

Evidence of Social Nesting in the *Ceratina* of Borneo (Hymenoptera: Apidae)

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ABSTRACT: The bee tribe Ceratinini is important for understanding early stages in social evolution. Their extant sister tribe Alloedapini contains no strictly solitary species, and while some Ceratinini are commonly regarded as solitary, little behavioural data exist to substantiate this. Studies on Asian congeners have shown recurrent sociality in temperate and subtropical ranges while behavioural data are lacking in tropical regions. Field work along the west coast of Borneo in Sarawak, Malaysia, has provided some insight into these tropical taxa. Here we describe the nesting biology and social behaviour of four taxonomically described yet behaviourally unclassified *Ceratina* species. These four species are from three subgenera, namely *Ceratina* (*Ceratinidia*) *accusator* Cockerell, *C. (Ceratinidia) nigrolateralis* Cockerell, *C. (Neoceratina) dentipes* Friese, *C. (Pithitis) smaragdula* Fabricius. Nests of all species were typically attended by an adult female while all species except *C. accusator* had a low frequency of multi-female nesting assemblages. The four tropical ceratinines described here and all other behaviourally classified species exhibit recurrent patterns of maternal care, maternal longevity, and nest protection. Prolonged parental care found across the genus and occasional transitions into sociality make *Ceratina* of future interest for the study of life history and social evolution.

KEY WORDS: social polymorphism, small carpenter bees, maternal care, maternal longevity, nest protection, Ceratinini, *Pithitis*, *Ceratinidia*, *Neoceratina*, Borneo

The evolution of eusociality is considered one of the major transitions in evolution (Maynard Smith and Szathamary, 1995). In solitary species, offspring disperse and reproduce independently whereas workers in eusocial societies remain at the natal nest and largely forego reproduction to aid the queen in rearing siblings. Socially polymorphic lineages, those containing both solitary and social species, retain the plasticity to allow intra-specific comparisons of solitary with social life. The key to understanding the transition to sociality requires a group of closely related taxa possessing broad social, taxonomic and geographic diversity. Bees provide numerous contrasts to offer insights into the origin of sociality with their range of solitary to social forms.

The small carpenter bees Hymenoptera: Apidae (Xylocopinae: Ceratinini) are commonly regarded as solitary (Michener, 1974). All behaviourally classified species share a relatively simple life history. Females disperse from their natal nests and find appropriate nesting substrate. These twig-nesting bees excavate linear burrows in the cores of dead exposed pithy stems. Subsequent to burrow construction, females forage for pollen and nectar provisions that they form into a pollen mass on which they lay an egg. After provisioning and oviposition, brood cells are capped with a partition of wood pith, and the process is repeated in a serial manner along the linear nest chamber.

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Some ceratinines exhibit the following four traits unusual in solitary bees (Sakagami and Maeta, 1977). First, mothers are nest loyal, ovipositing all of their brood in a single nest and remain at the nest to guard offspring from parasites and predators. Second, mothers exhibit prolonged parental care, periodically inspecting developing brood and incorporating faecal pellets and dead desiccated offspring into brood cell partitions to limit contamination of other developing brood. Third, females are remarkably long-lived, remaining with their developing brood, occasionally surviving to a second reproductive season, and sometimes even forming subsequent broods. Fourth, mothers and sometimes the eldest daughters, may forage for pollen and nectar to feed newly eclosed brood. Most importantly, multi-female nesting associations in which more than one adult female tends to the brood have been reported in several Japanese temperate *Ceratina* (*Ceratinidia*) species: *C. flavipes* Smith, *C. japonica* Cockerell, *C. megastigmata* Yasumatsu and Hirashima, and *C. okinawana* Matsumura and Uchida (Sakagami and Maeta, 1977; Maeta and Katayama, 1978).

Small carpenter bees are found on every continent except Antarctica, with all members classified into a single genus, *Ceratina*, comprising 17 Old World subgenera and six New World subgenera (Michener, 2007). Species are most abundant and diverse in the tropical regions in which they are considered to have originated (Iwata, 1971). Despite their taxonomic diversity, the social behaviour of most tropical ceratinines remains unknown, although there are descriptions of the nesting biology of *Ceratina* (*Ceratinula*) sp., *C. (Zadontomerus) ignara* Cresson (Michener and Eickwort, 1966), *C. (Neoceratina) propinqua* Cameron, *C. (Pithitis) smaragdula* Fabricius (Batra, 1976), and *C. (Ceratina) dentipes* Friese (Okazaki, 1992). Here we present the first account of the life history and nesting biology of four taxonomically described but behaviourally unclassified *Ceratina* from Borneo.

Methods

Nest Contents

Ceratina nests were collected at six locations in Sarawak, Malaysia (Fig. 1) between 8 and 17 August 2007. Broken stems with entrance holes in the exposed pithy ends were collected and the entrance holes sealed with masking tape. Most nests were found in *Mussaenda* sp. (Rubiaceae), a pink flowering shrub commonly referred to as Bangkok Rose. Nests were opened the day of collection, upon which their contents, including number and location of adults in the nest, brood developmental stages (Fig. 2), presence of parasites, and overall nest appearance, were recorded. Immatures were identified to sex from the pupal stage onward. In addition, elements of the nest architecture were recorded, including nest length, nest width, gallery length, and brood cell septum thickness.

