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ORIGINAL ARTICLE

The influence of social environment on cooperation and conflict in an incipiently social bee, *Ceratina calcarata*

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Abstract

How mutual tolerance is produced, and the role of social environment in inducing cooperation in social groups, remains unstudied in many simple societies. In particular, maternal and sibling care and conflict are challenging to manipulate experimentally for many species. Most bees are solitary, but mothers of the eastern small carpenter bee, *Ceratina calcarata*, engage in prolonged care of offspring, and are therefore subsocial. Females form social associations of parents and a single generation of offspring, including a smaller dwarf eldest daughter (DED) who forages and feeds her adult siblings. Adult assemblages of *C. calcarata* present a unique opportunity to study the effect of social environment on cooperation and sibling care in an otherwise subsocial bee. To study how social environment influences foraging and intranidal behaviors, observation nests were constructed, and unaltered as a control, or treated by removing either only mothers or both mothers and DEDs. Nests were video-recorded for 464 h during summer (July–August) parent-adult offspring cohabitation. Individual and interactive behaviors were scored. In the absence of mothers, offspring were more tolerant, suggesting that a hierarchy between mother and offspring produces less tolerance between offspring. Aggression was only significantly greater in the absence of both mother and DED. We found that foraging was the lowest in the absence of mothers, and persisted in the absence of both mother and DED. This study provides the first detailed account of the intranidal behaviors of this species and experimentally reveals how social environment influences cooperative behavior.

Significance statement

Understanding how particular life histories, such as extended parental care, may set the stage for more complex social behaviors, such as sibling cooperation, is critical to understanding how alloparental care evolves in group living organisms. Most species exhibiting parental care and sibling cooperation are difficult to manipulate experimentally. Though relatively uncommon in invertebrates, extended parental care is frequently found in small carpenter bees that can be carefully observed within their nests and foraging. Here, we examine how the absence of mothers and worker-like daughters influences the social behavior of related adults living in close group association of such bees. This experiment presents an intranidal study of a subsocial bee's behavior, and our results suggest that mothers play a dramatic role regulating social behavior. Furthermore, our results show that siblings are more tolerant in the absence of mothers, suggesting that mothers may maintain social hierarchies among offspring. Siblings may interact more aggressively and more frequently as they negotiate intranidal and foraging tasks in the absence of maternal care.

Keywords Aggression · Tolerance · Carpenter bee · Hymenoptera · Maternal care · Phenotypic plasticity

Introduction

Maternal care forms a key component of social environment, profoundly influencing behavior in both vertebrates (Caldji et al. 1998; Fish et al. 2006) and invertebrates (Costa 2006; Kramer et al. 2015). In its simplest form, social behavior in invertebrates consists of extended parental care of offspring, most frequently by mothers, termed subsocial behavior (West-

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Eberhard 1969; Wilson 1971; Michener 1974). In such cases, mothers may associate with offspring after they are no longer dependent on them for survival, even into fully adult stages of development (Tallamy and Wood 1986; Costa 2006). Subsocial behavior is frequently exhibited within the Hymenoptera, where mothers and, rarely, fathers, may engage in offspring provisioning or nest guarding (West-Eberhard 1969; Wilson 1971; Michener 1974; Rehan and Richards 2010; Mikát et al. 2019).

Within many species, extended parental care may result in more complex social interactions, including cooperative behaviors among offspring resulting from dominance hierarchies between parents and offspring. Adoption of such cooperative roles within a social group is thus sensitive to changes in social environment. Alloparental care of siblings, though relatively uncommon, occurs in some species of cooperatively breeding birds, several mammal and fish species, and many insects (Wilson 1971; Michener 1974; Emlen 1984; Taborsky 1984; Stacey and Koenig 1992; Koenig and Dickinson 2004; Costa 2006). In cotton-top tamarins, daughters that care for younger siblings are reproductively repressed in the presence of their family (Widowski et al. 1990). In many cases, aggression is the primary mechanism differentiating cooperative helpers from their siblings. In the Serengeti dwarf mongoose, subordinates acting as alloparents are the recipients of increased aggression during mating periods (Creel et al. 1992). Within the primitively eusocial sweat bee, *Lasioglossum zephyrum*, foraging and nest constructing bees are the recipients of frequent nudges from dominant reproductives (Michener and Brothers 1974). Aggressive interactions are known to play a role in determining social structure in a diversity of group living organisms, including social Hymenoptera (Gadagkar 1980; Reyer et al. 1986; Creel et al. 1992; Reeve 1992; Mulder and Langmore 1993; Wcislo 1997; Cameron and Jost 1998; Arneson and Wcislo 2003; Jandt et al. 2014).

Cooperative behaviors in the socially diverse Hymenoptera are frequently determined through social interactions and environment (Wilson 1971; Michener 1974). The subsocial small carpenter bee, *Ceratina calcarata*, has a simple social system that is ideal for testing the role of the social environment in group tolerance and conflict (Shell and Rehan 2018). *Ceratina calcarata* offspring are not only provided with provisions prior to development but also require additional feeding as adults to survive overwintering, as is found in several other temperate *Ceratina* species (Sakagami 1977; Mikát et al. 2016). The colony cycle of *C. calcarata* begins when dispersing males and females mate in early spring (Rehan and Richards 2013). Females establish nests by excavating a tunnel in the central pith of a dead plant stem, and then begin a period of mass provisioning in early June (Rehan and Richards 2013). After provisioning, brood mothers continue to associate with their nest and care for their developing offspring by cleaning and inspecting them (Rehan and Richards 2013). Mothers engage in a second period of foraging and provisioning of eclosed adults from late July to mid-September (Rehan and Richards 2013; Mikát et al. 2017). During this period, offspring typically remain in the nest, with the exception of a smaller, under provisioned daughter (also referred to as a dwarf eldest daughter (DED)) who forages to feed her siblings often alongside her mother (Johnson 1988; Mikát et al. 2017). This second period of foraging ensures adult offspring survival during overwintering, but the DEDs, which exhibit worker-like altruism in caring for their siblings, do not survive overwintering (Rehan and Richards 2010; Rehan et al. 2014).

The maintenance of adult assemblages from late summer through early spring within *C. calcarata* nests presents a unique opportunity in which to characterize social behavior and study the role of social environment in colony cooperation. Experiments studying individual encounters in forced association circle tube assays use a single length of plastic tube connected at either end such that bees are forced to interact (Breed et al. 1978; Packer 2006). Such studies suggest that bees from orphaned nests are more avoidant and aggressive, and that mothers preferentially direct aggression towards their daughters rather than non-nest mates (Rehan and Richards 2013; Arsenault et al. 2018). It is thus likely that maternal aggression has a significant effect on offspring behavior, though the social environment of a nest differs significantly from that of temporary forced association. Intranidal behavior has not been observed in this species, and it is not known if aggressive interactions are frequent under the conditions of extended cohabitation of multiple interacting related individuals. Experience is known to play a role in aggression in *C. calcarata*, and it is thus likely that the repeated interactions found within nests lead to different social dynamics than found in temporary encounters between females (Withee and Rehan 2016).

