## Original Article

# The price of insurance: costs and benefits of worker production in a facultatively social bee

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Kin selection theory is foundational in helping to explain the evolution of sociality; however, the degree to which indirect fitness benefits may underlie helping behavior in species of early stage sociality has received relatively little empirical attention. Facultatively social bees, which demonstrate multiple forms of social organization, provide prime systems in which to empirically test hypotheses regarding the evolutionary origins of sociality. The subsocial small carpenter bee, *Ceratina calcarata*, may establish a social nest by manipulating brood provisions to rear a worker daughter, which then assists in critical late-season alloparental care. Here, we combine nest demographic and behavioral data with genetic relatedness estimates to calculate the relative inclusive fitness of both subsocial and social reproductive strategies in *C. calcarata*. Social mothers benefit from improved likelihood of brood survivorship and have higher fitness than subsocial mothers. Worker daughters have low indirect fitness on average, and will not produce their own offspring. Among-sibling relatedness is significantly higher in social nests than subsocial nests, though mothers of either reproductive strategy may mate multiply. Though this study corroborates the ultimate role of indirect fitness and assured fitness returns in the evolution of social traits, it also offers additional support for maternal manipulation as the proximate mechanism underlying evolutionary transitions in early stage insect societies.

*Key words*: **assured fitness returns, *Ceratina*, facultative sociality, inclusive fitness, maternal manipulation, Social evolution.**

## INTRODUCTION

Eusociality is one of the most complex forms of social organization in nature (Wilson 1971). Although eusocial organisms are represented by a diverse suite of taxa (e.g., naked mole rats, Jarvis 1981; thrips, Crespi 1992; shrimp, Duffy 1996; termites, Thorne 1997), Hymenoptera collectively contain more eusocial species than any other group (Wilson 1971). Obligately eusocial bees (e.g., *Apis mellifera*) demonstrate complex reproductive division of labor. Each individual's role within the colony is irreversibly determined during development, and a reproductive queen's lifetime fitness depends on the collective effort of thousands of sterile workers (Wilson 1971; Wilson and Hölldobler 2005; Michener 2007). Despite their established ecological dominance, however, eusocial bees represent relatively few species. Most of the more than 20,000 bee species worldwide are solitary (Michener 2007) and the remainder demonstrate forms of noneusocial organization (Rehan and Toth 2015). Solitary nesting is ancestral in bees, but evidence suggests that lineages may undergo continuous evolutionary gains or losses in their social complexity (Szathmáry and Smith 1995; Danforth 2002; Rehan and Toth 2015). The evolutionary origins of an obligate and sterile worker caste

thus appear paradoxical: why would an individual sacrifice its direct fitness to assist in rearing another's offspring? Further, how might such a seemingly altruistic behavioral phenotype be selectively reinforced?

Inclusive fitness theory suggests that indirect fitness benefits to the altruist may be enough to account for the origin and elaboration of the advanced eusocial worker caste (Hamilton 1964; West-Eberhard 1975; Trivers and Hare 1976; Foster et al. 2006). As formalized by Hamilton (1964), if an altruist's helping behavior were to contribute to the direct fitness of close genetic relatives, its indirect fitness gains could outweigh the incurred costs of forgoing some or all of its own reproduction. Kin selection is thus considered a plausible explanation for the evolution of sociality in Hymenoptera, in which female siblings are expected to share significantly more of their genetic identity with each other compared to their mother or brothers (Hamilton 1972; Lin and Michener 1972). Though the multiple mating of advanced eusocial species appears to confound these expectations by reducing among-sibling relatedness (Palmer and Oldroyd 2000), monandry is thought to be ancestral to Hymenoptera and suggests indirect fitness could have facilitated the emergence of early stage social traits (Hughes et al. 2008).

The biological applicability of kin selection has been the subject of heated debate within the field of social evolution (West-Eberhard 1975; Wilson 2005; Gadagkar 2010; Nowak et al. 2010; Marshall 2011). While the maintenance and elaboration of

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social traits by inclusive fitness remains a widely accepted theory (Foster et al. 2006; Hughes et al. 2008; Bourke 2011; Quiñones and Pen 2017), relevant examinations within early stage social systems remain limited (Leadbeater et al. 2011; Rehan, Richards, et al. 2014; Kapheim et al. 2015). Similarly, though many insights into the evolution of sociality have been gained through the study of advanced eusocial taxa, reproductive divisions of labor are often obligate in such species, and provide only inferential insights regarding their evolutionary origins (Queller and Strassmann 1998; Wilson and Hölldobler 2005; Toth et al. 2007). Facultatively social species, by contrast, demonstrate a capacity for multiple degrees of social organization, and exhibit at least 2 distinct nesting phenotypes (e.g., solitary and eusocial nesting). Accordingly, such species provide a unique opportunity to empirically investigate whether theoretically predicted mechanisms of evolution have a biologically realized influence on gains or losses in social complexity across the social spectrum (Rehan and Toth 2015; Shell and Rehan 2017a; Toth and Rehan 2017; Quiñones and Pen 2017).

Divisions of labor among Hymenoptera are frequently based on differences in age or body size. For instance, larger and older daughters in *Polistes* paper wasp colonies are more likely to assert reproductive dominance than their smaller or younger siblings if given a viable opportunity to do so (Hughes and Strassmann 1988; reviewed in Jandt et al. 2014). Among facultatively eusocial bees, foundresses are the eldest in the family unit, and often able to manipulate the sex, size, and behavior of their offspring through selective fertilization, provision investment, and physical coercion, respectively (Sakagami and Maeta 1984; Yanega 1989; Aneson and Wcislo 2003; Smith et al. 2003; Rehan and Richards 2010b; Kapheim et al. 2011, 2015). In this way, mothers maximize their reproductive investment while minimizing potential conflict or competition from their offspring. Maternal manipulation of brood has consequently been proposed as a proximate mechanism of early divisions of labor across multiple lineages (Alexander 1974; Craig 1979; Ratnieks and Wenseleers 2008), and evidence shows that, in combination with high intracolonial relatedness, it likely reinforces early stage social organization (Crespi and Ragsdale 2000; Richards et al. 2005; Kapheim et al. 2015).

