

Evidence for social nesting in Neotropical ceratinine bees

S. M. Rehan¹ · S. M. Tierney² · W. T. Wcislo³

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Abstract Small carpenter bees (Ceratinini) are a key taxon to understanding the transition from subsocial to social behaviour, as all documented groups are long-lived and tend to their young periodically throughout development, though the behaviour of multiple lineages is little known. This study provides the first evidence for social nesting in three Neotropical species of *Ceratina* (*Ceratinula*) from Panama. Social nesting was associated with nest reuse, consistent with the hypothesis of kin associations, and the proportion of nests (per species) that displayed sociality was as follows: *C. buscki* 5 %; *C. rectangulifera* 0 %; *C. tricolor* 6 %; and *C. zeteki* 23 %. Sociality is always a low-frequency phenomenon in ceratinine bee populations, and generally represents a third or less of the population. The fact that the majority of colonies remain solitary indicates that solitary nesting is adaptive in the studied species.

Keywords Subsocial · Facultatively social · Small carpenter bee · Ceratinini · Xylocopinae

Introduction

Subsociality is defined as prolonged parental care with parent–offspring interactions (Tallamy and Wood 1986), and is arguably the simplest form of social behaviour

attained by animals. Since subsocial taxa are quite common yet eusocial taxa are relatively scarce the question persists, what rare conditions act to facilitate the evolution of eusociality? Conversely, what prevailing selective forces retain the remaining taxa in a subsocial state?

Presocial taxa are fundamental to understanding the origins of sociality and can give insights into the evolutionary steps from solitary to social life. The Xylocopinae are a monophyletic grouping of xylophilous bees consisting of four tribes: Xylocopini, Manuelliini, Allodapini and Ceratinini (Rehan et al. 2012). Xylocopini have shown that species display parasociality but never exhibit eusociality (Michener 1990). Manuelliini may represent a reversion to solitary life with only three extant solitary species (Flores-Prado et al. 2008). Allodapine bees show ancestral sociality with no reversions to solitary life (Schwarz et al. 2011), while the Ceratinini exhibit the full spectrum from solitary to eusocial behaviour within a narrow range of taxa (Sakagami and Maeta 1977; Michener 1985). The Ceratinini comprise a single genus *Ceratina*, and approximately 200 described species within 17 Old World subgenera and six New World subgenera (Michener 2007).

Ceratina were traditionally regarded as solitary, but many presocial behaviours including maternal longevity, nest loyalty and prolonged mother–offspring interaction are reported for species with behavioural data (reviewed by Rehan and Richards 2010). Growing evidence supports ancestral and recurrent sociality in tropical species (Rehan et al. 2009, 2011, 2014). Floral associations, nest architecture and brood productivity are reported from biofaunistic notes (Gonzalez et al. 2004; Michener and Eickwort 1966; Sakagami and Laroca 1971), but behavioural data are lacking for Neotropical species. Here, we provide nesting biology and evidence for social nesting in three of four Central American *Ceratina* (*Ceratinula*) species examined:

✉ S. M. Rehan
sandra.rehan@unh.edu

¹ Department of Biological Sciences, University of New Hampshire, Durham, USA

² School of Biological Sciences, The University of Adelaide, Adelaide, Australia

³ Smithsonian Tropical Research Institute, Panama, Panama

C. buscki (Cockerell), *C. rectangulifera* (Schwarz and Michener), *C. tricolor* (Michener) and *C. zeteki* (Cockerell).

Methods

Nest collections

Ceratina nests were collected in Panama during the 2011 dry season, between 7 January and 11 April. *Ceratina buscki* and *C. rectangulifera* were collected from Chiriqui province (8.78° N, 82.44° W, altitude 1276 m). *C. tricolor* and *C. zeteki* were collected from Panama province (8.59° N, 79.31° W, altitude 17 m). *Ceratina tricolor* were exclusively collected in stems of *Bougainvillea*, and the remaining *Ceratina* species were collected from stems of unknown plants species. Nest contents and architecture were recorded, detailing numbers of brood cells, developmental stage of brood, and sex from pupal stage onwards.