In order to study the influence of social environment on intranidal and foraging behavior in *C. calcarata*, two experimental removal treatments were compared to control observation nests. The two treatments were (i) mother removal and (ii) mother and DED removal. We predict that aggressive interactions will be frequent within nests (Withee and Rehan 2016). Given the formerly observed division of foraging labor between mothers and DEDs versus regular adult offspring, we further predict that foraging will be reduced when mothers are removed from nests, and more so when only regular offspring are present (Mikát et al. 2017). Finally, given the previous evidence for the role of maternal care in social behavior in *C. calcarata*, we hypothesize that aggression and avoidance will increase in the absence of mothers (Rehan and Richards 2013; Arsenault et al. 2018). The aims of this study are threefold: first, to document foraging rates and all observed behaviors within nests; second, to characterize the response of colony foraging behavior to mother and DED removal; and third, to examine interactive behaviors across different social environments. Here, we present the first intranidal observations and removal experiments to determine the role of social environment on cooperation and conflict in *C. calcarata*.

Methods

Observation nests

Nests were established by distributing a common *C. calcarata* nest substrate (cut raspberry branches, *Rubus idaeus*) mounted to bamboo stakes with zip-ties in the wild around Strafford County, NH, USA (43° 08′ N, 70° 55′ W). Established nests were identified by the presence of entrance holes excavated in the broken ends of stems. Control nests (C) were left undisturbed, and mother (M) and mother and DED removals (MD) were made when these bees first left the nest to forage. Stems were mounted vertically and enclosed within boxes to reduce ambient light within nests, and eight stems were mounted per box (Fig. 1a). A transparent plastic cup with a domed top was secured to the top of each stem to create an enclosed foraging arena, and bees were provided with flowers and feeding stations containing water and/or sugar (Fig. 1b). Observation was facilitated by longitudinally opening branches and applying a plastic window to exposed nests (Fig. 1c). Foraging arenas and nest interiors were video-monitored (Sony HDR-CX240/L for exterior, Sony HDR SR-11 Hybrid Nightshot for interior) with multiple observation periods of 50 min each between 25 July and 28 August 2018. Interiors of nests were monitored using infrared capable cameras (Fig. 1d). Videos were subsequently reviewed, with observers blinded to treatment, and novel behaviors were noted and recorded along with known behaviors identified in pre-existing ethograms (Rehan and Richards, 2013; Withee and Rehan, 2016). Duration of behaviors and number of bees present during an observation were also recorded. A total of 35 nests were included in the study, 13 control (C), 13 mother removal (M), and 9 mother and DED removal (MD) nests. A total of 557, 50-min recording periods were made, over 34 days: 273 in C, 152 in M, and 132 in MD ($N = 557$; total observation time = 464 h).

Nineteen distinct behaviors were identified from the video recordings (Table 1). Behaviors were considered events and counted for each observation period, and durations were recorded. Individual behaviors (Table 1) were novel to the ethogram for this bee and defined in this study. We defined two behavioral categories for this study: (i) individual behaviors, which involved one focal bee; and (ii) interactive behaviors, which involved two bees within two body lengths of each other. Interactive behaviors were considered a single event and as durations (Table 1). Interactive behaviors were classified as aggressive, avoidant, or tolerant (Rehan and Richards 2013). Because the social role of following behavior varies between species of bee, it was considered a separate behavioral classification (Withee and Rehan 2016). After behavioral assay, all bees were killed by freezing at −20 °C.

Statistical methods

First, to understand how social behaviors changed overall within nests, a PCA including interactive behaviors by type was created using the prcomp function in base R 3.6.1 (July 5,

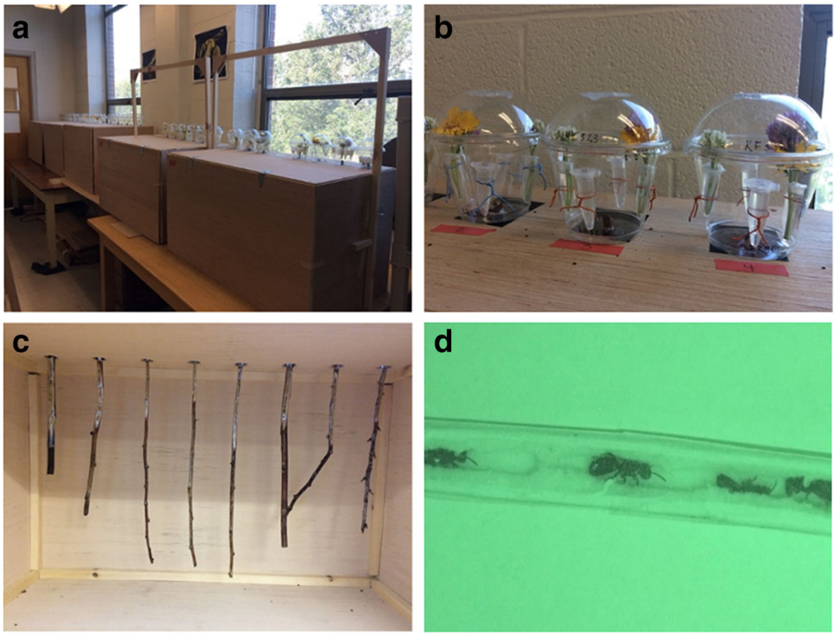


Fig. 1 Observation nests. **a** Experimental setup with boxes in which observation nests were housed and foraging arenas. **b** Foraging arenas with flowers, water, and sugar water. **c** Interior view of boxes with eight observation nests. **d** Still from infrared video showing interior of observation nest

Table 1 Ethogram of behaviors identified in study, with category of behavior and counts of behaviors observed

Category	Behavior	Description	Count
Individual	Bump	One individual bumps into another with its abdomen	376
	Cleaning	Individual collects pith, old pollen, or feces to carry outside the nest	532
	Cluster	Two or more individuals huddle together for more than a few seconds	2383
	Foraging	Active time outside of nest	261
	Grooming	Individual cleans self, may articulate abdomen	2759
	Guarding	Individual's abdomen is visible in entrance of nest, or individual pokes head/upper body outside of nest	753
	Pulsing	One individual's abdomen contracts and extends rapidly	48
	Walking	Individual moves forward away from another	193
Aggressive	Bite	On individual bites another	127
	C-posture	Aggressive posture where abdomen is bent towards second individual	39
	Nudge	One individual headbutts another individual's abdomen	7097
Avoidant	Back	Individual backs away from another	290
	Reverse	Individual reorients him/herself by turning around from another bee	399
Follow	Follow	One individual pursues another	79
Tolerant	Allogrooming	Individual(s) cleans another	361
	Antennation	In a frontal encounter, individuals stop and touch one another with antennae.	71
	Pass	One individual passes by another	279
	Dual pass	Two individuals pass each other	159
	Head to head	Two individuals sit with heads touching	181