Cost-benefit analyses are an effective means of evaluating different life histories (Hamilton 1964; Trivers 1971) and have been applied in facultatively social bees to compare the relative fitness of alternative reproductive strategies (Augusto and Garófalo 2004; Pech et al. 2008; Rehan, Richards, et al. 2014; Kapheim et al. 2015). Where brood loss to predation or parasitism may represent a significant natural problem for solitary nests, social nesting may provide a means of increasing fitness through improved resistance to these pressures (Rehan et al. 2011; Yagi and Hasegawa 2012; Rehan, Richards, et al. 2014). For example, social nests of the facultatively eusocial sweat bee, *Lasioglossum baleicum*, were better able to preserve developing larvae compared to solitary nests when under pressure from ant predation (Yagi and Hasegawa 2012). Consequently, higher fitness for social reproductive females and their sterile first-brood workers compared to solitary conspecifics supported the role of inclusive fitness in maintaining social traits (Yagi and Hasegawa 2012). Inclusive fitness benefits also likely contribute to the maintenance of division of labor in facultatively social orchid bees (Pech et al. 2008). However, persistent agonistic interactions among siblings or between a mother and her brood indicate that social behavior

may be prompted by physical aggression rather than sibling relatedness in this group (Augusto and Garófalo 2004; Pech et al. 2008). Taken together, studies suggest that while ecological pressures and genetic identity among family groups likely help to maintain and even reinforce social traits through inclusive fitness, 1) social nesting may not be an advantageous strategy for all individuals involved; and 2) physical and/or aggressive interactions among nestmates may be required to elicit and maintain sib-social care behaviors (Ratnieks and Wenseleers 2008). Cost-benefit analyses of facultatively social species are few, yet critical to understanding the underlying mechanisms for social organization in early stage societies.

The small carpenter bee, *Ceratina calcarata* demonstrates a form of facultative incipient sociality across its range in eastern North America (Rehan and Sheffield 2011; Shell and Rehan 2016a) where it produces one brood per year (Johnson 1988; Rehan and Richards 2010b). All reproductively active female *C. calcarata* nest subsocially by providing extended parental care for their maturing brood. Some mothers, however, establish social nests by producing a worker daughter to assist with late-season brood feeding and defense (Figure 1; Rehan, Berens et al. 2014; Lawson et al. 2016). Maternal manipulation is thought to play an important role in *C. calcarata*'s nesting biology (Rehan and Richards 2013). Specifically, a reproductive female can choose whether to fertilize her eggs and, with carefully controlled pollen provisioning, can determine both the sex and body size of her developing brood (Rehan, Richards, et al. 2014; Lawson et al. 2016). Socially nesting mothers initiate their nest by provisioning a fertilized egg with a relatively small amount of pollen (Lawson et al. 2016), which subsequently develops into a dwarf eldest daughter (Rehan and Richards 2010b). As differences in body size govern dominance hierarchies in *C. calcarata*, this particular daughter's small stature at adulthood is thought to facilitate her mother's ability to coerce her into a worker role (Rehan and Richards 2013; Rehan, Berens, et al. 2014; Withee and Rehan 2016).

Adult *C. calcarata* offspring must be fed in late summer to ensure their overwintering survival (Durant et al. 2016; Lewis and Richards 2017; Mikát et al. 2017). It is during this late summer feeding that the worker daughter may contribute to her siblings' survivorship by acting as a secondary forager: either gathering pollen and nectar alongside her mother, or acting as the sole forager in orphaned nests (Figure 1; Rehan, Berens, et al. 2014). Outside of its social context, the worker daughter's diminutive size effectively negates her chances of surviving the winter season to reproduce in the following year (Rehan and Richards 2010b; Mikát et al. 2017). Thus, though the decision to establish a brood with a dwarf daughter may initially appear to represent a needlessly high maternal cost, worker daughters are thought to represent an investment in late season brood insurance for some social mothers (Mikát et al. 2017). By contrast, though subsocial mothers rear only reproductively viable brood, their nests may fail when left without feeding services in the likely event of late-season maternal mortality. *C. calcarata* nests thus provide a natural system in which to empirically assess the inclusive fitness of an incipient form of social nesting. In this study, we estimate genetic relatedness within and between sympatric subsocial and social *C. calcarata* colonies. Next, we assess the potential influences of mate frequency and maternal body size on social phenotype. We then combine genetic and nest demographic data to calculate the relative costs and benefits of subsocial and social reproduction in a species capable of both reproductive strategies in sympatry.
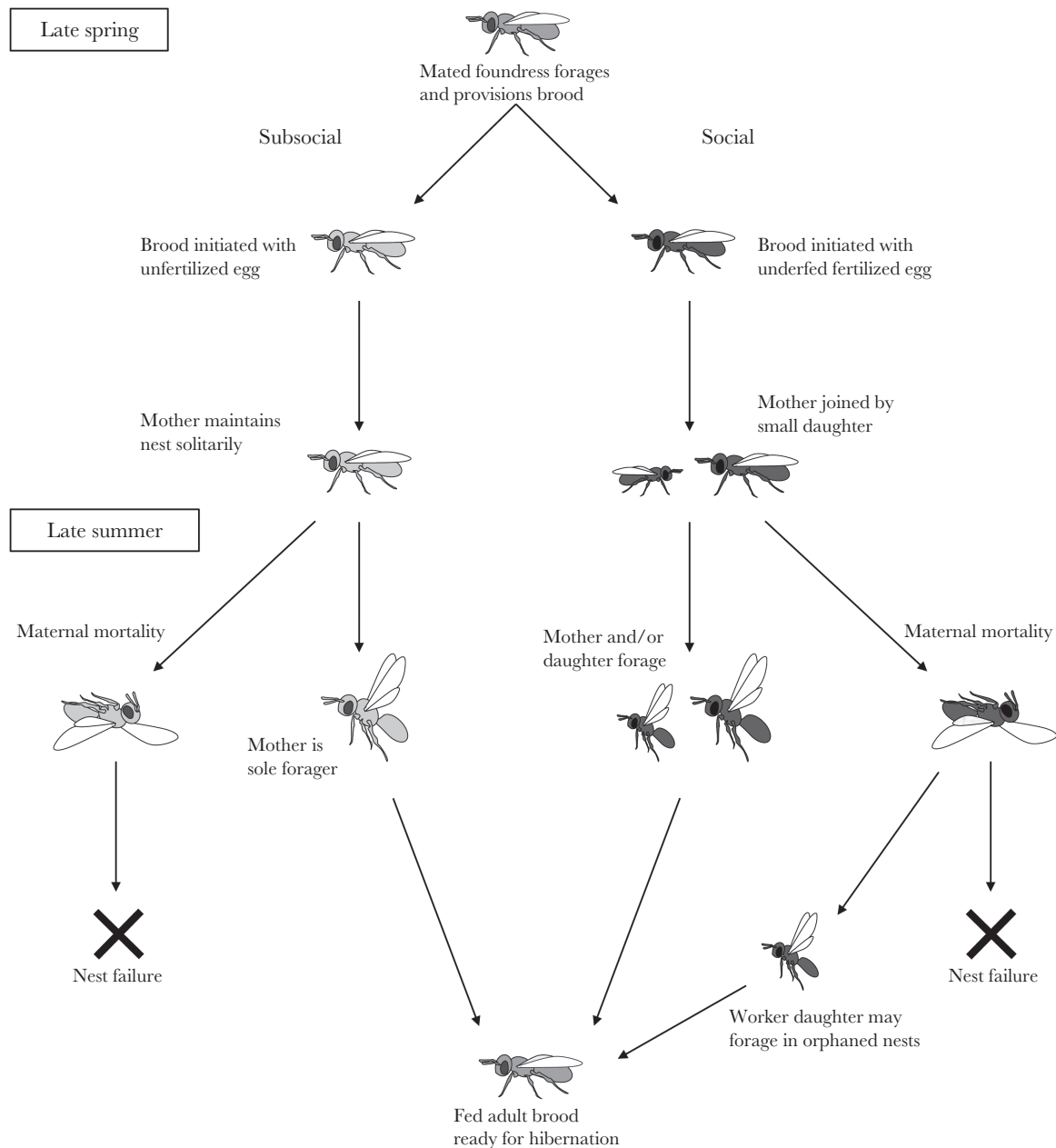
**Figure 1**