Nest classifications

The condition of each nest was assigned to categories following descriptions of Rehan et al. (2009) based on general appearance and contents. *Hibernacula* were old nests with faecal pellets and pollen residue containing adults with darkened wings and integument colouration. *Founding nests* did not contain pollen provisions or immature brood. *Active brood* nests contained one or more pollen mass or immature bee. *Full brood* nests contained immature offspring and the outermost brood cell nearest the nest entrance contained a larva or pupa. *Mature brood* nests contained adults but no pollen masses or immature offspring, rather these nests contained callow offspring with milky wings, pale integument colouration and these nests had soiled walls often with faecal pellets and pollen residue. Among active and full brood nests, ‘reused nests’ were soiled with pollen stains and faecal pellets and ‘new nests’ were devoid of refuse and had pristine interior walls. We considered a nest to be potentially social if there was more than one adult female in an active or full brood nest. The metric ‘percentage social nesting’ was calculated as the number of nests containing multiple adult females divided by the sum of active and full brood nests collected for each species.

Morphometrics

Adult morphometrics recorded were head width (body size), wing wear (foraging effort or age), ovarian development (reproductive status), and spermathecal contents (mating status). Head width (HW) was measured across the widest part of the face including both compound eyes. The size difference between all adult females in the same nest was

reported as a ratio: (largest HW – smallest HW)/largest HW. Wing wear was scored as a relative measure from zero to five. Unworn bees with no nicks or tears in the apical margin of the forewing received a score of zero and heavily worn bees with the apical margin of the forewing completely shredded received a score of five. Ovarian development was scored as the sum of the lengths of the three largest terminal oocytes. Mating status was determined by the presence of sperm in the spermatheca. A mated female has an opaque spermatheca and an unmated female has a transparent spermatheca. Callow adults were identified by milky wing condition and pale integument colouration.

Statistical analyses

Descriptive statistics and Chi squared goodness-of-fit tests were calculated in SPSS (Statistical Package for the Social Sciences) version 16.0 (SPSS Inc., Chicago).

Results

A total of 140 *Ceratina* (*Ceratinula*) nests were collected and all four species were reproductively active (Fig. 1), with some nests containing the full spectrum of immature developmental stages: 60 *C. buscki* nests (30 active brood nests, 13 full brood nests); 21 *C. rectangulifera* nests (8 active, 1 full brood); 40 *C. tricolor* nests (8 active, 8 full brood); and 19 *C. zeteki* nests (11 active, 2 full brood). No species displayed brood sex ratios significantly different from equality (Table 1). All four species formed linear burrows in pithy stems and nest dimensions are summarized in Table 2. All species had similar nest dimensions ranging from 40 to 65 mm long and 2.5–3.0 mm wide. Nest reuse was observed in all four species: *C. buscki* (38 % nests), *C. rectangulifera* (57 %), *C. tricolor* (65 %), *C. zeteki* (32 %).

Ceratina (*Ceratinula*) *buscki*

The 60 colonies collected comprised 11 founding nests, 30 active brood, 13 full brood, 2 mature brood, and 4 hibernacula. All founding nests were new nests and hibernacula reused nests and all contained a single adult female. Of the 30 active brood nests, 19 were new nests and 11 were reused. The number of active and full brood nests containing multiple adult females was 5 % (2/43).

One reused nest (F157) contained two adult females and five brood cells. The innermost four brood cells contained larvae and the outermost brood cell contained an egg. The smaller female (HW = 1.39 mm) had unworn wings (WW = 0), was unmated and had little ovarian development, each oocyte being ~0.2 mm in length, with a total

Fig. 1 Brood developmental stages of all *Ceratina* nests collected in the January to April 2011. *Ceratina* are mass provisioning bees and thus pollen in brood cells proceeds oviposition, eggs are found on completed pollen masses, larvae are found consuming pollen masses, pupae have completely consumed their pollen mass, callow offspring are newly emerged offspring with milky wings, and empty cells are devoid of pollen and immature offspring. *N* = number of nests