2019). Behaviors were categorized as aggressive, avoidant, and tolerant, and averaged for each nest prior to normalization for PCA plotting using the ggbiplot function in the dplyr package (Wickham et al. 2020). To test for changes in overall activity, we built two general linear mixed models (GLMMs) with binomial distributions, with total behaviors per 50-min observation period as response variable and treatment as predictor, one with and one without the number of bees as an offset on the binomial natural log scale to account for variation in number of bees in an observation (Zuur 2009). Removal treatments were modeled as fixed effects and nests as a random factor. To test the effect of removal treatments on the response variables of 10 interactive behaviors, and four interactive behavioral categories (aggressive, avoidant, tolerant, and follow), we built two GLMMs for the frequency of each behavior or category. Treatment was modeled as a fixed effect and nests as a random factor, to account for varying number of nests subjected to each treatment, and differing numbers of repeated observations between nests. In the analyses of frequency of behaviors, frequencies of each behavior were determined as the number of a behavior in an observation. Distributions were modeled as Poisson or, to account for overdispersion, negative binomial (linear or quadratic). As the number of bees varied among observations, frequencies were modeled with the natural logarithm of number of bees in an observation as an offset, to maintain the log scale of the Poisson or negative binomial distributions. Due to the fact that all behaviors were not observed in each period, data for some behaviors were zero-inflated (Zuur 2009; Zuur and Ieno 2016). Models for frequencies and duration of foraging behavior were fit, considering frequency and durations per bee, or duration of activity in an observation, using log offsets. Distributions and choice of inclusion of a zero-inflation parameter were selected to minimize BIC values, and models were validated by visually inspecting residual plots using the package DHARMa (Zuur 2009; Hilbe 2011; Hartig 2020). All models showed good overall fit and passed Kolmogorov-Smirnov tests and tests for overdispersion (Hartig 2020).

All statistical tests were conducted in R version 3.6.1 (2019-07-05). Models were produced with the glmmTMB package (Brooks et al. 2017). Type-II Wald chi-square tests of the effect of treatment were conducted for each model. As behaviors were observed from the same nests, a Holm-Bonferroni correction was applied to an $\alpha = 0.05$ for behavioral categories ($N = 4$), as well as interactive behaviors considered frequencies and durations ($N = 10$). Adjusted P-values are presented throughout. Marginal means of each frequency per bee were estimated from models, and post hoc Tukey pairwise comparisons between treatments were conducted using the emmeans package in R (Lenth 2020).

Data availability

The datasets generated during and/or analyzed during the current study are available in the Dryad repository: https://doi.org/10.5061/dryad.np5hqbzs6.

Results

Intranidal and foraging behaviors

Across treatments, bees were most frequently engaged in individual and aggressive behaviors, followed by tolerant and then avoidant behaviors (Fig. 2). Individual behaviors were the most frequent per observation (10, 7–14; mean, 95% CI), followed by aggressive behaviors (8, 5–13). Tolerant (1.33, 0.85–2.07) and avoidant (0.58, 0.33–1.02) behaviors were much less frequent per 50-min observation period. Following was least frequent (0.069, 0.03–0.14). Frequency of overall behaviors was similarly greater than control in both removal treatments, both as raw frequencies ($\chi^2_2 = 34.145$, $N = 557$, $P < 0.0001$; Table 2) and as frequencies per bee ($\chi^2_2 = 34.495$, $N = 557$, $P < 0.0001$; Table 2).

Foraging trips were observed 0.28 times (0.14–0.56) per observation period. Frequency of foraging per bee differed significantly among treatments ($\chi^2_2 = 6.710$, $N = 557$, $P = 0.03$). Foraging trips were more frequent in control (C) treatments as compared to mother-removal treatments (M) (C > M; Tukey test: $t_{551} = 2.456$, $P = 0.0381$) and were intermediate between treatments in mother-removal and DED-removal treatments (MD). Durations of foraging also differed between treatments ($\chi^2_2 = 6.846$, $N = 557$, $P = 0.03$). Bees spent less time foraging in M treatments compared to that in C (Tukey test: M < C, $t_{552} = 2.484$, $P = 0.03$).

Social environment

The first and second PCs of our PCA accounted for 65.5% and 17.6% of variation respectively (Fig. 3). PC1 was positively correlated with all behavioral types (eigenvectors: aggressive = 0.40; tolerant = 0.52; avoidant = 0.55). PC2 was positively correlated with aggression (eigenvector = 0.89) and negatively with other types (eigenvectors: tolerant = −0.05; avoidant = −0.25; follow = −0.38). Control nests were primarily negatively associated with aggressive behaviors, whereas M and MD treatments primarily positively associated with these behaviors (Fig. 3). Control nests were also negatively associated with tolerant, avoidant, and following behaviors, whereas M and D treatment nests tended to be average or uncorrelated with these behaviors, with the exception of two M treatments nests which were strongly positively associated with them (Fig. 3).

The frequency of aggressive behaviors differed significantly among treatments ($\chi^2_2 = 8.3973$, $N = 557$, $P = 0.045$) was slightly but not significantly more frequent in M treatments than in control (Tukey test: C < M, $t_{551} = -2.318$, $P = 0.0541$), and was significantly more frequent in MD treatments (MD > C, $t_{541} = -2.686$, $P = 0.02$; Fig. 4). C-posture was more frequent in M treatment (M > C, $t_{552} = -3.168$, $P = 0.0046$; Table 3, Fig. 4).

Table 2 Total behaviors per observation period and total behaviors per bee. Contrasts are post hoc Tukey tests of treatments effects estimated from GLMM. Df = 552. Bold demarcates statistically significant results

	Contrast	Estimate	SE	*T* ratio	*P*-value
Total behaviors	**C - M**	**−2.011**	**0.4578**	**−4.392**	**< 0.0001**
	C - MD	**−2.678**	**0.4961**	**−5.398**	**< 0.0001**
	M - MD	−0.6673	0.5050	−1.294	0.3839
Total behaviors per bee	**C - M**	**−1.5343**	**0.3358**	**−4.570**	**< 0.0001**
	C - MD	**−1.9353**	**0.3627**	**−5.336**	**< 0.0001**
	M - MD	−0.4010	0.3688	−1.087	0.5224

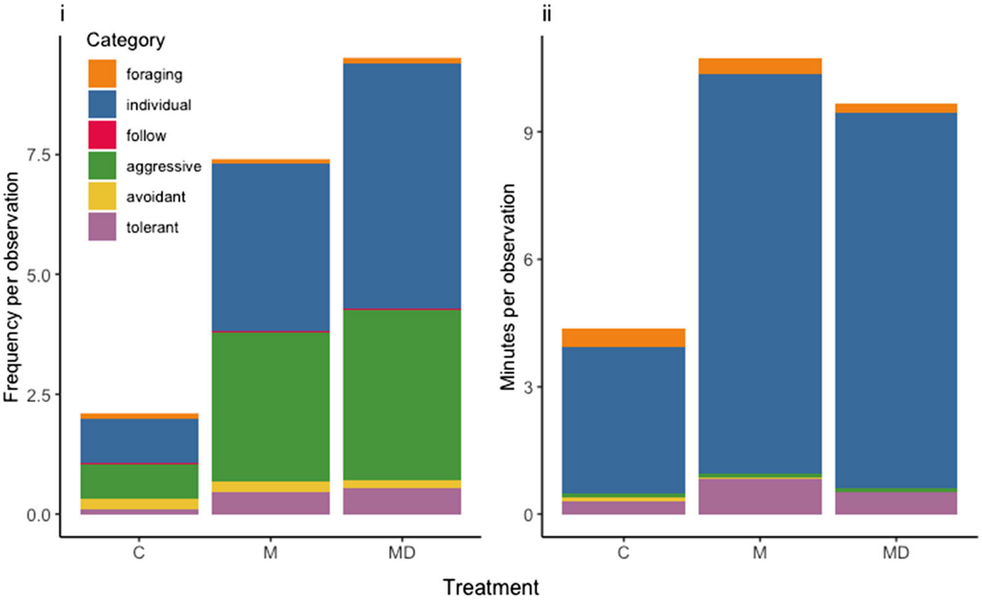


Fig. 2 Multiple bar plot of behaviors by category. **i** Mean frequency of behavior per observation per individual bee. **ii** Mean durations of behaviors per bee. C control, M mother removed, MD mother and DED removed