Annual life cycle and reproductive strategies of *Ceratina calcarata*. In late spring, each female disperses, mates, and establishes a new nest either subsocially (light gray) or socially (dark gray). During the late summer adult brood feeding period, social mothers and/or their worker daughters forage. A worker daughter may save her orphaned nest from failure in the event of maternal mortality. Sufficiently fed adult brood of either nest type survive a lengthy winter hibernation to disperse the following spring.

## METHODS

### Nest collections and assessment

A total of 167 *C. calcarata* nests were collected from Durham, New Hampshire during the 2014 through 2016 summer field seasons. *Ceratina* nests were identified in the field using burrow entrance holes in dead broken stems of staghorn sumac (*Rhus typhina*) and berry brambles (*Rubus* spp.). Nests were gathered at dawn, while adults were still dormant, as this ensured collection of foraging individuals (i.e., mother and worker daughter) and prevented offspring escape. Nests were refrigerated to sedate individuals, and were then dissected lengthwise to reveal nest architecture and brood composition. Total brood cells, pollen provisions, brood parasitism, and mortality were recorded, along with the developmental stage, brood cell position, and sex of each offspring (assessable at the pupal developmental stage or later); mothers were then measured for overall body size using head width as an accurate proxy (Rehan and Richards 2010b). As worker daughters are reared in the first brood cell, social nests were defined as those which contained a female offspring in the first brood cell; and subsocial nests were defined as those with a male offspring in the first position (Johnson 1988; Rehan and Richards 2010b; Lawson et al. 2016; Lewis and Richards 2017). Sex ratio (male and female brood count), social category (subsocial vs. social), total clutch size (all provisioned

brood cells), live brood (offspring alive at time of measurement), and maternal body size were assessed in all nests for which relevant data was available.

## DNA extraction, amplification, and allelic profiles

Nests used for genotyping were collected during the full brood stage, wherein the reproductive female has finished laying eggs and has assumed a brood guarding and cleaning role. Gathering nests at this stage thus ensures that both the reproductive female and her complete brood are collected. Twenty-seven full brood nests of mixed-sex brood, containing 257 individuals in total, were selected for DNA extraction and genotyping. Of the 27 nests, 19 were social and the remaining 8 were subsocial. A modified Phenol-Chloroform Isolation protocol (Kirby 1956) was used to extract DNA from the abdomen and 3 legs of each adult, and from the full body of each late-stage pupa. Each individual was then screened at 8 polymorphic microsatellite loci (Shell and Rehan 2016b) using the fluorescent M13-tail methodology described in Schuelke (2000). PCR reactions were mixed to a volume of 11 μL as follows: 5.45 μL ddiH$_2$0; 2.0 μL 5× HF Buffer (Thermo Scientific); 0.2 μL [10 mM] dNTPs; 0.1 μL Phusion HF Taq Polymerase (Thermo Scientific); 0.25 μL [10 mM] forward primer; 0.5 μL Fluorescent M13 oligo [10 mM], 0.5μL [10 mM] reverse primer; 2.0 μL DNA template. Thermocycler programs were run following primer annealing specifications from Shell and Rehan (2016b). After amplification was confirmed via gel electrophoresis (1% agarose gel) PCR product was mixed with Hi-Di Formamide (Applied Biosystems, Foster City, CA) and submitted to the DNA Analysis Facility at Yale University for fragment analysis on a 3730xl Analyzer (Applied Biosystems).

Individual allelic profiles were called via manual inspection of peaks in Peak Scanner 2 (Applied Biosystems). Intra- and intercolony maximum likelihood relatedness scores between mothers and their offspring, as well as among all siblings and non-nestmates, were then assessed using ML-Relate (Kalinowski et al. 2006). Pairwise statistical tests of relatedness were then performed using one randomly sampled female per nest to account for variation in brood counts among nests (Rehan, Richards, et al. 2014). Variation among female sibling allelic profiles also allowed for assignment of an estimated sire count for each genotyped nest (as in Richards et al. 2005). Statistical analyses of demography and relatedness were then performed in JMP Pro 13 (SAS Institute Inc., Cary, NC).

## Fitness calculations

Mature brood which are not fed before the end of the blooming season do not survive the winter (Durant et al. 2016), and orphaned nests with no additional foraging activity will fail (Lewis and Richards 2017). Foraging activity by a mother or worker daughter indicates successful brood feeding and can therefore be used as a proxy for expected brood overwintering survivorship. Empirical frequencies of nest orphanage, and foraging activity by mothers and worker daughters, were made available by a comprehensive study of foraging behavior (Mikát et al. 2017). These values were used to calculate average expected survivorship for subsocial and social foundresses, which were then combined with relatedness and demography datasets to calculate the relative fitness of subsocial and social nesting strategies.