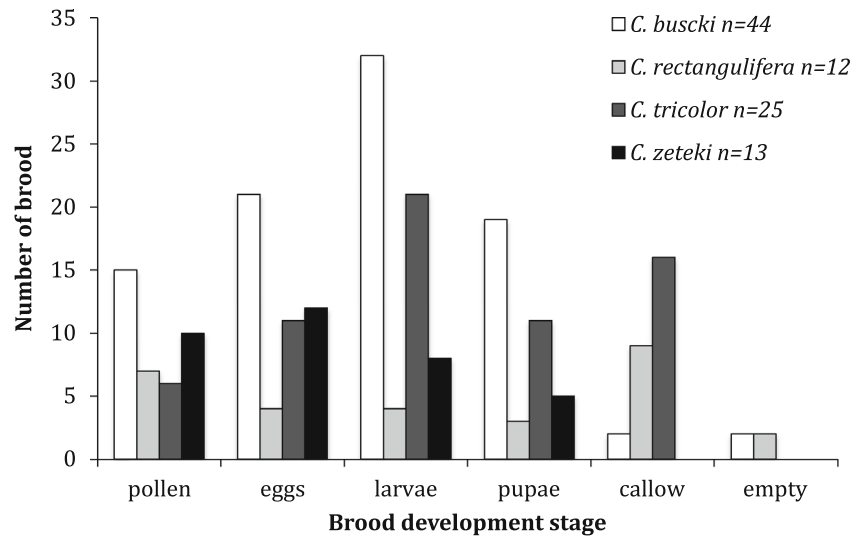


Table 1 Population sex ratios based on active and full brood nests by species

Subgenus	Species	<i>N</i>	Females	Males	Total	χ^2	<i>p</i>
<i>Ceratinula</i>	<i>buscki</i>	16	13	21	34	0.54	0.46
<i>Ceratinula</i>	<i>rectangularifera</i>	1	2	4	6	0.34	0.56
<i>Ceratinula</i>	<i>tricolor</i>	12	18	11	29	0.42	0.51
<i>Ceratinula</i>	<i>zeteki</i>	4	5	5	10	0.00	1.00

N = number of nests. The mean numerical sex ratio for each species was compared to the expectation of equal sex ratios using Chi squared goodness-of-fit tests for each species

Table 2 Nest architecture measurement data (mm)

Species	Nest class	Tunnel length	tunnel width	Stem diameter	<i>N</i>
<i>Ceratinula buscki</i>	Single female	61.3 ± 28.0	2.8 ± 0.6	5.4 ± 1.0	58
	Two female	113.5 ± 27.6	3.0 ± 0.0	5.0 ± 0.0	2
<i>Ceratinula rectangularifera</i>	Single female	61.3 ± 46.3	2.4 ± 0.6	4.9 ± 1.5	21
	Two female	n/a	n/a	n/a	0
<i>Ceratinula tricolor</i>	Single female	42.6 ± 24.2	2.8 ± 0.4	6.9 ± 1.6	38
	Two female	65.0 ± 46.7	2.5 ± 0.7	8.0 ± 4.2	2
<i>Ceratinula zeteki</i>	Single female	45.9 ± 25.7	2.6 ± 0.4	4.4 ± 0.6	16
	two female	39.3 ± 16.6	3.0 ± 0.0	5.7 ± 1.2	3

Mean ± one standard deviation. *N* = number of nests

ovarian score of 0.58 mm. The second female was slightly larger (HW = 1.41 mm), had worn wings (WW = 2), was mated and had three half-developed oocytes, ranging from 0.7 to 1.0 mm in length, with a score of 2.46 mm. The proportional size difference between the larger reproductive female and the smaller non-reproductive female was 1 %.

Thirteen full brood nests were collected for *C. buscki*. Six of these were new and seven were reused. One reused nest (F85) contained two adult females and five brood cells. The

two innermost brood cells contained pupae, and the three outermost brood cells contained larvae. The smaller female (HW = 1.31 mm) had unworn wings (WW = 0), was unmated and had undeveloped ovaries. Each of her oocytes was 0.1 to 0.3 mm in length, with a total score of 0.69 mm. The larger female (HW = 1.44 mm) had worn wings (WW = 3), was mated and had moderately developed ovaries, with oocytes ranging from 0.5 to 1.0 mm in length, for an ovarian score of 2.22 mm. The proportional size

difference between the larger reproductive female and the smaller non-reproductive female was 9 %.