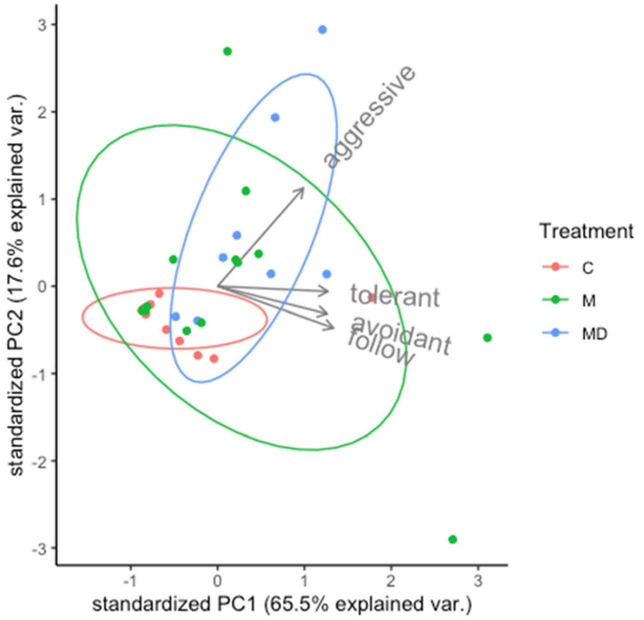


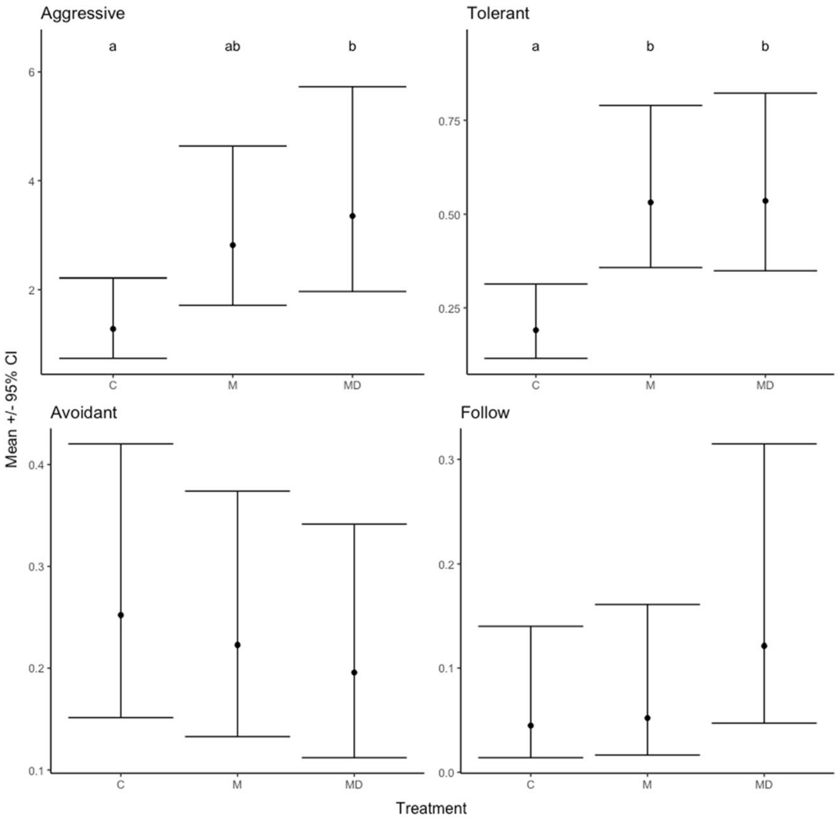
Fig. 3 PCA of social behaviors by type, aggressive, tolerant, avoidant, and follow. Each point represents an observation nest. Counts of behaviors averaged per observation for each nest. Ellipses represent 0.68 normal probabilities for each treatment

Table 3 Results of Wald type-II chi-square tests of frequencies of behaviors per individual bee. Bold demarcates statistically significant results. Df = 2

Category	Behavior	χ^2	*P*-value
Aggressive	Bite	6.560	0.1505
	C-posture	**10.41**	**0.04482**
	Nudge	8.793	0.08182
Avoidant	Backing	8.898	0.08182
	Reverse	0.7991	0.6706
Tolerant	Allogrooming	**25.00**	**< 0.0001**
	Antennation	3.344	0.3757
	Dual pass	**13.49**	**0.01061**
	Head to head	5.526	0.1893
	Passing	7.559	0.1122
Follow	Follow	2.5484	0.5594

Frequencies of tolerant behaviors also differed significantly among treatments (χ^2_2 = 14.69, *N* = 557, *P* = 0.002), being significantly more frequent in M and MD treatments (M > C, $t_{552} = -3.378$, $P = 0.0029$; MD > C, $t_{552} = -3.419$, $P = 0.002$; Fig. 4). Allogrooming and dual pass were significantly more frequent in both removal treatments (allogrooming: M > C, $t_{552} = -4.332$, $P = 0.0001$; MD > C, $t_{552} = -4.843$, $P < 0.0001$; dual pass: M > C, $t_{552} = -3.188$, $P = 0.0043$; MD > C, $t_{552} = -3.462$, $P = 0.0017$; Table 3, Figs. 4 and 5).

Fig. 4 Estimated marginal means of categories of behavior per observation per individual bee. Differing letters identify significant differences from post hoc Tukey tests. Treatments: C control, M mother removed, MD mother and DED removed