Adult bees were assessed in terms of body size and reproductive status. Head width (HW) was measured across the widest part of the face, including both compound eyes. The proportional size difference between adult females from the same nest was calculated as (larger HW – smaller HW)/larger HW. Wing lengths were measured along the costal vein from the base of the wing to the proximal tip of the stigma. Wing wear scores were used to assess age and foraging effort: unworn bees with no nicks or tears along the apical margins of their forewings received a

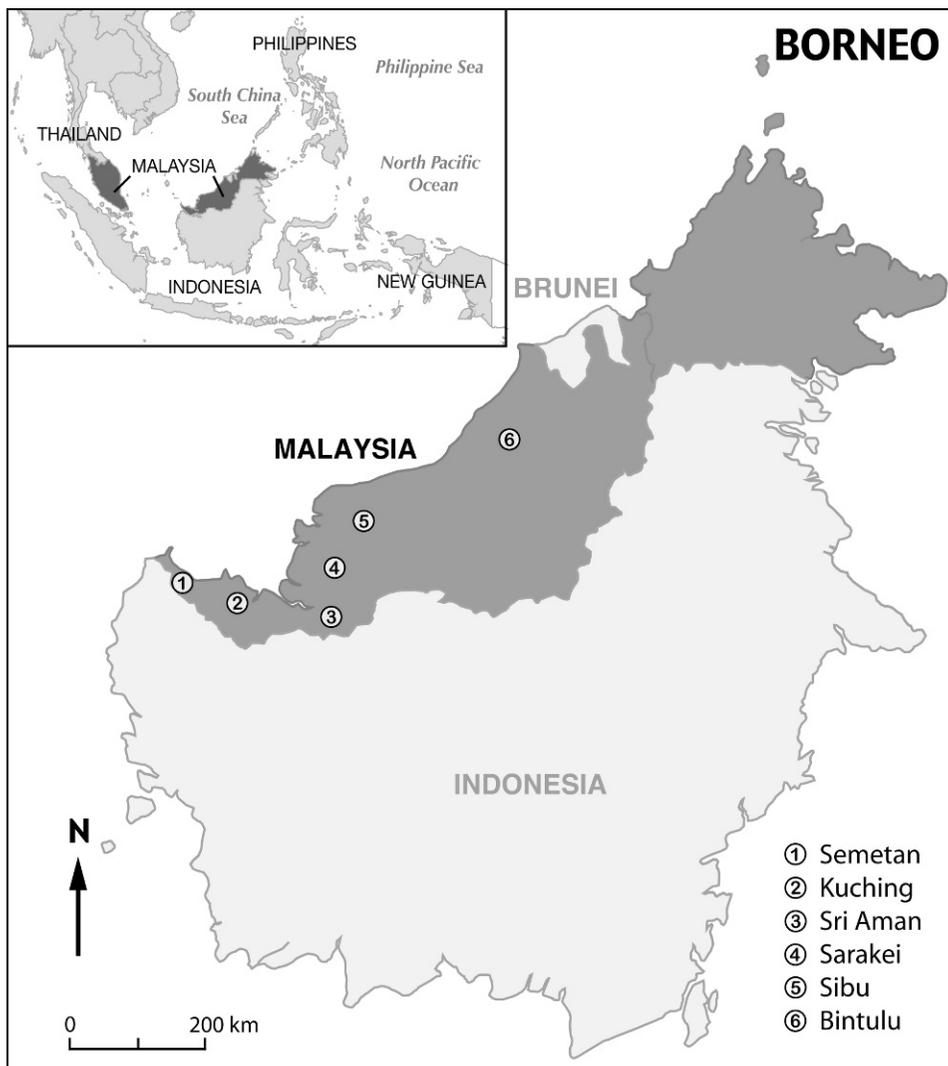


Fig. 1. Map of Sarawak, Malaysia showing *Ceratina* collection locations.

score of zero, and highly worn bees with completely shredded apical margins received a score of five.

Adult female nest occupants were dissected to determine mating status and ovarian development. Ovarian development was scored as the sum of the lengths of the three largest terminal oocytes (accuracy ± 0.01 mm). Insemination status was determined by the presence of sperm in the spermatheca (the spermatheca of a mated female is opaque, whereas an unmated female has a transparent spermatheca).

Nest Classification

Nests were assigned to categories modified from similar descriptions by Daly (1966), based on their contents and the reproductive status of the adult females found inside.

