



Original Article

Large fitness benefits of social nesting in a small carpenter bee

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Facultatively social insects are an optimal model group for the study of the emergence of cooperation between individuals. Factors influencing the fitness benefits of social nesting are still debated. Nonreproductive subordinates can benefit from indirect fitness benefits due to increasing reproductive success of related individuals or direct fitness benefits due to direct future reproduction. Here, we studied the costs and benefits of social nesting in the small carpenter bee *Ceratina albosticta*. From demographic data and within-nest relatedness, we obtained key parameters for assessing the fitness of solitary females, social primaries, and social secondaries. *C. albosticta* were found to usually mate with one male and multiple mating is not common although exists at low frequencies. Social nests usually contain two females, which were found to be related (full sisters), but also some females were unrelated to each other. Patterns of parentage from microsatellite loci revealed that only one female reproduces in social nests. Our results show that relatedness, per capita brood productivity, and offspring survival strongly increase the fitness benefits of social nesting strategies. Social secondaries, when related to the social primaries, have higher inclusive fitness than solitary females, but unrelated social nesting females had no indirect fitness and much reduced inclusive fitness compared to solitary females. Interestingly, average fitness benefits of the social secondary were higher than solitary females. This study provides important empirical data on the costs and benefits of sociality in a facultatively social bee and sets the stage for future comparative studies.

Key words: *Ceratina*, facultatively sociality, inclusive fitness, relatedness.

INTRODUCTION

The stability of social nesting in facultatively social species is dependent on subordinate female(s) remaining in the group (Cahan et al. 2002; Hughes et al. 2008; Griesser et al. 2017). Selection for subordinate individuals to remain in the colony is dependent on their actual or potential fitness benefits (Kokko et al. 2002; Riehl 2013; Korb and Heinze 2016). There are multiple parameters that influence these benefits, most importantly are relatedness to offspring of the reproductive female, overall productivity of social nesting, possibility for direct reproduction, and possibility for inheriting the dominant reproductive position (Smith et al. 2007; Hughes et al. 2008; Schwarz et al. 2011; Korb and Heinze 2016). These parameters are strongly influenced by local resources and social environment (Hirata and Higashi 2008; Field et al. 2010; Purcell 2011; Shen et al. 2017; Ohkubo et al. 2018; Vickruck and Richards 2021; Ostwald et al. 2022).

Relatedness is a key parameter that can influence the selection for individuals to cooperate. If individuals in a colony are related, lifetime forgoing of one's own reproduction can be beneficial because

a subordinate individual receives indirect fitness through the reproduction of the dominant colony member (Hamilton 1964; Bourke 2014). The scale of these indirect fitness benefits is dependent on relatedness between subordinate individual(s) and the offspring of the dominant individual (Hamilton 1964). Nonreproductive subordinates have the highest indirect fitness in eusocial colonies, with one singly mated reproductive dominant, which is the mother of all reproductive subordinates. However, nature presents a wide variety of social organizations (Jennions and Macdonald 1994; Sherman et al. 1995; Schwarz et al. 2007; Riehl 2013; Korb and Heinze 2016). In Hymenoptera, within-nest relatedness can be influenced by four factors: 1) exchange of individuals between colonies such as drifting workers or pleometrotic nest founding (Nonacs 2001; Field and Leadbeater 2016; Pennell and Field 2020); 2) presence of generation overlap (Reeve and Keller 1995; Hearn et al. 2022); 3) mating frequency (Hughes et al. 2008); and 4) turnover of the dominant reproductive individual (Bolton et al. 2006; Andrade et al. 2016; Pennell and Field 2020).