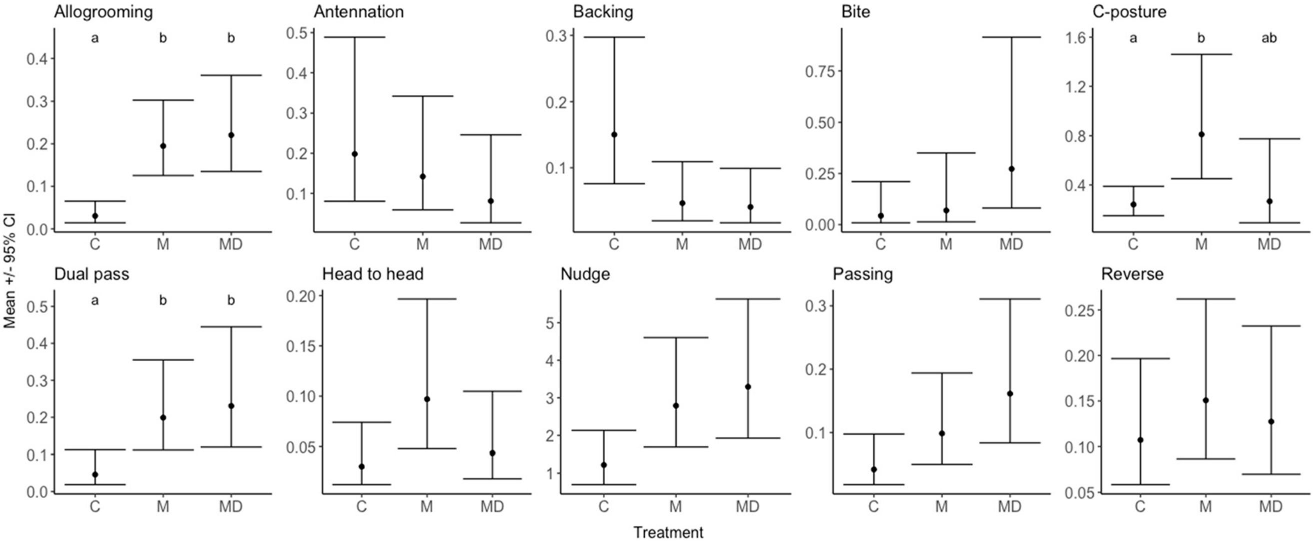


Fig. 5 Frequencies of interactive behaviors. Estimated marginal means of interactive behaviors as frequencies per individual bee, per observation. Differing letters distinguish significantly different results of pairwise comparisons of estimates from GLMM with post hoc Tukey tests. Treatments: C control, M mother removed, MD mother and DED removed

Discussion

Social environment influences intranidal behavior and foraging in adult assemblages of *C. calcarata.* Our multivariate analysis found that behavior in nests with mothers and both mothers and DED removed were associated with higher aggression. We found that overall activity within nests increased significantly in the absence of mothers. Maternal presence lowered overall tolerance within nests. As aggression was only greater in the absence of both mothers and DEDs, the presence of DEDs also plays a significant role in social behavior. Both maternal and DED presence thus appear to play a significant role in maintaining social organization within nests. Foraging behavior was also the lowest in nests lacking mothers but where DEDs were still present. This is likely explained by a lack of stimulus to forage directed from mothers to DEDs along with the quiescence of regular daughters waiting to receive provisions. In the absence of both mothers and DEDs, foraging behavior was observed in regular daughters indicating that they are capable of foraging, but this is not observed in the presence of mothers and DEDs (Rehan et al. 2014; Mikát et al. 2017).

Mothers: primary foragers and regulators of nesting activity

Our results indicate that aggressive interactions are frequent within *C. calcarata* during the social nesting period (44% of observed behaviors). Aggressive behaviors were more frequent than the individual behaviors, such as cleaning and grooming, that comprise the next most frequent activity within nests (43% of observed behaviors). Aggression thus appears to be a regular part of social nesting behavior, confirming previous observations that aggression is directed towards nestmates (Rehan and Richards 2013). This could support the existence of a dominance hierarchy based on aggression within *C. calcarata.* Such dominance hierarchies are typical of species exhibiting simple cooperative behaviors. Foraging helpers of cooperatively breeding pied kingfishers and superb fairy-wrens are frequently the recipients of aggressive behavior from their breeding parents (Reyer et al. 1986; Mulder and Langmore 1993). Within Hymenoptera alloparental care, reproductive roles and dominance hierarchies are frequently determined through aggressive interactions (West 1967; West-Eberhard 1969; Michener and Brothers 1974; Gadagkar 1980; Chandrashekara and Gadagkar 1991; Wcislo 1997; Arneson and Wcislo 2003; Jandt et al. 2014). Dominance hierarchies in the polistine paper wasps, established through repeated aggressive interactions, determine reproductive or foraging and nest maintenance roles (Pardi 1948; West-Eberhard 1969; Chandrashekara and Gadagkar 1991), and aggression is similarly correlated with division of labor in some primitively eusocial halictine sweat bees (Michener 1990). Within these species, foraging and nest-building females are frequently smaller, and are the recipients of aggressive nudging and backing from their mothers, the primary egg-layers and reproductive dominant in nests (Michener and Brothers 1974; Greenberg and Buckle 1981; Buckle 1982; Michener 1990; Boomsma and Eickwort 1993; Richards and Packer 1994). Within the *Polistes*, paper wasps subordinate co-foundresses are the primary foragers, and dominant individuals are more likely to conduct low-risk tasks, such as collecting nest materials (West-Eberhard 1969; Gamboa et al. 1978; Reeve and Gamboa 1987; Pratte 1989). Given that unmarked colony members were observed in this study, further research tracking individuals is a necessary next step. Such observations would further test previous finding from circle tube assays that aggression is typically directed from mothers to daughters (Rehan and Richards 2013).

The overall increase in activity under both removal treatments suggests that behavior within nests undergoes reorganization in the absence of mothers, not significantly mitigated by the presence of DEDs. Given this dramatic change, it is unlikely that DEDs substitute for mothers in the overall stability of colony functioning. Within *Ropalidia* paper wasps, removal of dominant individuals results in temporary loss of distinguishable behavioral roles, including reproductive and foraging distinctions (Chandrashekara and Gadagkar 1991). In the absence of both mothers and DEDs, foraging by regular daughters for supplemental provisions represents a significant risk, as well as an energetic cost for orphaned *C. calcarata* females. The dramatic change in overall colony behavior in the absence of mothers, and the increase in aggression in the absence of mothers and DEDs, may be the result of conflict over foraging roles among size-matched siblings (Smith and Parker 1976). While previous studies suggest that DEDs are both the primary forager in the absence of mothers, and that they forage as frequently as mothers, these results suggest that ordinary siblings may contribute to intranidal activities and the overall social environment that may also be important to fitness in the absence of mothers (Mikát et al. 2017).

Mothers reduce intranidal aggression and mutual tolerance

Higher aggression in the absence of mothers and DEDs suggests both maternal and alloparental care from DEDs reduce aggression between offspring. This supports prior studies that found daughters from nests retaining mothers show negligible aggressive interactions (Rehan and Richards 2013) and increased aggression between unfamiliar females from orphaned nests (Arsenault et al. 2018). This pattern of aggression from mothers resulting in reduced aggression from offspring suggests that there is a rudimentary dominance heirarchy between mothers and offspring in *C. calcarata* (Breed et al. 1978; Wcislo 1997). Dominance hierarchies result when repeated aggressive encounters establish dominant winners and subordinant losers (Smith and Parker 1976; Rutte et al. 2006). Subordinant individuals are less likely to escalate conflicts, and thus, an overall decrease in aggression may be observed when a hierarchy exists (Hemelrijk 2000). Within primitively eusocial sweat bees, aggression of reproductively dominant individuals, frequently foundresses, towards nestmates is thought to orchestrate intranidal tasks, such as nest construction (Brothers and Michener 1974; Michener and Brothers 1974; Michener 1990). In other closely related species of *Ceratina*, *C. japonica* and *C. flavipes*, smaller individuals engage in foraging, while larger individuals are primarily occupied with nest guarding and reproduction (Sakagami and Maeta 1989; Sakagami et al. 1993). Maternal aggression in *C. calcarata* is thus most likely a mechanism by which DEDs are ejected from nests to conduct foraging (Rehan et al. 2014).