On average, *C. calcarata* nests have a 70% chance of being orphaned during the mature brood feeding stage (Mikát et al. 2017). Therefore, likelihood of survivorship in a subsocial nest was calculated as one minus the average probability of a mother dying or abandoning her brood ($S_{sub} = 1 - 0.70$). A worker daughter was

observed to adopt foraging responsibilities in 18% of orphaned social nests (Mikát et al. 2017). Thus, likelihood of survivorship in social nests was calculated as one minus the probability that a nest is orphaned and not then fed by a worker daughter ($S_{soc} = 1 - (0.70 * 0.82)$). Average subsocial and social maternal fitness values were then calculated as a mother's relatedness to her offspring ($r_m$) multiplied by the average number of live brood for her nesting type ($subsocial = \mathcal{N}_{sub}; social = \mathcal{N}_{soc}$), then multiplied by the probability of offspring survival for that nesting strategy ($S_{sub}$ or $S_{soc}$). The average live brood count for social mothers was penalized by 1 to represent the cost of rearing a nonreproductive daughter:

Equation 1. Maternal inclusive fitness (IF): subsocial

$$IF_{sub} = [(r_m) * (\mathcal{N}_{sub})] * (S_{sub})$$

Equation 2. Maternal inclusive fitness (IF): social

$$IF_{soc} = [(r_m) * (\mathcal{N}_{soc} - 1)] * (S_{soc})$$

A worker daughter is active in 29% of social nests, either foraging alongside her mother (11%) or operating as the sole remaining forager in an orphaned nest (18%) (worker daughters do little to no foraging in the remaining social nests, Mikát et al. 2017). Mothers that survive to the end of the blooming season are expected to be able to provide sufficient late-season feeding for their brood's overwintering survival. Interestingly, in the 11% of cases where a worker daughter aids her mother in foraging, both individuals forage at half the rate of a lone forager (Mikát et al. 2017). As such, a worker daughter may be expected to receive a halved return on her total potential indirect fitness when she aids her mother in a nonorphaned nest ($wD_{coop} = 0.30 * 0.11$), and her full indirect fitness in the 18% of cases where she assumes the role of sole forager for her siblings in the event of nest orphanage ($wD_{solo} = 0.70 * 0.18$). The average inclusive fitness for the worker daughter ($IF_{wD}$) is thus calculated as 1) her total expected average indirect fitness ($IndF_{wD}$) multiplied by the likelihood of her contributing as the sole remaining forager ($wD_{solo}$), plus 2) half her expected average indirect fitness ($IndF_{wD} * 0.5$), multiplied by the likelihood of her foraging alongside her mother ($wD_{coop}$).

Equation 3. Worker daughter inclusive fitness (IF$_{wD}$)

$$IF_{wD} = [(IndF_{wD}) * (wD_{solo})] + [(IndF_{wD} * 0.5) * (wD_{coop})]$$

## RESULTS

### Demography

Genotyped nests ranged in clutch size from 4 to 15 individuals, with female offspring comprising between 18 and 89% of the total brood. In the population-wide dataset of 167 nests there were 122 social and 45 subsocial nests. Social nests contained significantly larger clutch sizes on average (*t*-test, $t = 2.10$, df = 80, $P = 0.04$), however, the percentage of live brood was not significantly different between nest types ($t = -0.068$, df = 71, $P = 0.95$; Figure 2), nor was the frequency of brood parasitism (social = $0.11 \pm 0.54$; subsocial = $0.27 \pm 1.03$; $t = -1.29$, df = 53, $P = 0.33$). Brood sex ratios were significantly more female biased in social than subsocial nests (mean female %$_{Social}$ = $57 \pm 0.02$; %$_{subsocial}$ = $36 \pm 0.038$; $t = -4.47$, df = 31, $P < 0.0001$). There was a significant positive correlation between maternal body size and clutch size in social nests (social; $F_{1, 95} = 3.93$, $r^2 = 0.04$, $P = 0.05$), but not for subsocial nests
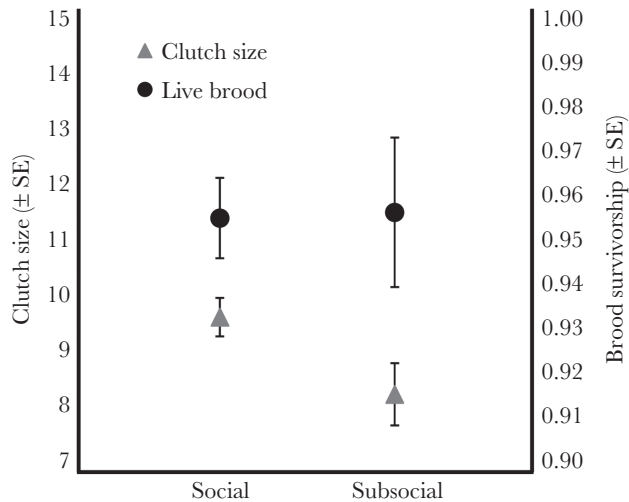
**Figure 2**

Mean clutch size and live brood in social and subsocial nests of *Ceratina calcarata*. Though social mothers produce significantly more brood ($t = 2.10$, df = 80, $P = 0.04$), average brood survivorship to adulthood is nearly identical for social and subsocial nest types ($t = -0.068$, df = 71, $P = 0.95$).

(subsocial; $F_{1, 28} = 0.001$, $r^2 < 0.001$, $P = 0.99$). Similarly, a significant positive correlation between maternal body size and percent female sex investment was found across social nests ($F_{1, 94} = 5.3492$, $r^2 = 0.05$, $P = 0.02$), but not subsocial nests ($F_{1, 28} = 0.0222$, $r^2 < 0.001$, $P = 0.88$). The frequency of social nest formation was found to increase with maternal body size (Logistic regression, $\chi^2 = 4.04$, df = 1; $P = 0.04$) such that for each 0.1 mm increase in maternal head width the likelihood of forming a social nest increased by 4%.