The productivity of social nests is an important factor and closely connected with relatedness. The benefits of helping for reproductive subordinate, in terms of the number of additional

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offspring in social versus solitary nests, are multiplied by relatedness of the helper to the reproductive and have to exceed costs of giving up one's own reproduction (Hamilton 1964). Therefore, social nesting could be favored by the reproductive subordinate when related to the social primary, which produces at least the same offspring per capita as solitary nests. In bees, nest productivity is traditionally measured as the number of brood cells provisioned or the number of offspring per adult female (Michener 1964; Rehan et al. 2014). Studies performed on social Hymenoptera have shown decreasing, stable, and also increasing trends of nest productivity with increasing numbers of colony members (Jeanne et al. 2022). Moreover, in some facultatively social allodapine bees, a unimodal trend is reported with the largest per capita productivity in moderate-sized colonies (Joyce and Schwarz 2006; Chenoweth and Schwarz 2007; Bernauer et al. 2021). However, per capita productivity can underestimate the benefits of social nesting because fitness estimates are typically derived only from successful nests as destroyed nests may be difficult to detect. Social nesting can substantially increase the probability of nest survival (Smith et al. 2007).

Selection for subordinate individual(s) to stay in social groups is influenced by their direct reproduction. In obligately eusocial systems, usually only one female reproduces, and the remaining adult females have no direct reproduction (Wilson 1971). The total reproductive dominance of a single female is known in several small insect societies (Hogendoorn and Velthuis 1999; Freiria et al. 2017; Hearn et al. 2022). However, most data show that small societies experience high variance of reproductive skew (Lucas et al. 2011; Andrade et al. 2016; Dew et al. 2016; Silva et al. 2016; Friedel et al. 2020; Pennell and Field 2020) and that a continuum between total skew and total reproductive equality is observed in these societies (Sherman et al. 1995). Great between-species variability in reproductive skew exists in carpenter bees (subfamily Xylocopinae). In large carpenter bees, genus *Xylocopa*, reproduction is dominated by only one female (Hogendoorn and Velthuis 1999), or possibly serial monogamy can be present when one female dominates reproduction at time (Steen 2000). On the other hand, several species of allodapine bees have revealed casteless societies, where multiple females reproduce at the same time (Silva et al. 2016; Dew et al. 2018). Variability among species also exists in the small carpenter bees, genus *Ceratina*. In Japanese *Ceratina* from the subgenus *Ceratinidia*, often multiple females participate in reproduction (Sakagami and Maeta 1984; Maeta and Sakagami 1995), but reproduction is dominated by one female in Australian *Ceratina* (*Neoceratina*) *australensis* (Rehan et al. 2011) and European *Ceratina* (*Euceratina*) *chalybea* (Mikát et al. 2021).

Reproductive skew is more complicated when the potential for future reproduction is also considered. Reproductive subordinates can stay in social groups and not reproduce but may inherit the dominant's status and gain direct reproduction (Kokko et al. 2002; Bridge and Field 2007; Schwarz et al. 2011). In most social insects, the chance for inheriting a dominant position is relatively small because subordinates have high mortality rates if engaged in intensive foraging behavior (Wilson 1971). However, in carpenter bees, a nontraditional reproductive division of labor is common, in which the reproductive dominant female also performs foraging and subordinate female(s) serve as guards (Hogendoorn and Velthuis 1995, 1999; Rehan et al. 2011). Therefore, subordinate behavior and social colonies are common due to the benefits from waiting to inherit the nest and dominant reproductive position (Schwarz et al. 2011).

Colony productivity, relatedness, and reproductive skew are key factors influencing if social nesting is beneficial for social primaries and also social secondaries (Dunn and Richards 2003). However, all these factors are rarely studied together in a single analysis, and although there are a lot of theoretical literature comparing costs and benefits of sociality, empirical studies are scarce.

In Xylocopinae bees, there are several studies that assess reproductive skew and relatedness using indirect methods, such as observations of egg-laying or genealogy (Stark 1992; Hogendoorn and Leys 1993; Hogendoorn and Velthuis 1995, 1999; Maeta and Sakagami 1995), but there are very few studies which use genetic markers (Steen 2000; Langer et al. 2006; Rehan et al. 2014).

Ceratina small carpenter bees are an excellent genus to study simple forms of social behavior. Although most females have a solitary lifestyle, facultatively sociality is known across most behaviorally studied species (Groom and Rehan 2018; Mikát et al. 2022). Within-nest relatedness has been studied rarely in facultatively social *Ceratina* species. Within-nest relatedness was examined for *C. australensis* (Rehan et al. 2014; Oppenheimer and Rehan 2020), *C. calcarata* (Shell and Rehan 2017), and *C. chalybea* (Mikát et al. 2021).

