signify the maximum and minimum observations. Unrelated M-D: non-nestmate dyad of females from different generations; related M-D: nestmate dyad of females from different generations; related D-D: nestmate dyad from the same newly eclosed age cohort; unrelated D-D: non-nestmate dyad of newly eclosed females. Significant differences in behavioural tendency are noted with an asterisk.

considered both as vestigial (West-Eberhard 1987) and as rudimentary (Michener 1985; Sakagami & Maeta 1995). The difference here is important to determine whether social phenotypes are truly emergent behaviours or whether they are based on earlier selection for social traits. In the latter case, all it means is that the capacity for reproductive division of labour and mutual tolerance among individuals has not been lost, even if recent selection has not maintained multiple-female cohabitation (Wcislo 1997; Wcislo & Danforth 1997).

### Conclusions

In addition to mutual tolerance and prolonged cohabitation, subsociality is quite frequent in many organisms, yet further elaboration into eusocial life clearly requires very specific selective environments that are rare in nature. The ceratinines are quite capable of forming eusocial colonies, as this behaviour is observed naturally in some species and can be provoked in others (Chandler 1975; Sakagami & Maeta 1977, 1995; Rehan 2011). Although eusociality has led to the great ecological success of some lineages, for others, including the small carpenter bees, social organization has disadvantages preventing further elaboration of this trait.

It would be interesting through experimental manipulation, such as the circle tube assays employed in this study, to test the seasonal plasticity of recognition and the range of behavioural profiles across a wider range of bees and wasps. Recent data suggest sociality may be disappearing from *Ceratina* (Rehan et al. 2012), but there is a dearth of behavioural observations across this and other facultatively social lineages. Understanding the nature of behavioural plasticity across a wide range of taxa will provide the phylogenetic contrasts to qualify the importance of mutual tolerance

and reproductive aggression during evolutionary origins versus losses of eusocial behaviour.

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### Supplementary Material

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