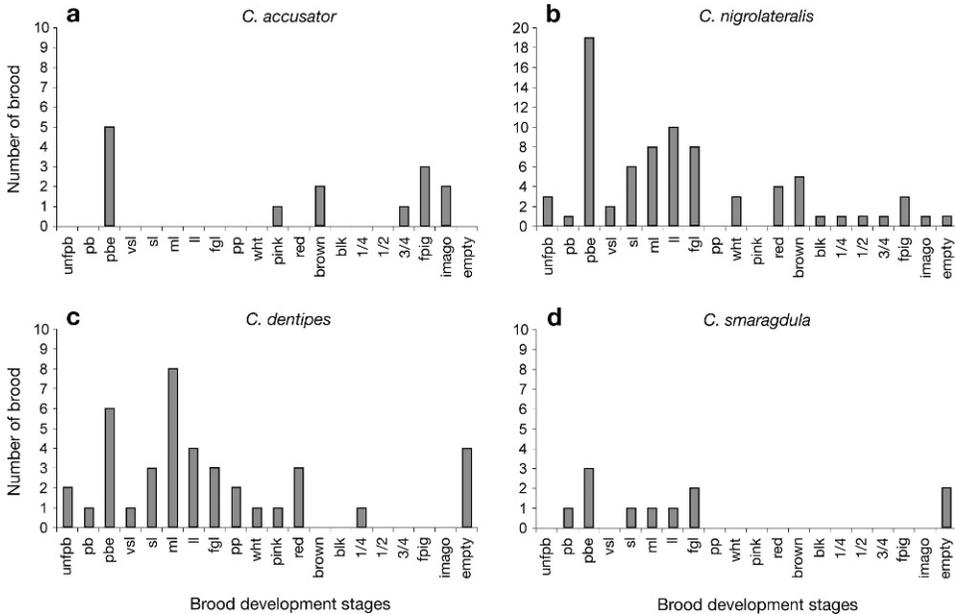


Fig. 2. Brood developmental stages of all *Ceratina* nest collections in August 2007. **a)** *C. accusator* brood from 2 active brood and 6 full brood nests. **b)** *C. nigrolateralis* brood from 20 active brood and 5 full brood nests. **c)** *C. dentipes* brood from 12 active brood and 3 full brood nests. **d)** *C. smaragdula* brood from 3 active brood nests. Brood cell provisioning and offspring developmental stages were recorded as follows: unfinished pollen mass in brood cells not forming a complete loaf (*unfpb*), completed pollen mass without an egg (*pb*), pollen mass with an egg (*pbe*), very small larva 1/3 to 2/3 the length of the pollen mass (*vs1*), small larva 2/3 to 7/8 the length of the pollen mass (*sl*), medium larva the length of the pollen mass (*ml*), large larva 1.5 times the length of the pollen mass (*ll*), full grown larva 2 times the length of the pollen mass (*fgl*), and prepupa on the verge of pupation with defined head capsule (*pp*). Pupal stages were recorded based on the darkening pigmentation of their eyes from white to black (*wht*, *pink*, *red*, *brown*, *blk*), followed by increasing integumental pigmentation from one quarter to full (1/4, 1/2, 3/4, *fpig*). Upon final moult, newly emerged offspring have milky wings (*imago*).

Founding nests formed in newly excavated pith are indicated by light interior walls; they are devoid of faecal pellets or pollen residue. These nests contain adults but do not contain pollen masses or immature brood and are considered to be at a stage prior to pollen mass provisioning, oviposition, and brood cell construction. *Active brood nests* contain one or more pollen masses or immature bees. *Full brood nests* contain brood cells, with the outer-most (youngest) cell containing a larva or pupa. Active and full brood nests with an adult female are termed ‘complete’ and those without an adult female ‘orphaned’. *Mature brood nests* contain adults but do not contain pollen masses or immature brood. Instead, these nests contain callow brood, have darkened interior walls and often contain faecal pellets and pollen residue. These nests are considered to be at a stage between brood development and dispersal.

Statistical Analyses

Descriptive statistics, correlations and one sample *t*-tests were calculated in SPSS (Statistical Package for the Social Sciences) version 11.0 (SPSS Inc., Chicago). Simulated random sampling was performed with Resampling Stats, version 4.1 for Macintosh (www.statistics.com).

Table 1. Population sex ratio (based on pupae) by species. The mean numerical sex ratio over colonies of each species was compared to the expectation of an equal sex ratio using a one-sample *t*-test for each species (*N* = number of nests).

| Subgenus | Species | <i>N</i> | Females | Males | Total | % Male | <i>P</i> |
|--------------------|-----------------------|----------|---------|-------|-------|--------|----------|
| <i>Ceratinidia</i> | <i>accusator</i> | 3 | 8 | 1 | 9 | 11 | 0.073 |
| | <i>nigrolateralis</i> | 11 | 17 | 4 | 21 | 19 | 0.002 |
| <i>Neoceratina</i> | <i>dentipes</i> | 5 | 5 | 1 | 6 | 17 | 0.208 |

Results

A total of 77 nests containing *Ceratina* species were collected in Borneo, comprising 22 nests of *Ceratina* (*Ceratinidia*) *accusator* Cockerell, 32 of *C. (Ceratinidia)* *nigrolateralis* Cockerell, 19 of *C. (Neoceratina)* *dentipes* Friese, and four of *C. (Pithitis)* *smaragdula* Fabricius.

Nest Contents

In mid-August, all four species were reproductive and had nests containing eggs and developing brood (Fig. 2). Nests of *C. accusator*, *C. nigrolateralis*, and *C. dentipes* contained the full spectrum of brood stages from eggs to pupae, whereas *C. smaragdula* nests contained eggs and larvae but no pupae. Nests containing one or more empty brood cells were observed in all species except *C. accusator*. Brood cells with a pollen mass but no egg, were less frequent than empty brood cells (Fig. 2).

The total numbers of male versus female pupae of each colony was tallied to estimate the numerical sex ratio (% male) for each species. All three had female-biased numerical sex ratios among pupae: *C. accusator* 11%, *C. nigrolateralis* 19%, and *C. dentipes* 17% (Table 1). None of the four *C. smaragdula* nests contained pupae, so sex ratios could not be calculated. In all species, adult females were larger than males (Table 2). Intraspecific body size, as measured by head width, is more variable in females than males for each species.

Nest Architecture

All four species formed single linear burrows in pithy stems and nest dimensions are summarised in Table 3. *Ceratina nigrolateralis* had especially long nest burrows and left about three quarters of the nest's length as an entrance gallery. Conversely, the three remaining species formed shorter nesting burrows and left galleries approximately half their nest's length.

Given the variability in nest dimensions and nest contents, the correlation between nest length and the number of nest occupants for each species was examined, but longer nests did not house more adult bees than shorter nests (*C. accusator* $r = 0.30$, $n = 15$, $P = 0.28$; *C. dentipes* $r = 0.40$, $n = 18$, $P = 0.10$; *C. nigrolateralis* $r = 0.32$, $n = 29$, $P = 0.09$; *C. smaragdula* $r = 0.82$, $n = 4$, $P = 0.18$) but the small sample size for *C. smaragdula* entails very low power. There was also no relationship between adult female body size and nest burrow length for any species (*C. accusator* $r = 0.17$, $n = 15$, $P = 0.57$; *C. dentipes* $r = 0.10$, $n = 17$, $P = 0.64$; *C. nigrolateralis* $r = 0.14$, $n = 29$, $P = 0.44$; *C. smaragdula* $r = 0.32$, $n = 3$, $P = 0.80$), but again small sample sizes need to be taken into account. In addition, there were no consistent differences between single female and multiple female nest dimensions for each species (Table 3).