## Relatedness and inclusive fitness estimates

Average intercolony relatedness was low ($R = 0.07$), though average relatedness between mothers and their brood was identical in both social and subsocial nests ($R = 0.55$; $t$-test, $t = 0.08$ df = 10, $P = 0.94$; Supplementary Table S1). Average relatedness among female siblings in social nests ($R = 0.70$), however, was significantly higher than subsocial nests ($R = 0.48$) ($t = -3.11$, df = 18, $P = 0.0061$). Within social nests, average worker daughter relatedness to sisters ($R = 0.698$) was not significantly different from the relatedness among nonworker sisters ($R = 0.701$) ($t = -0.045$, df = 34, $P = 0.96$). Across all genotyped nests, relatedness values between female offspring and their male siblings was 0.25. Genotyping colonies also revealed that 22 of the 27 nests were singly sired. The relatedness among female siblings of singly mated nests ($R = 0.71$) was significantly higher than multiply mated nests ($R = 0.47$) ($t = -4.158$; df = 5.34; $P = 0.008$). Intracolony allelic profiles indicate that 16% (3/19) of social nest mothers and 25% (2/8) of subsocial nest mothers were multiply mated, though the frequency of multiple mating was not significantly different between nest types (Fisher's Exact Test, $P = 0.62$; Supplementary Table S1).

Though social mothers provisioned larger clutches than subsocial on average, accounting for the rearing of a nonreproductive worker daughter resulted in similar direct fitness values for both reproductive strategies (subsocial = 4.29, social = 4.51; Wilcoxon, $\div^2 = 0.2218$, df = 1, $P = 0.64$). Higher expected offspring survivorship in social colonies ($S_{soc} = 0.426$) compared to subsocial nests ($S_{sub} = 0.30$) resulted in higher inclusive fitness for social

mothers ($IF_{soc} = 1.92$; vs. $IF_{sub} = 1.29$). Though worker daughters do not produce offspring, their average indirect fitness was high ($IndF_{wD} = 4.06$). The likelihood that a worker daughter becomes solely responsible for the survival of the brood, however, is relatively low ($wD_{solo} = 0.126$). Further, her indirect fitness is halved in the few cases where she forages alongside her mother ($wD_{coop} = 0.033$; Equation 3). This low overall probability of indirect fitness gains through helping, combined with her lack of direct fitness, results in low average inclusive fitness for worker daughters ($IF_{wD} = 0.58$). Comparing fitness values calculated for the 3 reproductive strategies across the total assessed population, social mothers have significantly higher fitness than subsocial mothers, and subsocial mothers significantly higher fitness than worker daughters on average (Kruskal–Wallis, df = 2, $\div^2 = 154.53$, $P < 0.0001$; Figure 3).

## DISCUSSION

*C. calcarata* demonstrates a relatively simple form of facultative social organization, and one that is characteristic of bees in the earliest stages of social evolution (Rehan and Richards 2010b; Rehan, Berens, et al. 2014; Rehan and Toth 2015). This study is the first empirical assessment of inter- and intracolony relatedness in *C. calcarata*, and a cost-benefit analysis of a bee representative of both subsocial and incipient social reproductive strategies, in which a single worker daughter may be produced. Demographic analyses confirmed positive relationships between maternal body size and brood composition (Johnson 1988; Rehan and Richards 2010b; Rehan, Berens, et al. 2014), and revealed that socially nesting females provision larger clutch sizes and invest in a significantly greater proportion of female offspring (Figure 2). As the probability of social nesting increased with female body size, this study provides further support for the role of maternal body size in determining division of labor in *C. calcarata* (Rehan and Richards 2010b; Withee and Rehan 2016), other *Ceratina* small carpenter bees (e.g., *C. japonica*, Sakagami and Maeta 1984), and among facultatively social bees more broadly (e.g., *Halictus rubicundus*, Field et al. 2012; *Megalopta genalis*, Kapheim et al. 2011). Screening at 8 polymorphic microsatellite loci revealed higher average intracolony relatedness among female siblings in social nests compared to subsocial, and indicates that *C. calcarata* is capable of limited polyandrous mating. Inclusive fitness calculations reveal that while social nesting is advantageous in *C. calcarata*, worker daughters receive few fitness benefits for their role. Social nests are initiated though careful control of pollen provisions and sib-social care is likely enforced through differences in body size. *C. calcarata* provides a clear example of how an early division of labor may be initiated by maternal manipulation and consequently maintained through maternal inclusive fitness benefits.

## Variations in *C. calcarata* relatedness and implications of multiple mating

Microsatellite screening revealed no difference in maternal relatedness to brood between social and subsocial nests. The higher average relatedness among social female siblings compared to subsocial, however, represents a notable asymmetry, and one which has been detected in other facultatively social bees (e.g., *Ceratina australensis*, Rehan, Richards, et al. 2014; and *Megalopta genalis*, Kapheim et al. 2015). High intracolony relatedness, particularly within female-biased broods, is thought to play an important role in the emergence of social organization (Trivers and Hare 1976; Hughes et al. 2008). However, the degree to which social nesting may be
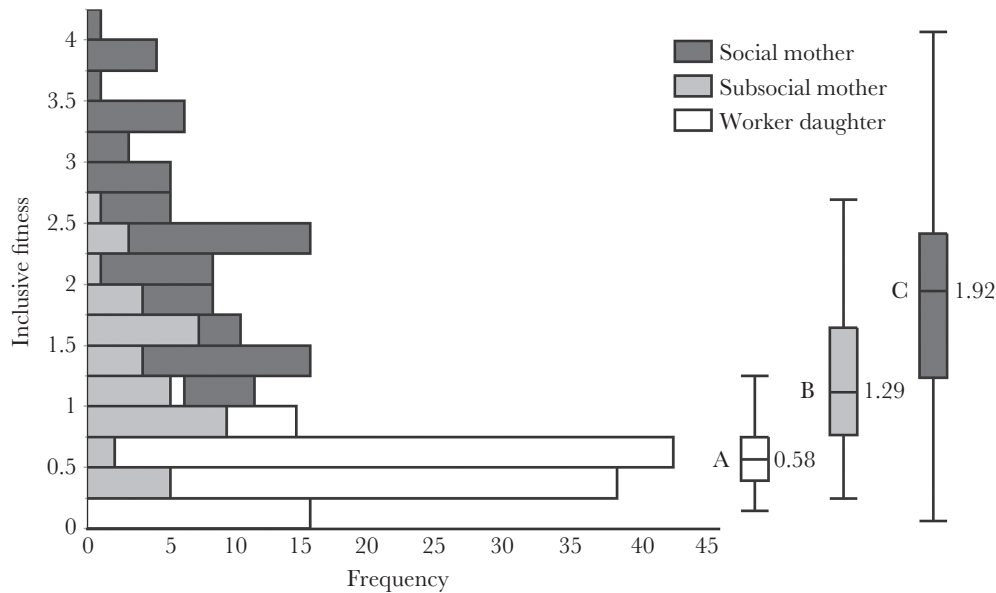
**Figure 3**
Histogram and box plots of population-wide distribution of estimated inclusive fitness values for social mothers (dark gray; $N = 122$), subsocial mothers (light gray; $N = 45$), and worker daughters (white; $N = 122$). Average inclusive fitness values for worker daughters (A), subsocial mothers (B), and social mothers (C) (Kruskall–Wallis, df = 2, $\chi^2 = 154.53$, $P < 0.0001$; Wilcoxon for Each Pair, social mother versus subsocial mother $P < 0.0001$, social mother versus worker daughter $P < 0.0001$, subsocial mother versus worker daughter $P < 0.0001$). All values were calculated using nest demography data and imputed relatedness scores.