Comparable levels of aggression between nests with mothers and those with only DEDs may not be explained by DEDs assuming a dominant role, as they are the smallest individual in the nest (Hogendoorn and Velthuis 1999; Smith et al. 2009; Rehan and Richards 2013). Production of smaller, more easily dominated offspring through manipulation of pollen mass provisions and developmental nutrition is thought to be a method of ensuring subordinance in many incipiently and primitively eusocial Hymenoptera (Hogendoorn and Velthuis 1999; Smith et al. 2009; Kapheim et al. 2011; Rehan and Richards 2013). Examples of such size-based divisions of labor are not restricted to invertebrates (Reeve 1992). Dominants within naked mole-rat colonies subject larger subordinates to more frequent shoving, eliciting increased foraging (Reeve 1992). When dominant individuals are removed from nests of naked mole-rats, smaller individuals typically perform most of the work within nests, foraging and expanding nests (Reeve 1992). Such size-related dominance interactions are particularly common to social Hymenoptera. Within primitively eusocial sweat bees, body size is frequently predictive of dominance and division of labor (Packer 1986; Kukuk and May 1991; Boomsma and Eickwort 1993). Similarly, within eusocial paper wasps, size frequently predicts competitive ability and worker versus reproductive roles (Dropkin and Gamboa 1981; Sullivan and Strassmann 1984; Tibbetts and Sheehan 2012). Higher aggression in the absence of mothers and DEDs may thus result from escalated conflict among more evenly size-matched individuals (Smith and Parker 1976). Such intensified conflicts in size symmetrical individuals are found in both vertebrates in invertebrates (Enquist et al. 1987; Smith et al. 1994; Pratt et al. 2003).

Size, however, is not always associated with dominance in contests between female *C. calcarata*, with experience being the decisive factor after repeated encounters (Withee and Rehan 2016). During extended cohabitation, experience is thus likely to influence the outcome of conflicts between similarly sized individuals (Withee and Rehan 2016). DEDs are not only the smallest daughter but also the eldest daughter. Within the related allodapine bee, *Exoneura robusta*, size is only weakly associated with dominance, with the eldest individuals typically assuming this role (Schwarz and Woods 1994). Aside from the influence of size and age, individual variation in sensitivity, e.g., to deprivation of supplementary provisions, may result in differential levels of foraging and potential food sharing among regular daughters (Calabi 1988; Page and Robinson 1991). Such variation, differences in response thresholds, may be influenced by social environment within solitary sweat bees, resulting in a rudimentary division of labor among otherwise physiologically similar individuals (Jeanson et al. 2008). While increased aggression in the absence of mothers and DEDs implies conflict over division of labor, the degree to which regular daughters share food is not clear. In the absence of mothers and DEDs, age, experience, and individual response thresholds likely influence foraging and food sharing among regular siblings. Examples of food sharing between siblings within diverse taxa are common. For example, in the absence of maternal provisioning, food sharing between siblings increases in earwigs (Kramer et al. 2015). It is thus possible that regular, non-dwarf, siblings may be able to maintain their own inclusive fitness through food sharing while contributing to other tasks within the nest, such as nest guarding and cleaning.

Reduced mutual tolerance between nestmates in the presence of mothers may also result from an established social hierarchy. Within primitively eusocial sweat bees, subordinate individuals are more avoidant of dominant individuals, responding to forced encounters with characteristic reversal or withdrawal behaviors (Buckle 1982, 1985; Wcislo 1997). After repeated encounters between females, avoidant behavior decreases in *C. calcarata* (Withee and Rehan 2016); thus, decreased avoidance in removal treatments could be the result of increased overall activity and resultant repeated interactions (Withee and Rehan, 2016). Greater tolerance within removal treatments is consistent with an overall difference in social structure within nests lacking mothers. It is also possible that in the absence of mothers, who typically prevent intruders and nest usurpation by guarding nests, nestmate recognition through chemical cues may facilitate increased tolerance (Rehan and Richards 2010).

Together, our results suggest that aggressive behaviors are frequent within nests and function to maintain division of labor among age and size classes, much as in group living vertebrates, in particular birds and mammals (Reyer et al. 1986; Creel et al. 1992; Reeve 1992; Mulder and Langmore 1993). The role of aggression in adult nests of *C. calcarata* is also similar to that commonly found in primitively eusocial paper wasps and sweat bees (West-Eberhard 1969; Brothers and Michener 1974; Michener and Brothers 1974; Gamboa et al. 1978; Buckle 1982, 1985; Reeve and Gamboa 1987; Pratte 1989; Michener 1990). Behavior within *C. calcarata* is highly plastic, and through removal experiments, we have shown that group dynamics in this species is sensitive to changes in social environment. How foraging and pre-overwintering feeding is negotiated among regular daughters in the absence of provisioning from both mothers and DEDs remains to be investigated. In particular, further study could focus on how individual variation in response thresholds, age, and experience may result in differential foraging and, potentially, sibling food sharing (i.e., trophallaxis) (Calabi 1988; Breed and Page 1989; Page and Robinson 1991; Fewell and Page 2000). Such threshold responses may explain allocation of reproductive division of labor found among adult *Ceratina flavipes* during forced cohabitation (Sakagami and Maeta 1987). Understanding how changes in social environment interact with individual differences to affect cooperation and conflict, and how group cohesion is maintained and modulated, is critical to understanding why some individuals cooperate and others do not.

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References

Arneson L, Wcislo WT (2003) Dominant-subordinate relationships in a facultatively social, nocturnal bee, *Megalopta genalis* (Hymenoptera: Halictidae). J Kansas Entomol Soc:183–193

Arsenault SV, Brendan HG, Rehan SM (2018) The effect of maternal care on gene expression and DNA methylation in a subsocial bee. Nat Commun 9:1–9. https://doi.org/10.1038/s41467-018-05903-0

Boomsma JJ, Eickwort GC (1993) Colony structure, provisioning and sex allocation in the sweat bee *Halictus ligatus* (Hymenoptera: Halictidae). Biol J Linn Soc 48:355–377. https://doi.org/10.1111/j.1095-8312.1993.tb02097.x

Breed MD, Page RE Jr (eds) (1989) Genetic basis for division of labor in an insect society. In: The genetics of social evolution. Westview Press, Boulder, pp 61–80

Breed MD, Silverman JM, Bell WJ (1978) Agonistic behavior, social interactions, and behavioral specialization in a primitively eusocial bee. Insect Soc 25:351–364. https://doi.org/10.1007/BF02224299

Brooks ME, Kristensen K, van Benthem KJ et al (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J 9:378–400

Brothers DJ, Michener CD (1974) Interactions in colonies of primitively social bees: III. Ethometry of division of labor in *Lasioglossum zephyrum* (Hymenoptera: Halictidae). J Comp Physiol 90:129–168. https://doi.org/10.1007/BF00694482

Buckle GR (1982) Queen-worker behavior and nestmate interactions in young colonies of *Lasioglossum Zephyrum*. Insect Soc 29:125–137. https://doi.org/10.1007/BF02228746

Buckle GR (1985) Increased queen-like behaviour of workers in large colonies of the sweat bee *Lasioglossum zephyrum*. Anim Behav 33:1275–1280. https://doi.org/10.1016/S0003-3472(85)80187-1

Calabi P (1988) Behavioral flexibility in Hymenoptera: a re-examination of the concept of caste. Advances in Myrmecology. E.J. Brill, New York, pp 237–258

Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, Meaney MJ (1998) Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. Proc Natl Acad Sci U S A 95:5335–5340. https://doi.org/10.1073/pnas.95.9.5335

Cameron SA, Jost MC (1998) Mediators of dominance and reproductive success among queens in the cyclically polygynous Neotropical bumble bee *Bombus atratus* Franklin. Insect Soc 45:135–149. https://doi.org/10.1007/s000400050075

Chandrashekara K, Gadagkar R (1991) Behavioural castes, dominance and division of labour in a primitively eusocial wasp. Ethology 87: 269–283. https://doi.org/10.1111/j.1439-0310.1991.tb00252.x

Costa JT (2006) The other insect societies. Belknap Press of Harvard University Press. Mass, Cambridge

Creel S, Creel N, Wildt DE, Monfort SL (1992) Behavioural and endocrine mechanisms of reproductive suppression in Serengeti dwarf mongooses. Anim Behav 43:231–245. https://doi.org/10.1016/S0003-3472(05)80219-2

Dropkin JA, Gamboa GJ (1981) Physical comparisons of foundresses of the paper wasp, *Polistes metricus* (Hymenoptera: Vespidae). Can Entomol 113:457–461. https://doi.org/10.4039/Ent113457-6

Emlen ST (1984) Cooperative breeding in birds and mammals. In: Krebs JR, Davies NB (eds) Behavioural ecology: an evolutionary approach, 2nd edn. Blackwell Science, Cambridge, pp 305–339

Enquist M, Ljungberg T, Zandor A (1987) Visual assessment of fighting ability in the cichlid fish *Nannacara anomala*. Anim Behav 35: 1262–1264. https://doi.org/10.1016/S0003-3472(87)80189-6

Fewell JH, Page RE (2000) Colony-level selection effects on individual and colony foraging task performance in honeybees, *Apis mellifera L*. Behav Ecol Sociobiol 48:173–181. https://doi.org/10.1007/s002650000183

Fish EW, Shahrokh D, Bagot R et al (2006) Epigenetic programming of stress responses through variations in maternal care. Ann N Y Acad Sci 1036:167–180. https://doi.org/10.1196/annals.1330.011

Gadagkar R (1980) Dominance hierarchy and division of labour in the social wasp *Ropalida Marginata (Lep.)*. Curr Sci India 772–775

Gamboa GJ, Heacock BD, Wiltjer SL (1978) Division of labor and subordinate longevity in foundress associations of the paper wasp, *Polistes metricus* (Hymenoptera: Vespidae). J Kansas Entomol Soc 343–352

Greenberg L, Buckle GR (1981) Inhibition of worker mating by queens in a sweat bee, *Lasioglossum zephyrum*. Insect Soc 28:347–352. https://doi.org/10.1007/BF02224192

Hartig F (2020) Residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.3.3.0. http://florianhartig.github.io/DHARMa/. Accessed 8 Jan 2021

Hemelrijk CK (2000) Towards the integration of social dominance and spatial structure. Anim Behav 59:1035–1048. https://doi.org/10.1006/anbe.2000.1400

Hilbe JM (2011) Negative binomial regression. Cambridge University Press. Cambridge University Press, Cambridge, UK

Hogendoorn K, Velthuis HHW (1999) Task allocation and reproductive skew in social mass provisioning carpenter bees in relation to age and size. Insect Soc 46:198–207. https://doi.org/10.1007/s000400050135

Jandt JM, Tibbetts EA, Toth AL (2014) *Polistes* paper wasps: a model genus for the study of social dominance hierarchies. Insect Soc 61: 11–27. https://doi.org/10.1007/s00040-013-0328-0

Jeanson R, Clark RM, Holbrook CT et al (2008) Division of labour and socially induced changes in response thresholds in associations of solitary halictine bees. Anim Behav 76:593–602. https://doi.org/10.1016/j.anbehav.2008.04.007

Johnson MD (1988) The relationship of provision weight to adult weight and sex ratio in the solitary bee, *Ceratina calcarata*. Ecol Entomol 13:165–170. https://doi.org/10.1111/j.1365-2311.1988.tb00344.x

Kapheim KM, Bernal SP, Smith AR, Nonacs P, Wcislo WT (2011) Support for maternal manipulation of developmental nutrition in a facultatively eusocial bee, *Megalopta genalis* (Halictidae). Behav Ecol Sociobiol 65:1179–1190. https://doi.org/10.1007/s00265-010-1131-9

Koenig WD, Dickinson JL (2004) Ecology and evolution of cooperative breeding in birds. Cambridge University Press, Cambridge

Kramer J, Thesing J, Meunier J (2015) Negative association between parental care and sibling cooperation in earwigs: a new perspective on the early evolution of family life? J Evol Biol 28:1299–1308. https://doi.org/10.1111/jeb.12655

Kukuk P, May B (1991) Colony dynamics in a primitively eusocial halictine bee *Lasioglossum (Dialictus) zephyrum* (Hymenoptera: Halictidae). Insect Soc 38:171–188. https://doi.org/10.1007/BF01240967

Lenth R (2020) Emmeans: estimated marginal means, aka least-squares means. https://CRAN.R-project.org/package=emmeans. Accessed 8 Jan 2021

Michener CD (1974) The social behaviour of the bees: a comparative study. Harvard University Press, Cambridge

Michener CD (1990) Reproduction and castes in social halictine bees. In: Social insects: an evolutionary approach to castes and reproduction. Springer, Berlin, pp 77–121

Michener CD, Brothers DJ (1974) Were workers of eusocial Hymenoptera initially altruistic or oppressed? Proc Natl Acad Sci U S A 71:671–674. https://doi.org/10.1073/pnas.71.3.671

Mikát M, Černá K, Straka J (2016) Major benefits of guarding behavior in subsocial bees: implications for social evolution. Ecol Evol 6:6784–6797. https://doi.org/10.1002/ece3.2387

Mikát M, Franchino C, Rehan SM (2017) Sociodemographic variation in foraging behavior and the adaptive significance of worker production in the facultatively social small carpenter bee, *Ceratina calcarata*. Behav Ecol Sociobiol 71:135. https://doi.org/10.1007/s00265-017-2365-6

Mikát M, Janošík L, Černá K, Matoušková E, Hadrava J, Bureš V, Straka J (2019) Polyandrous bee provides extended offspring care biparentally as an alternative to monandry based eusociality. Proc Natl Acad Sci U S A 116:6238–6243. https://doi.org/10.1073/pnas.1810092116

Mulder RA, Langmore NE (1993) Dominant males punish helpers for temporary defection in superb fairy-wrens. Anim Behav 45:830–833. https://doi.org/10.1006/anbe.1993.1100

Packer L (1986) Multiple-foundress associations in a temperate population of *Halictus ligatus* (Hymenoptera; Halictidae). Can J Zool 64: 2325–2332. https://doi.org/10.1139/z86-346

Packer L (2006) Use of artificial arenas to predict the social organisation of halictine bees: data for fourteen species from Chile. Insect Soc 53: 307–315. https://doi.org/10.1007/s00040-006-0873-x

Page RE, Robinson GE (1991) The genetics of division of labour in honey bee colonies. Advances in insect physiology. Elsevier, In, pp 117–169