Table 2. Head width (mm) of each species by sex.

| Subgenus | Species | Females | | | | Males | | | |
|--------------------|-----------------------|---------|------------------|-----------|----------|--------|------------------|-----------|----------|
| | | CV (%) | Mean (\pm SD) | Min-Max | <i>N</i> | CV (%) | Mean (\pm SD) | Min-Max | <i>N</i> |
| <i>Ceratinidia</i> | <i>accusator</i> | 15.7 | 2.16 (0.34) | 1.57–2.97 | 46 | 11.6 | 1.89 (0.22) | 1.72–2.20 | 4 |
| | <i>nigrolateralis</i> | 13.0 | 1.92 (0.25) | 1.53–2.73 | 49 | 1.9 | 1.55 (0.03) | 1.53–1.57 | 2 |
| <i>Neoceratina</i> | <i>dentipes</i> | 5.2 | 1.54 (0.08) | 1.36–1.68 | 22 | n/a | n/a | n/a | 0 |
| <i>Pithitis</i> | <i>smaragdula</i> | 8.2 | 2.44 (0.20) | 2.29–2.77 | 6 | 5.5 | 2.19 (0.12) | 2.05–2.33 | 5 |

Evidence for nest reuse was observed twice. One *C. dentipes* nest (SRI47) (Fig. 3) that contained an adult female, had darkened interior walls and an empty, soiled basal chamber 75 mm long capped with a pith septum 2 mm thick. Above the septum there was an egg on a pollen mass in a closed brood cell. A second nest (B18) had a 68 mm long basal chamber capped with a 2 mm pith septum; an adult female *C. nigrolateralis* was found in the antechamber but there was no brood or pollen within the nest.

Colony Structure

The 22 dissected colonies of *C. accusator* comprised seven founding, two active brood, six full brood, and seven mature brood nests. All the founding nests were newly formed burrows with clean pith walls, each containing a single adult female. Both active brood nests also contained a single adult female. Of the six full brood nests, five were complete and one was orphaned, lacking an adult female. Among the seven mature brood nests five were complete and two were orphaned. All complete nests contained only a single adult female.

In total, 19 *C. dentipes* nests were collected, consisting of four founding nests, twelve active brood nests, and three full brood nests. All four founding nests were newly formed burrows with a single adult female. Eleven of the twelve active brood nests contained a single adult female, although one nest (B49) contained two adult females and two brood cells, each with a pollen mass and egg. The smaller female (head width = 1.38 mm, wing length = 1.31 mm) was unmated and had very little ovarian development with a score of 0.50 mm. The second female was considerably larger (head width = 1.55 mm, wing length = 1.51 mm), and contained two partially developed eggs (0.99 and 0.65 mm long), as well as one fully developed egg (1.21 mm long). This female was mated and retained nurse cells from recent oviposition,

Table 3. Nest architecture measurement data (mm). Mean \pm one standard deviation (*n* = sample size).

| Species | Nest class | Nest measurements | | | | Brood cell | | |
|---|---------------|-------------------|---------------|-----------------|----------|---------------|---------------|----------|
| | | Length | Width | Gallery | <i>n</i> | Length | Septa Width | <i>n</i> |
| <i>Ceratinidia</i> <i>accusator</i> | single female | 77.8 \pm 28.8 | 3.0 \pm 0.0 | 40.0 \pm 4.2 | 22 | 5.3 \pm 0.5 | 2 \pm 0.0 | 11 |
| | two female | n/a | n/a | n/a | 0 | n/a | n/a | 0 |
| <i>Ceratinidia</i> <i>nigrolateralis</i> | single female | 102.9 \pm 49.3 | 3.3 \pm 0.7 | 76.6 \pm 52.8 | 30 | 9.4 \pm 6.7 | 2.7 \pm 1.1 | 78 |
| | two female | 132.5 \pm 13.4 | 3.3 \pm 1.1 | 111.5 \pm 2.1 | 2 | 5.3 \pm 1.0 | 1.7 \pm 0.5 | 4 |
| <i>Neoceratina</i> <i>dentipes</i> | single female | 64 \pm 33.4 | 2.9 \pm 0.3 | 37.2 \pm 21.6 | 19 | 7.1 \pm 6.8 | 2 \pm 1.4 | 42 |
| | two female | 52 | 3 | 33 | 1 | 7.5 \pm 0.7 | 1.5 \pm 0.7 | 2 |
| <i>Pithitis</i> <i>smaragdula</i> | single female | 81.3 \pm 5.2 | 3.5 \pm 0.7 | 40.0 \pm 22.3 | 4 | 8.6 \pm 1.3 | 2.9 \pm 1.4 | 11 |
| | two female | 74 | 4 | 64 | 1 | 8 | 2 | 1 |