heritable or influenced by individual condition remains an aim for further investigation (Hamilton 1964; Crozier and Pamilo 1996).

Colony relatedness data additionally indicate *C. calcarata* females are primarily singly mated (22/27 mothers), with a capacity for occasional multiple mating with 2 or more males (5/27 mothers). Most species of bees are thought to be monandrous (Strassmann 2001; Wilson 2005), though many demonstrate a facultative capacity for polyandry (e.g., *Bombus* species with 2 to 4 sires, Estoup et al. 1995; *Lasioglossum malachurum* reporting up to 3 sires, Paxton et al. 2002). Polyandry is taken to an extreme degree in some advanced eusocial bees (e.g., *Apis mellifera* with over 17 sires on average, Laidlaw and Page 1984), but may be disadvantageous in non-*Apis* bees, in which intermediate levels of colony genetic heterogeneity may incur a fitness cost. For instance, *Bombus terrestris* females mated to 2 males had reduced reproductive output compared to females mated to either 1 or 4 males (Baer and Schmid-Hempel 2001). Limited polyandry among social nesting *C. calcarata* suggests that strict monandry may not be necessary for the persistence of early social traits. Additional studies in *C. calcarata* and other early stage social bees are needed to better understand the degree to which mating frequency may affect the emergence and maintenance of social phenotypes.

## The costs and benefits of social and subsocial nesting in *C. calcarata*

According to kin selection theory, genes underlying social care behavior are more likely to be passed on when an altruist's gains in indirect fitness outweigh its direct fitness losses incurred by forgoing reproduction (Hamilton 1964). The low average inclusive fitness of worker daughters compared to both social and subsocial mothers thus seems to contradict theoretical expectation (Figure 3). Although a worker daughter's foraging behavior is comparable to that of her mother (Mikát et al. 2017), surviving late-season

mothers can sufficiently feed their mature brood alone (Lewis and Richards 2017). For this reason, on the occasion a worker daughter provisions alongside her mother, her contributions may represent a supplemental rather than necessary resource for brood survival. Additionally, although sib-social provisioning may be critical to preventing brood mortality, worker daughters assume their role of sole forager with relatively low frequency in orphaned nests (Mikát et al. 2017). Therefore, despite high relatedness between worker daughters and their siblings, reinforcement of sib-social care behavior in *C. calcarata* does not appear to be fully explained by indirect fitness benefits to the helping individual (Hamilton 1964). Although she may be making the best of a bad situation by foraging for her siblings, in the absence of inclusive fitness benefits to the worker daughter maintenance of division of labor in *C. calcarata* appears best explained by maternal manipulation (Johnson 1988; Crespi and Ragsdale 2000; Rehan and Richards 2013; Rehan, Berens, et al. 2014; Withee and Rehan 2016; Mikát et al. 2017).

Maternal manipulation of brood has long been theorized as a proximate mechanism underlying the emergence of social traits (Alexander 1974; Craig 1979) and has been suggested as an explanation for facultative sociality in other species (e.g., *Lasioglossum malachurum*, Richards et al. 2005; *M. genalis*, Kapheim et al. 2016). For instance, *M. genalis* queens manipulate pollen provisions to reduce the body size of their offspring (Kapheim et al. 2011). As observed in *C. calcarata*, *M. genalis* workers have low inclusive fitness, and it is thought that their small adult body size facilitates physical coercion into their worker roles (Arneson and Wcislo 2003; Kapheim et al. 2016). Body size affects behavioral roles in *C. calcarata* (Withee and Rehan 2016), and appears to predict the probability of social nesting. Larger *C. calcarata* mothers may thus be better-equipped to manipulate pollen during the mass provisioning period (Rehan and Ricahrds 2010b; Lawson et al. 2016), and may be more capable of establishing a social hierarchy during the adult offspring feeding stage (Withee and Rehan 2016; Mikát et al. 2017).

*C. calcarata*'s social and subsocial nesting polyphenism has gone previously unreported across its range (Rau 1928; Johnson 1988; Rehan and Richards 2010a; Lawson et al. 2016; Lewis and Richards 2017); this could be attributable to the subtle differences between nest types. Both social and subsocial mothers produce similar clutch sizes, and relatively infrequent brood losses to parasitism do not appear to affect one nesting type more than the other (Figure 2). Further, while mothers of either strategy are capable of independently feeding their brood for a successful overwintering period, all mothers are also equally likely to orphan their adult offspring at the end of the brood rearing season (Mikát et al. 2017). In the event of late season orphanage, a social mother's direct fitness may be insured through sib-social brood provisioning. Similar insurance-based advantages to social nesting have also been detected in the facultatively social hover wasp, *Liostenogaster flavolineata*, in which the fitness of brood rearing adults is likely to be assured through the collective effort of multiple helpers (Field et al. 2000). Assured fitness returns have been proposed as an important ultimate mechanism for the maintenance of social reproduction in facultatively eusocial bees (Smith et al. 2007), and among social allodapine bees (Schwarz et al. 2010) and wasps (Gadagkar 1990, 2001). The fitness advantage observed in social nesting *C. calcarata* thus suggests assured fitness returns may operate in selecting for group living and early divisions of labor (Gadagkar 1990).