Ceratina (Ceratinula) rectangulifera

In total, 21 nests were collected comprising 6 hibernacula, 3 founding nests, 8 active brood, 1 full brood, and 3 mature brood nests. Among reproductive, active and full brood, nests (0/9) 0 % were social nests. All hibernacula and founding nests contained only a single adult female. Of the active brood nests, 6 were new and 2 reused. The full brood nest (F79) was reused, contained 5 brood cells and a single adult female. All brood and the mother were dead at the time of nest dissection as the contents had been completely consumed by mold.

Ceratina (Ceratinula) tricolor

Of the 40 nests collected, 9 were hibernacula, 6 founding nests, 8 active brood, 8 full brood and 9 mature brood nests. Of active and full brood reproductive nests (1/16), 6 % were social nests with two adult females. Seven of 8 active brood nests contained a single adult female, but one reused nest (P54) contained two adult females. The smaller female (HW = 1.01 mm) had unworn wings, was unmated and had undeveloped ovaries (0.35 mm). The larger female (HW = 1.19 mm) had worn wings, was mated and had developed ovaries, with one fully developed oocyte 1.18 mm in length and two partially developed oocytes 0.52 and 0.26 mm in length, for a total ovarian development score of 1.96 mm. The proportional size difference between the larger reproductive and smaller non-reproductive females was 15 %.

Ceratina (Ceratinula) zeteki

Of the 19 nests collected, 2 were hibernacula, 4 founding nests, 11 active brood, 2 full brood. Eight out of 11 active brood nests contained a single adult female, but 3 reused nests contained two adult females. Of reproductive nests, both active and full brood, (3/13) 23 % were social nests.

One active brood reused nest (P7) contained two adult females in which the smaller (HW = 1.29 mm) had unworn wings, was unmated and had undeveloped ovaries (0.60 mm). The larger female (HW = 1.43 mm) had worn wings, was mated and had developed ovaries, with one fully developed oocyte 1.57 mm in length and two partially developed oocytes 0.32 and 0.44 mm in length, for a total ovarian development score of 2.33 mm. The proportional size difference between the larger reproductive and smaller non-reproductive females was 10 %.

The second reused multi-female active brood nest (P16) also contained two adult females. The smaller

(HW = 1.05 mm) was unmated and had unworn wings (WW = 0) and undeveloped ovaries (0.26 mm total ovarian development). The larger female (HW 1.40 = mm) was mated, with worn wings (WW = 1) and had developed ovaries (1.35 mm total ovarian development). The proportional size difference between females was 25 %.

The third reused multi-female nest (P137) was also reused and contained two adult females. The smaller female (HW = 1.29 mm) was again unworn (WW = 0) and unmated with a total ovarian development of 0.66 mm. The larger female (HW = 1.32 mm) was worn (WW = 2), mated and had a total ovarian development of 1.47 mm. The proportional size difference between females was 3 %.

Discussion

Our study provides the first evidence for social nesting in the *Ceratina* of Panama. Three of four species examined revealed two female reproductive nesting associations and reproductive division of labour was observed in all social nests. We found no evidence for communal nesting in these species. Based on morphometrics, in all multifemale nests the larger female was a reproductive forager and the smaller female a non-reproductive guard, supporting the notion that division of labour is consistent with all observations of social colony formation in *Ceratina* species reported to date (this study; Sakagami and Maeta 1995; Rehan et al. 2009, 2014).

Social nesting is recurrent among tropical species of this genus (Michener 1962; Sakagami and Maeta 1989; Rehan et al. 2009, 2011). Tropical species tend to have multiple reproductive cycles per year and usually mature in less than a month (Michener and Eickwort 1966; Sakagami and Laroca 1971; Rehan et al. 2010). Given their propensity to more reproductive bouts and quicker maturation time with decreasing latitude, it is likely that Neotropical *Ceratina* are multivoltine (two or more broods per annum). In addition to the longevity and nest loyalty of ceratine mothers, the ability for brood to mature rapidly allows for the overlap of generations, traits which all contribute to the formation of multi-female nesting associations (Sakagami and Maeta 1977; Rehan et al. 2009).

Genetic data and intranidal colony observations are lacking for Neotropical species, but social nests were all found in reused nesting substrates suggesting that colonies form between kin, either closely related sororal (semisocial) or matrilineal (eusocial) groups. Future studies quantifying genetic relatedness among nestmates are needed to determine the exact nature of these social colonies and how social nests form, and will reveal the prevalence of oophagy and potential conflicts limiting more frequent social colony formation.

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