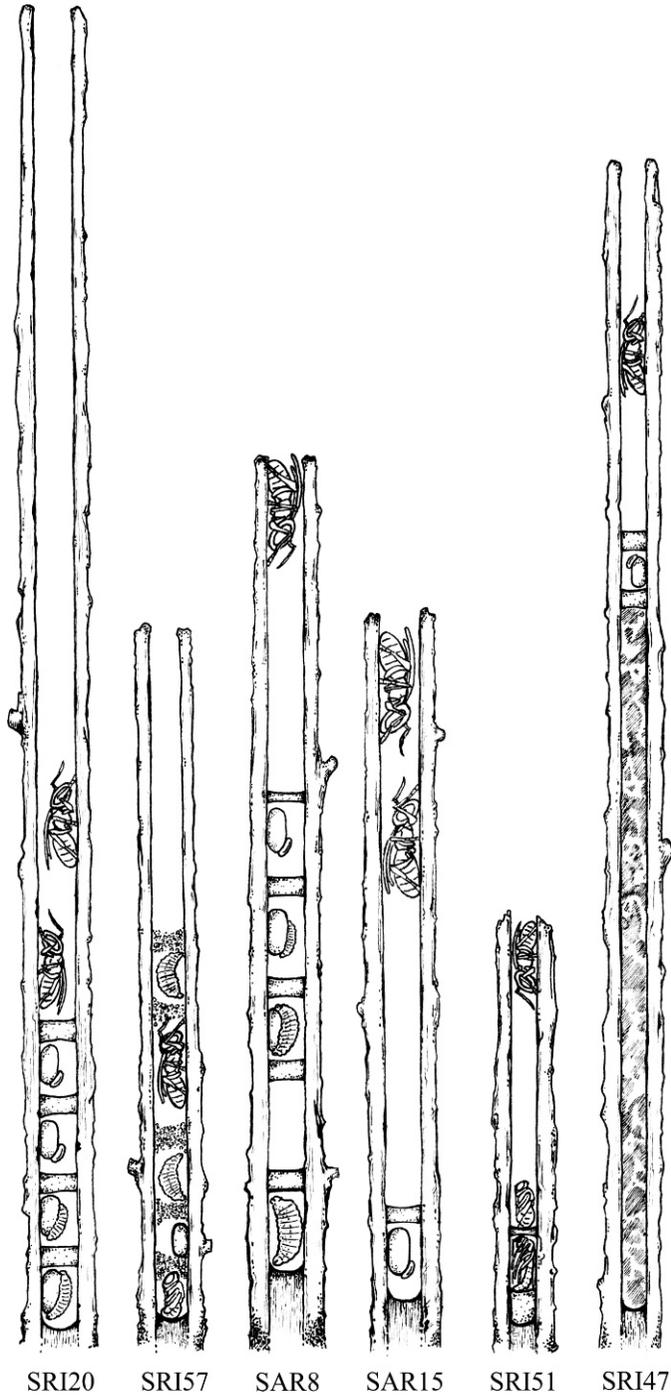


Fig. 3. Scale drawings of *Ceratina* nests collected in Sarawak, Malaysia. Each nest represents a different aspect of unusual nest architecture or female behaviour in these species. Two *C. nigrolateralis* nests exhibiting a multi-female nest (SRI20) and maternal nest inspection (SRI57). Two *C. smaragdula* nests showing an empty brood cell (SAR8) and multi-female nesting (SAR15). Two *C. dentipes* nests demonstrating brood cell parasitism (SRI51) and nest reuse (SRI47).

observed as yellow bodies at the pedicel of the ovaries. The proportional size difference between the larger, reproductive female and the smaller, non-reproductive female was 11%. Both females were likely of the same recently emerged generation, as neither had a single nick in her wings. All three full brood nests contained an adult female assumed to be the brood's mother. No mature brood nests were collected for this species.

Of the 32 nests of *C. nigrolateralis*, seven were classified as founding nests, 20 as active brood nests, five as full brood nests and two as mature brood nests. All founding nests contained a single adult female. Six of the seven founding nests were newly formed, whereas one nest (B18) was reused, with dark soiled interior walls. This nest had a basal chamber 68 mm long, capped with a pith septum 2 mm wide. An adult female was found in the antechamber, but no brood or pollen was found within the nest.

Of the 20 active brood nests of *C. nigrolateralis*, 17 were complete, containing a single adult female with her brood; one was orphaned, lacking an adult female; and two were multi-female nests, each containing two adult females. The first multi-female nest (SRI20) (Fig. 3) contained two adult females and four brood cells that housed two small larvae and two eggs. One female was smaller (head width = 2.07 mm, wing length = 1.77 mm) and had three equivalently sized oocytes (~0.4 mm each) accumulating to an ovarian score of 1.29 mm. This female had completely unworn wings and was also unmated. The second female was larger (head width = 2.13 mm, wing length = 1.98 mm), mated and had slightly worn wings with a wing wear score of two. This female had one fully developed egg (2.24 mm in length) and two large oocytes (1.98 and 1.68 mm) and yellow bodies in the pedicel of her ovaries. The proportional size difference between the larger, reproductive female and the smaller, non-reproductive female was 3%.

The second multi-female *C. nigrolateralis* nest (SRI66), contained two adult females and two brood cells, each housing a pollen mass and egg. One female was smaller (head width = 1.87 mm, wing length = 1.83 mm), unmated and had unworn wings. This female had undeveloped ovaries, each oocyte ~0.3 mm in length, combining to an ovarian score of 0.99 mm. The second female was larger (head width = 2.13 mm, wing length = 1.85 mm) and had unworn wings. Dissection of this female revealed three partially developed eggs, the largest oocytes 0.67, 1.01, and 0.80 mm in length, summing to an ovarian score of 2.48 mm. This female was mated and had yellow bodies in the pedicel of her ovaries, indicating recent oviposition. The proportional size difference between the larger, reproductive female and the smaller, non-reproductive female was 12%.

Five full brood nests were collected for *C. nigrolateralis*. Four broods were complete with a mother present, and one incomplete, lacking an adult female. Finally, two mature nests were collected. One (B74) contained a wing-worn, mated mother, and one male and five female imagos and the second (B59) contained one wing-worn, mated mother, in addition to one imago of each sex.