It therefore appears that, while the emergence of social traits likely requires high relatedness and inclusive fitness benefits, selection on physiological and behavioral traits that maximize maternal fitness may represent recurring proximate mechanisms for early evolutionary transitions towards social organization. Additional empirical assessments of colony demography and the relative fitness of alternative reproductive strategies in other facultatively social species are necessary to understand lineage specific and recurrent processes underlying evolutionary gains and losses of social complexity across taxa.

## SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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## REFERENCES

Alexander RD. 1974. The evolution of social behavior. Annu Rev Ecol Evol Syst. 5:325–383.

Arneson J, Wcislo WT. 2003. Dominant-subordinate relationships in a facultatively social, nocturnal bee, *Megalopta genalis* (Hymenoptera: Halictidae). J Kans Entomol Soc. 76:183–193.

Augusto SC, Garófalo CA. 2004. Nesting biology and social structure of *Euglossa (Euglossa) townsendi* Cockerell (Hymenoptera, Apidae, Euglossini). Insect Soc. 51:400–409.

Baer B, Schmid-Hempel P. 2001. Unexpected consequences of polyandry for parasitism and fitness in the bumblebee, *Bombus terrestris*. Evolution. 55:1639–1643.

Bourke AFG. 2011. The validity and value of inclusive fitness theory. Proc R Soc B. 278:3313–3320.

Craig R. 1979. Parental manipulation, kin selection, and the evolution of altruism. Evolution. 33:319–334.

Crespi BJ, Ragsdale JE. 2000. A skew model for the evolution of sociality via manipulation: why it is better to be feared than loved. Proc Biol Sci. 267:821–828.

Crespi BJ. 1992. Eusociality in Australian gall thrips. Nature. 359:724–726.

Crozier RH, Pamilo P. 1996. Evolution of social insect colonies. Oxford (UK): Oxford University Press.

Danforth BN. 2002. Evolution of sociality in a primitively eusocial lineage of bees. Proc Natl Acad Sci USA. 99:286–290.

Duffy JE. 1996. Eusociality in a coral-reef shrimp. Nature. 381:512.

Durant DR, Berens AJ, Toth AL, Rehan SM. 2016. Transcriptional profiling of overwintering gene expression in the small carpenter bee, *Ceratina calcarata*. Apidologie. 47:572–582.

Estoup A, Scholl A, Pouvreau A, Solignac M. 1995. Monoandry and polyandry in bumble bees (Hymenoptera; Bombinae) as evidenced by highly variable microsatellites. Mol Ecol. 4:89–93.

Field J, Paxton R, Soro A, Craze P, Bridge C. 2012. Body size, demography and foraging in asocially plastic sweat bee: a common garden experiment. Behav Ecol Sociobiol. 66:743–756.

Field J, Shreeves G, Sumner S, Casiraghi M. 2000. Insurance-based advantage to helpers in a tropical hover wasp. Nature. 404:869–871.

Foster KR, Wenseleers T, Ratnieks FL. 2006. Kin selection is the key to altruism. Trends Ecol Evol. 21:57–60.

Gadagkar R. 1990. Evolution of eusociality: the advantage of assured fitness returns. Phil Trans R Soc B. 329:17–25.

Gadagkar R. 2001. The social biology of *Ropalidia marginata*: toward understanding the evolution of eusociality. Cambridge, MA: Harvard University Press.

Gadagkar R. 2010. Sociobiology in turmoil again. Curr Sci. 99:1036–1041.

Hamilton WD. 1964. The genetical evolution of social behavior. J Theor Biol. 7:1–52.

Hamilton WD. 1972. Altruism and related phenomena, mainly in social insects. Annu Rev Ecol Evol Syst. 3:193–232.

Hughes CR, Strassmann JE. 1988. Age is more important than size in determining dominance among workers in the primitively eusocial wasp, *Polistes instabilis*. Behaviour. 107:1–14.

Hughes WO, Oldroyd BP, Beekman M, Ratnieks FL. 2008. Ancestral monogamy shows kin selection is key to the evolution of eusociality. Science. 320:1213–1216.

Jandt JM, Tibbets EA, Toth AL. 2014. *Polistes* paper wasps: a model genus for the study of social dominance hierarchies. Insect Soc. 61:11–27.

Jarvis JUM. 1981. Eusociality in a mammal: cooperative breeding in naked mole-rat colonies. Science. 212:571–573.

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.

Kalinowski ST, Wagner AP, Taper ML. 2006. ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. Mol Ecol Notes. 6:576–579.

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.

Kapheim KM, Chan T-Y, Smith AR, Wcislo WT, Nonacs P. 2016. Ontogeny of division of labor in a facultatively eusocial sweat bee *Megalopta genalis*. Insect Soc. 63:185–191.

Kapheim KM, Nonacs P, Smith AR, Wayne RK, Wcislo WT. 2015. Kinship, parental manipulation and evolutionary origins of eusociality. Proc Biol Sci. 282:20142886.

Kirby KS. 1956. Isolation and characterization of ribosomal ribonucleic acid. Biochem J. 96:266–269.

Laidlaw HH, Page RE. 1984. Polyandry in honey bees (*apis mellifera* L.): sperm utilization and intracolony genetic relationships. Genetics. 108:985–997.

Lawson SP, Ciaccio KN, Rehan SM. 2016. Maternal manipulation of pollen provisions affects worker production in a small carpenter bee. Behav Ecol Sociobiol. 70:1891–1900.

Leadbeater E, Carruthers JM, Green JP, Rosser NS, Field J. 2011. Nest inheritance is the missing source of direct fitness in a primitively eusocial insect. Science. 333:874–876.

Lewis V, Richards MH. 2017. Experimentally induced alloparental care in a solitary carpenter bee. Anim Behav. 123:229–238.

Lin N, Michener CD. 1972. Evolution of sociality in insects. Q Rev Biol. 47:131–159.

Marshall JA. 2011. Group selection and kin selection: formally equivalent approaches. Trends Ecol Evol. 26:325–332.

Michener CD. 2007. The bees of the world, 2nd edn. Baltimore (MD): John Hopkins University Press.

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.

Nowak MA, Tarnita CE, Wilson EO. 2010. The evolution of eusociality. Nature. 466:1057–1062.