Pardi L (1948) Dominance order in *Polistes* wasps. Physiol Zool 21:1–13. https://doi.org/10.1086/physzool.21.1.30151976

Pratt AE, McLain DK, Lathrop GR (2003) The assessment game in sand fiddler crab contests for breeding burrows. Anim Behav 65:945–955. https://doi.org/10.1006/anbe.2003.2152

Pratte M (1989) Foundress association in the paper wasp *Polistes dominulus* Christ. (Hymen. Vesp.). Effects of dominance hierarchy on the division of labour. Behaviour 208–219. https://doi.org/10.1163/156853989X00664

Reeve HK (1992) Queen activation of lazy workers in colonies of the eusocial naked mole-rat. Nature 358:147–149. https://doi.org/10.1038/358147a0

Reeve HK, Gamboa GJ (1987) Queen regulation of worker foraging in paper wasps: a social feedback control system (*Polistes fuscatus,* Hymenoptera: Vespidae). Behaviour 102:147–167. https://doi.org/10.1163/156853986X00090

Rehan SM, Berens AJ, Toth AL (2014) At the brink of eusociality: transcriptomic correlates of worker behaviour in a small carpenter bee. BMC Evol Biol 14:260. https://doi.org/10.1186/s12862-014-0260-6

Rehan SM, Richards MH (2010) Nesting biology and subsociality in *Ceratina calcarata* (Hymenoptera: Apidae). Can Entomol 142:65–74. https://doi.org/10.4039/n09-056

Rehan SM, Richards MH (2013) Reproductive aggression and nestmate recognition in a subsocial bee. Anim Behav 85:733–741. https://doi.org/10.1016/j.anbehav.2013.01.010

Reyer H-U, Dittami JP, Hall MR (1986) Avian helpers at the nest: are they psychologically castrated? Ethology 71:216–228. https://doi.org/10.1111/j.1439-0310.1986.tb00585.x

Richards MH, Packer L (1994) Trophic aspects of caste determination in *Halictus ligatus,* a primitively eusocial sweat bee. Behav Ecol Sociobiol 34:385–391. https://doi.org/10.1007/BF00167329

Rutte C, Taborsky M, Brinkhof MWG (2006) What sets the odds of winning and losing? Trends Ecol Evol 21:16–21. https://doi.org/10.1016/j.tree.2005.10.014

Sakagami SF (1977) Some presumably presocial habits of Japanese *Ceratina* bees, with notes on various social types in Hymenoptera. Insect Soc 24:319–343. https://doi.org/10.1007/BF02223784

Sakagami SF, Maeta Y (1987) Sociality, induced and/or natural, in the basically solitary small carpenter bees *(Ceratina).* In: Ito Y, Brown J, Kikkawa J (eds) Animal societies: theories and facts. Japanese Scientific Societies Press, pp 1–16

Sakagami SF, Maeta Y (1989) Compatibility and incompatibility of solitary life with eusociality in two normally solitary bees *Ceratina japonica* and *Ceratina okinawana* (Hymenoptera, Apoidea), with notes on the incipient phase of eusociality. Jpn J Entomol 57:417–439

Sakagami SF, Maeta Y, Saito K, Hyodo K (1993) Diapause and non-delayed eusociality in a univoltine and basically solitary bee, *Ceratina japonica* (Hymenoptera, Anthophoridae): I. Diapause Termination by cooling and application of juvenile hormone analog. Jpn J Entomol 61:443–457

Schwarz MP, Woods RE (1994) Order of adult eclosion is a major determinant of reproductive dominance in the allodapine bee *Exoneura bicolor*. Anim Behav 47:373–378. https://doi.org/10.1006/anbe.1994.1051

Shell WA, Rehan SM (2018) The price of insurance: costs and benefits of worker production in a facultatively social bee. Behav Ecol 29:204–211. https://doi.org/10.1093/beheco/arx146

Smith IP, Huntingford FA, Atkinson RJA, Taylor AC (1994) Strategic decisions during agonistic behaviour in the velvet swimming crab, *Necora puber* (L.). Anim Behav 47:885–894. https://doi.org/10.1006/anbe.1994.1120

Smith AR, Kapheim KM, O'Donnell S, Wcislo WT (2009) Social competition but not subfertility leads to a division of labour in the facultatively social sweat bee *Megalopta genalis* (Hymenoptera: Halictidae). Anim Behav 78:1043–1050. https://doi.org/10.1016/j.anbehav.2009.06.032

Smith JM, Parker GA (1976) The logic of asymmetric contests. Anim Behav 24:159–175. https://doi.org/10.1016/S0003-3472(76)80110-8

Stacey PB, Koenig WD (1992) Stacey, P. B. and Koenig, W. D. 1990. Cooperative breeding In Birds: long term studies of ecology and behaviour. Cambridge University Press, Oxford, UK

Sullivan J, Strassmann J (1984) Physical variability among nest foundresses in the polygynous social wasp, *Polistes annularis*. Behav Ecol Sociobiol 15:249–256. https://doi.org/10.1007/BF00292986

Taborsky M (1984) Broodcare helpers in the cichlid fish *Lamprologus brichardi:* their costs and benefits. Anim Behav 32:1236–1252. https://doi.org/10.1016/S0003-3472(84)80241-9

Tallamy DW, Wood TK (1986) Convergence patterns in subsocial insects. Annu Rev Entomol 31:369–390. https://doi.org/10.1146/annurev.en.31.010186.002101

Tibbetts EA, Sheehan MJ (2012) The effect of juvenile hormone on *Polistes* wasp fertility varies with cooperative behavior. Horm Behav 61:559–564. https://doi.org/10.1016/j.yhbeh.2012.02.002

Wcislo WT (1997) Social interactions and behavioral context in a largely solitary bee, *Lasioglossum (Dialictus) figueresi* (Hymenoptera, Halictidae). Insect Soc 44:199–208. https://doi.org/10.1007/s000400050041

West MJ (1967) Foundress associations in polistine wasps: dominance hierarchies and the evolution of social behavior. Science 157:1584–1585. https://doi.org/10.1126/science.157.3796.1584

West-Eberhard MJ (1969) The social biology of polistine wasps. Museum of Zoology, University of Michigan, Michigan

Widowski TM, Ziegler TE, Elowson AM, Snowdon CT (1990) The role of males in the stimulation of reproductive function in female cotton-top tamarins, *Saguinus o. oedipus*. Anim Behav 40:731–741. https://doi.org/10.1016/S0003-3472(05)80702-X

Wickham H, Romain F, Lionel H, Kirill Müller (2020) dplyr: A grammar of data manipulation. R package version 0.8.4. https://CRAN.Rproject.org/package=dplyr. Accessed 8 Jan 2021

Wilson EO (1971) The insect societies. Belknap Press of Harvard University Press. Mass, Cambridge

Withee JR, Rehan SM (2016) Cumulative effects of body size and social experience on aggressive behaviour in a subsocial bee. Behaviour 153:1365–1385. https://doi.org/10.1163/1568539X-00003382

Zuur AF (2009) Mixed effects models and extensions in ecology with R. Springer, New York

Zuur AF, Ieno EN (2016) A protocol for conducting and presenting results of regression-type analyses. Methods Ecol Evol 7:636–645. https://doi.org/10.1111/2041-210X.12577