Only four nests of *C. smaragdula* were collected, comprising one founding nest and three active brood nests. The founding nest was newly formed, with clean interior walls, and contained a single unmated female. Two of the three active brood nests contained a solitary mother with her developing brood. The third active brood nest (SAR15) (Fig. 3) had 2 adult females and at the base of the nest was one capped brood cell containing a pollen mass and egg. The larger female (head width =

Table 4. Comparison of the mean observed and expected ovarian score and head width differences (mm) between multi-female nests. Expected differences were generated through Monte Carlo resampling for each species and *P* is the proportion of simulated differences that were greater than the observed differences, and which can be interpreted as the level of statistical significance.

| Subgenus | Species | Mean ovarian score difference | | Mean head width difference | |
|--------------------|-----------------------|-------------------------------|----------|----------------------------|----------|
| | | Observed | <i>P</i> | Observed | <i>P</i> |
| <i>Ceratinidia</i> | <i>nigrolateralis</i> | 2.67 | 0.026 | 0.159 | 0.696 |
| <i>Neoceratina</i> | <i>dentipes</i> | 2.35 | 0.017 | 0.168 | 0.103 |
| <i>Pithitis</i> | <i>smaragdula</i> | 0.932 | 0.317 | 0.037 | 1.00 |

2.33 mm, wing length = 2.07 mm) was unmated, had unworn wings, and her three largest oocytes were incompletely developed, each being ~0.4 mm in length. The second female was slightly smaller (head width = 2.29 mm, wing length = 1.98 mm), mated, and had unworn wings. She contained three large oocytes, each ~0.75 mm in length. The head width difference between the larger, non-reproductive female and the smaller, reproductive female was 10%.

In the absence of behavioural data, reproductive differentiation was assessed by the ovarian score difference among cohabiting females. In each case of multi-female nesting there seemed to be marked differences in ovarian development. To assess this we used a Monte Carlo simulation resampling technique (Sokal and Rohlf, 1995). For each species we calculated the mean difference in ovarian score for nestmates in multi-female colonies (Table 4). We then randomly sampled pairs of females from single-female colonies, so that the number of pairs was the same as the number of multi-female colonies in our collections, and then calculated mean difference in ovarian scores for these resampled 'colonies'. This procedure was repeated 1000 times for each species to give a null distribution to determine whether the observed mean difference in ovarian score between nestmates was due to stochastic variation alone. Only 26 of the 1000 simulated mean ovarian size differences were greater than that observed for *C. nigrolateralis* multi-female nests. For *C. dentipes* only 17 of the 1000 simulated ovarian size differences were greater than that observed between cohabiting females. These simulations suggest that reproductive differentiation in multi-female nests of both species is greater than would be expected from variance among solitary-nesting females. Conversely, in the simulation for *C. smaragdula* over 300 of the 1000 simulated ovarian size differences were greater than that observed in the lone multi-female nest. *C. smaragdula* was the least sampled in the study with only four nests collected in total, so the power of our analyses to detect reproductive differentiation here is very low, and assessment of reproductive differentiation will require further study with larger sample sizes.

Size variation, as measured by mean head width difference, among multi-female nests of each species was explored using the same procedures as above, but none of the simulations suggested that size variation was due to anything more than random variation alone (Table 4).

Maternal Behaviour

When nests were dissected, the locations of adult females and any evidence of guarding or grooming behaviour, including the rearrangement of the pith in the nest, were observed as signs of maternal care. Those females recovered from nests were

typically found guarding their brood with their abdomens blocking the nest entrances. Evidence of maternal care was exhibited in three nests. One *C. accusator* nest (B37) contained a single adult female with three pupae, one pink-eyed, one brown-eyed, and one fully pigmented. The pith partitions in this nest were completely loosened, and the mother was found in the second brood cell with the brown-eyed pupa. A second *C. nigrolateralis* nest (SRI57) (Fig. 3) contained a single adult female and four offspring ranging in age from full-grown larvae to a white-eyed pupa. Again the pith partitions in this nest were completely disrupted and the mother was found among loosened pith between her two youngest larvae. These observations show that females remain in their nests during juvenile development and also check on their developing offspring from time to time. Finally, a third *C. nigrolateralis* nest of interest (SRI10) contained a dead adult female who was highly wing worn (5+) and found in the gallery above an empty brood cell and a developing red-eyed pupa. This nest is consistent with the nest loyalty of a *Ceratina* mother who, after completing her nest, stayed with her brood throughout her life and guarded the nest entrance until her death.

To assess the effect of maternal longevity on offspring survival, all active brood, full brood, and mature brood nests were compared for the presence or absence of an adult female. Of 15 *C. accusator* nests, one full brood and two mature brood nests had been orphaned (20%). None of these orphaned broods showed any sign of desiccation or parasitism. Of 27 *C. nigrolateralis* nests, one active brood and one full brood nest were orphaned (7%), and again neither orphaned nest contained dead or parasitized offspring. The remaining 15 *C. dentipes* and 3 *C. smaragdula* nests contained immature brood (active and full brood classes respectively). Among these nests not a single orphaned brood was discovered. However, despite the presence of the maternal guard, 3/15 (20%) of the *C. dentipes* nests were parasitized by a chalcid wasp.

Discussion

Social Organization

The major finding of this study is that *Ceratina* is not a strictly solitary bee. It is noteworthy that none of the multi-female nests appeared to be communal (equally reproductive) assemblages. All active and full brood assemblages containing cohabiting females exhibited reproductive differentiation, with one female mated and the second unmated, suggesting these are semisocial or eusocial nests. It was difficult to assess whether cohabiting females differed in age as these bees had little to no wing wear. Thus, whether nests contained semisocial (adults of the same generation) versus eusocial (mother-daughter) pairs remains unknown. Sakagami and Maeta (1989) examined multi-female nests of *C. okinawana* in relation to adult female body size. The largest head width difference between females was accompanied by greatest reproductive skew. In these eusocial and semisocial associations, the larger female behaved as the guard and primary reproductive, and the smaller female took on a foraging non-reproductive role. When size differences were relatively small, reproductive skew diminished and role reversion of the smaller and larger females took place. Quasisocial nests, where both females are reproductive, were most common between similar sized associations. Size-based reproductive dominance is also recorded for *C. flavipes* (Sakagami and Maeta, 1987) and *C. japonica* (Sakagami and Maeta, 1984).