Palmer KA, Oldroyd BP. 2000. Evolution of multiple mating in the genus *Apis*. Apidologie. 31:235–248.

Paxton RJ, Ayasse M, Field J, Soro A. 2002. Complex sociogenetic organization and reproductive skew in a primitively eusocial sweat bee, *Lasioglossum malachurum*, as revealed by microsatellites. Mol Ecol. 11:2405–2416.

Pech MEC, May-Itzá WdeJ, Medina LAM, Quezadoa-Euán JJG. 2008. Sociality in *Euglossa (Euglossa) viridissima* Friese (Hymenoptera, Apidae, Euglossini). Insect Soc. 55: 428–433.

Queller DC, Strassmann JE. 1998. Kin selection and social insects. BioScience. 48:165–175.

Quiñones AE, Pen I. 2017. A unified model of Hymenopteran preadaptations that trigger the evolutionary transition to eusociality. Nat Commun. 8:1–13. doi:10.1038/ncomms15920

Ratnieks FL, Wenseleers T. 2008. Altruism in insect societies and beyond: voluntary or enforced? Trends Ecol Evol. 23:45–52.

Rau P. 1928. The nesting habits of the little carpenter-bee, *Ceratina calcarata*. Ann Entomol Soc Am. 21:380–396.

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.

Rehan SM, Richards MH, Adams M, Schwarz MP. 2014. The costs and benefits of sociality in a facultatively social bee. Anim Behav. 97:77–85.

Rehan SM, Richards MH. 2010a. Nesting biology and subsociality in *Ceratina calcarata* (Hymenoptera: Apidae). Can Entomol. 142:65–74.

Rehan SM, Richards MH. 2010b. The influence of maternal quality on brood sex allocation in the small carpenter bee, *Ceratina calcarata*. Ethology. 116:876–887.

Rehan SM, Richards MH. 2013. Reproductive aggression and nestmate recognition in a subsocial bee. Anim Behav. 85:733–741.

Rehan SM, Sheffield CS. 2011. Morphological and molecular delineation of a new species in the *Ceratina dupla* species-group (Hymenoptera: Apidae: Xylocopinae) of eastern North America. Zootaxa. 2873:35–50.

Rehan SM, Toth AL. 2015. Climbing the social ladder: the molecular evolution of sociality. Trends Ecol Evol. 30:426–433.

Richards MH, French D, Paxton RJ. 2005. It's good to be queen: classically eusocial colony structure and low worker fitness in an obligately social sweat bee. Mol Ecol. 14:4123–4133.

Sakagami SF, Maeta Y. 1984. Multifemale nests and rudimentary castes in the normally solitary bee *Ceratina japonica* (Hymenoptera: Xylocopinae). J Kans Entomol Soc. 57:639–656.

Schuelke M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol. 18:233–234.

Schwarz MP, Tierney SM, Rehan SM, Chenoweth LB, Cooper SJ. 2010. The evolution of eusociality in allodapine bees: workers began by waiting. Biol Lett. 7:277–280.

Seeley TD. 1982. Adaptive significance of the age polyethism schedule in honeybee colonies. Behav Ecol Sociobiol. 11:287–293.

Shell WA, Rehan SM. 2016a. Recent and rapid diversification of the small carpenter bees in eastern North America. Biol J Linnean Soc. 117:633–645.

Shell WA, Rehan SM. 2016b. Development of multiple polymorphic microsatellite markers for the small carpenter bee, *Ceratina calcarata* (Hymenoptera: Apidae) using genome-wide analysis. J Insect Sci. 16:1–4.

Shell WA, Rehan SM. 2017a. Behavioral and genetic mechanisms of social evolution: insights from incipiently and facultatively social bees. Apidologie. 1–18. doi:10.1007/s13592- 017-0527-1

Shell WA, Rehan SM. 2017b. Data from: the price of insurance: costs and benefits of worker production in a facultatively social bee. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.v3462

Smith AR, O'Donnell S. 2003. Assured fitness returns favor sociality in a mass- provisioning sweat bee, *Megalopta genalis* (Hymenoptera: Halictidae). Behav Ecol Sociobiol. 54:14–21.

Smith AR, Wcislo WT, O'Donnell S. 2007. Survival and productivity benefits to social nesting in the sweat bee *Megalopta genalis* (Hymenoptera: Halictidae). Behav Ecol Sociobiol. 61:1111–1120.

Strassmann J. 2001. The rarity of multiple mating by females in the social Hymenoptera. Insect Soc. 48:1–13.

Szathmáry E, Smith JM. 1995. The major evolutionary transitions. Nature. 374:227–232.

Thorne BL. 1997. Evolution of eusociality in termites. Annu Rev Ecol Syst. 28:27–54.

Toth AL, Rehan SM. 2017. Molecular evolution of insect sociality: an eco-evo-devo perspective. Annu Rev Entomol. 62:419–442.

Toth AL, Varala K, Newman TC, Miguez FE, Hutchison SK, Willoughby DA, Simons JF, Egholm M, Hunt JH, Hudson ME, et al. 2007. Wasp gene expression supports an evolutionary link between maternal behavior and eusociality. Science. 318:441–444.

Trivers RL, Hare H. 1976. Haploidploidy and the evolution of the social insect. Science. 191:249–263.

Trivers RL. 1971. The evolution of reciprocal altruism. Q Rev Biol. 46:35–57.

West-Eberhard MJ. 1975. The evolution of social behavior by kin selection. Q Rev Biol. 50:1–33.

Wilson EO, Hölldobler B. 2005. Eusociality: origin and consequences. Proc Natl Acad Sci USA. 102:13367–13371.

Wilson EO. 1971. The insect societies. Cambridge (MA): Belknap Press of Harvard University Press.

Wilson EO. 2005. Kin selection as the key to altruism: its rise and fall. Soc Res. 72:159–166.

Withee JR, Rehan SM. 2016. Cumulative effects of body size and social experience on aggressive behaviour in a subsocial bee. Behaviour. 153:1365–1385.

Yagi N, Hasegawa E. 2012. A halictid bee with sympatric solitary and eusocial nests offers evidence for Hamilton's rule. Nat Commun. 3:939.

Yanega D. 1989. Caste determination and differential diapause within the first brood of *Halictus rubicundus* in New York (Hymenoptera: Halictidae). Behav Ecol Sociobiol. 24:97–107.