In many social species where morphological castes are not present, body size is an important factor contributing to dominance (Batra, 1966; Packer, 1986; Hogendoorn and Velthuis, 1999). Size dimorphism within nesting assemblages of female bees typically suggests reproductive differentiation (Michener, 1974). The three multi-female nests belonging to *C. dentipes* and *C. nigrolateralis*, each contained one large female that had a high degree of ovarian development and was mated, and a smaller female that was non-reproductive and unmated. These data suggest that larger body size contributes to reproductive dominance; smaller females were reproductively subordinate to larger, reproductively dominant females. Conversely, in the single multi-female nest of *C. smaragdula* (SAR15), the larger female had no ovarian development and was unmated, while the smaller female had fully developed ovaries and was mated. However, both *C. smaragdula* females were unworn and a lone egg was found at the base of the nest with newly excavated pith and light interior walls, suggesting that this was a pleometrotic colony resulting from two adult females cofounding rather than reusing a nest burrow.

Body size data are limited for males of this genus but taxonomic records describing both sexes indicate that *Ceratina* species are sexually dimorphic with females consistently larger than males (Van der Vecht, 1952; Yasumatsu and Hirashima, 1969; Daly, 1973; Daly, 1988, Rehan and Richards unpubl. data). Moreover, the female-biased numerical sex ratios found in this study are consistent with studies on other socially polymorphic ceratinines including newly emerged full broods of *C. megastigmata* which are reported to have a 59.0% female-bias (Katayama and Maeta, 1979). The numerical sex ratio (% male) in mature brood populations is also predominantly female-biased in other Old World *Ceratina*: *C. (Neoceratina) australensis* Perkins, 27% (Michener, 1962); *C. (Ceratinidia) flavipes*, 37% (Tano, 1964) and 32% (Shiokawa, 1969); and *C. (Ceratinidia) japonica*, 13% (Shiokawa, 1969). Conversely, studies on a persistently solitary New World species, *C. (Zadontomerus) calcarata* Robertson, have reported male-biased numerical sex ratios: 54% (Johnson, 1988) and 57% (Rehan and Richards, unpubl. data).

Female-biased numerical sex ratios are often associated with sociality in halictine and allodapine bees (Schwarz *et al.*, 2007) and are most likely due to local resource enhancement (deriving from increased per capita brood production in multi-female nests) or production of workers, who do not count as investment in female reproductive function. Evidence for female-biased sex ratios reported here and in other *Ceratina* species is therefore somewhat puzzling given the low frequency of social colonies. Further study is clearly required to quantify this bias, and the possibility of further sex ratio biasing mechanisms, such as partial bivoltinism (Seeger, 1983) need to be examined. Partial bivoltinism seems particularly promising as a source of bias in *Ceratina* because of the reported adult longevity in some species (Sakagami and Maeta, 1977).

Maternal Behaviour

The transition from solitary to eusocial life requires: 1. maternal care, in that mothers must remain at the natal nest in order to interact with their offspring; 2. maternal longevity, so that mothers survive to associate with callow offspring after eclosion; and 3. mutual tolerance, as females must accept one another in the nest in order to coexist and produce successive brood (Lin and Michener, 1972; Michener, 1985). In general, xylocopine bees are known for their longevity and nest loyalty

(Michener, 1990). Some *Ceratina* adult females have been observed to live upwards of three years in greenhouse cages and produce three successive broods (Sakagami and Maeta, 1977). Maternal longevity is thought to increase brood survival by allowing a guard at the nest entrance to protect the brood from parasitism. Maternal care is also important for newly emerged offspring. Mature brood remain in the natal nest while the mother forages and feeds the offspring via trophallaxis (Sakagami and Maeta, 1977).

Evidence of brood cell inspection was exhibited twice: in one *C. accusator* (B37) and one *C. nigrolateralis* (SRI57) nest. Observations of the intranidal behaviour of Japanese species of the subgenus *Ceratinidia* have revealed that mothers periodically enter brood cells and inspect brood for desiccation, incorporating faecal pellets and dead brood into pith partitions (Sakagami and Maeta, 1977). Moreover, all behaviourally described *Ceratina* are nest loyal and remain with their mature brood (Rau, 1928; Michener, 1962; Daly, 1966; Sakagami and Laroca, 1971; Kislow, 1976; Katayama and Maeta, 1979; Johnson, 1990), even foraging and feeding them (Sakagami and Maeta, 1977). The nest loyalty of adult females with their brood allows for interaction with their newly eclosed brood and the persistence of occupants in the natal nest reveals mutual tolerance between mother and juveniles and among siblings.

Social colonies are thought to be selected for due to the benefits of lowering predator and parasite pressure (Lin and Michener, 1972; Evans, 1977; Andersson, 1984). Parasites were found in three of 19 *Ceratina dentipes* nests collected, and in each case a single chalcid pupa was found in a nest attended by an adult female assumed to be the mother of the developing brood. Nest orphaning was low to moderate (0–20%) across species, but did not coincide with parasitism. Hence the presence of an adult female in the nest seems ineffective against these chalcid parasites. Sakagami and Maeta (1977) also found that the presence of mothers provided no protection from fungi or large ichneumonid parasites. However, *C. flavipes* and *C. japonica* nests exhibit 25–50% brood cell mortality when orphaned versus 3–19% when guarded (Sakagami and Maeta, 1977) revealing that the presence of a mother at the nest entrance was effective in preventing mortality from small wasp and fly parasites, which were only present in orphaned nests.

Colony Structure

The diversity of brood developmental stages among nests for each species (Fig. 2) suggests two possible reproductive patterns. First, females may found nests and provision brood completely asynchronously. Thus, nests in which brood had matured to the pupal and callow adult stages must have been founded earlier than those nests containing eggs and small larvae. A second but not mutually exclusive explanation is that these species are multivoltine. Mature brood assemblages could represent the end of Brood 1, whereas founding and active brood nests could represent the beginning of Brood 2.

Temperate ceratinines tend to emerge in spring and produce a single brood prior to hibernation (univoltine), although they occasionally produce a second brood (bivoltine), and have prolonged developmental times from egg to adulthood, averaging 1.5 to 2 months (reviewed in Sakagami and Laroca, 1971). In contrast, subtropical species tend to have multiple reproductive cycles per year and usually mature in less than a month (reviewed in Sakagami and Laroca, 1971). Tropical taxa

do not experience a quiescent period and are thought to reproduce year round and these species are also reported to have rapid development, maturing from egg to adult in less than a month (Michener and Eickwort, 1966). Given the trend of more reproductive bouts and quicker maturation time with decreasing latitude, it is likely that the tropical *Ceratina* described herein are multivoltine (two or more broods per annum). In addition to the longevity and nest loyalty of ceratinine mothers, the ability for brood to mature rapidly allows for the overlap of generations which all contribute to the formation of multi-female nesting associations.

Constraints on Social Nesting

Ceratina nest in linear burrows with a single nest entrance and brood are provisioned one at a time in a serial manner. Thus, the inability of females to concurrently provision and oviposit in their own brood cells might create an impediment to communal nesting. Social nesting is unstable in all *Ceratina* species in which it has been reported; this is probably due to the constant disruption multiple females present each other while provisioning and constructing brood cells within a linear nest.

Multiple female nest occupancy requires females to remain at the natal nest or found a new stem. Evidence for nest reuse was observed twice in this study: once in a *C. dentipes* nest (SRI47) and second in a *C. nigrolateralis* nest (B18). Nest reuse is recurrent in Japanese ceratinines. In *C. japonica*, 203 (47%) of 433 nests examined were reused and 63 of these (31.0%) were multi-female nests. Conversely, of the 230 newly built nests, only three (1.3%) contained multi-female associations (Sakagami and Maeta, 1984). High rates of multi-female nesting were also recorded for *C. okinawana* as 57/276 reused nests contained multiple females, whereas only 1/133 newly founded nests contained a multi-female association (Sakagami and Maeta, 1989). Likewise, in *C. megastigmata*, 4/5 multi-female nests were discovered in reused nests (Katayama and Maeta, 1979). These data suggest multi-female nests predominantly arise when females stay in a natal nest rather than joining a new nest.

Further oddities within ceratinine nests arise from the inconsistency of females when provisioning their brood cells. Empty brood cells have been reported in nests of numerous *Ceratina* species (reviewed in Sakagami and Laroca, 1971; this study). There are multiple explanations for these empty spaces, including spacers for emerging offspring to pass one another within the linear nest (Malyshev, 1913). However, siblings have been observed to pass over developing siblings without injury in nests lacking empty spaces (Michener and Eickwort, 1966; Tano, 1964). Observations of trap nesting megachild bees have shown that empty spaces or false cells help to minimize brood mortality due to parasitic wasps (Tepedino *et al.*, 1979; Munster-Swendsen and Calabuig, 2000). This is a plausible explanation for ceratinines as they are known to have numerous parasites (Daly, 1967). Empty spaces have also been interpreted as interruptions in the brood rearing activities (Michener, 1962). Observations on *Ceratinidia* species have shown that females only begin foraging and oviposition activities following the formation of the pith septa (Sakagami and Maeta, 1987). Given this brood rearing sequence, an interruption such as bad weather or floral resource limitation could result in the formation of a brood cell septum and the omission of pollen provisions. Further physiological constraints such as egg-limitation (Linsley, 1958; Rosenheim, 1996) could result in a female producing a brood cell containing a pollen provision but no egg. Carpenter

bees are known for their disproportionately large eggs compared to other bees (Iwata and Sakagami, 1966) and brood cells containing egg-less pollen provisions are recurrent in the ceratinines (Johnson, 1988; Rehan and Richards unpub. data; this study). The prevalence of empty brood cells and egg-less pollen masses in this and other studies suggest that parasite pressure as well as egg and resource limitation are pervasive across temperate and tropical *Ceratina* in all behaviourally described subgenera.

Conclusions

Accumulating evidence from different regions and species all suggest that *Ceratina* are consistently socially polymorphic across Old World taxa. Whether the *Ceratina* of Borneo are semisocial or eusocial remains unknown, however our data strongly suggest that when multiple females nest together, some form of reproductive division of labour occurs.

North American species of the subgenus *Zadontomerus* have been documented as solitary across all aforementioned studies. Conversely, some Old World taxa show recurrent social polymorphism with all behaviourally classified species exhibiting multi-female nesting. Although typically regarded a solitary genus, some Old World *Ceratina* are socially polymorphic; within a population a few females form multi-female nests while the majority of females of the same population remain in a single foundress solitary state. Across all taxa we see recurrent maternal care and longevity, a broad range in adult female body size, and possible parasite avoidance. Prolonged longevity of queens is a prerequisite to the establishment of eusocial life in Hymenoptera (Sakagami and Maeta, 1977). Lin and Michener (1972) argued that the amount of size variation among individuals of solitary species was enough to account for the caste-linked size differences found in primitively eusocial species. Furthermore, extrinsic parasite pressure and strong selection to defend a nest may be a driving factor to social nesting (Crespi, 1994).

Future studies with larger sample sizes and prolonged study durations are needed to assess each species' behavioural repertoire and life history traits. Moreover, the composite of behavioural data suggest geographic and taxonomic variation in life history traits and social behaviour of the ceratinines, but these findings lack an evolutionary context. A phylogenetic framework is needed to compile and contrast the frequency and circumstance of sociality in these 'solitary' bees.

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