Ceratina albosticta is a North-African species for which nesting biology was recently described (Mikát and Rehan 2022). This species is facultatively social with the frequency of social nesting around 16% of reproductively active colonies. Most social nests contain two females, although nests containing up to four females are reported. Two-female nests contained around four times higher number of brood cells than solitary nests, therefore, social nests are much more productive. Social groups can form before the nesting season starts, as social nests were frequently detected in May, before any offspring of the new generation emerged (Mikát and Rehan 2022). We suppose that social nests can form from females that overwinter together, as groups of nonreproductive adult females were detected at the end of the nesting season just before overwintering (Mikát and Rehan 2022) and also after overwintering before the reproductive season starts (Daly 1983). In social nests, evidence for reproductive division of labor was clear based on differences in ovarian development (Mikát and Rehan 2022). Here, we examine the within-nest relatedness in *Ceratina albosticta*. We evaluated these features: 1) mating frequency of females, 2) relatedness between adult females in social nests, and 3) maternity of offspring in social and solitary nests. Moreover, we compare inclusive fitness among solitary nesting females, social primaries, and social secondaries from two-female nests. Taken together, this study advances our understanding of social evolution by providing empirical fitness data for facultatively sociality and the natural variation of reproductive skew in simple insect societies.

METHODS

Nests were collected in southern Morocco around the town of Kelaat M'Gouna (31.2365256N, 6.1347164W) in May 2019. Nests were collected from dead pruned rose stems (*Rosa damascena*) in the evenings or early mornings to ensuring that all inhabitants were present in nests. Nests were opened lengthwise, and the stage and position of brood as well as adults were noted. Brood and adults were preserved in 96% ethanol for subsequent morphological and molecular analyses. For each female, wing wear was scored using a relative score described in Mueller and Wolf-Mueller (1993). Demographic data from nests were taken from Mikát and Rehan (2022). This study focuses on solitary and two-female nests collected in Kelaat Mgouna in May 2019. However, we also collected

three-female ($N = 2$), four-female nests ($N = 2$), and a bisex nest ($N = 1$) in May 2019 and some solitary ($N = 2$) and social nests ($N = 1$) in several locations in September 2019. We also examined relatedness in these nests, but due to small sample sizes, it was not possible to include them in quantitative analyses and the results from three to four female nests are presented in the [Supplementary Materials](#) in the section *Relatedness in interesting nests*.

Microsatellite markers and in-nest relatedness

We used microsatellite loci originally developed for species *C. nigrolabiata* (Mikát et al. 2019). Cross-amplification of loci on other species from the subgenus *Euceratina* was previously confirmed (Mikát et al. 2019, 2021). We tested 25 possible primers and, for final analysis, selected 14 loci that had consistent amplification and were polymorphic. These microsatellite loci had 8.07 alleles on average (range 2–15, SD = 4.12). For more detailed primers and polymorphism of loci, see [Supplementary Tables S1 and S2](#).

DNA was isolated using the Chelex protocol (Coombs et al. 1999). Each sample was put into a strip microcentrifuge vial with a volume of 0.2 μL and dried for at least three hours. We then added 6 μL of proteinase K and 40 μL of 10% Chelex solution. The mixture was put into a thermocycler (Eppendorf flexlid mastercycler) where samples were heated to 55 $^{\circ}\text{C}$ per 50 min, followed by 97 $^{\circ}\text{C}$ for 8 min, and finally cooled down to 12 $^{\circ}\text{C}$. The mixture was vortexed, centrifuged, and the supernatant was pipetted into a 96-well plate. Samples were subsequently processed in 96-well plates. The mixture for PCR contained 5.5 μL of Multiplex PCR Master Mix (Qiagen), 2 μL of diluted DNA, 0.5 μL mix of diluted primers (primers had concentration between 0.05 and 0.15 $\mu\text{mol/L}$) and 2 μL of sterile H_2O . We used the following settings for PCR: 95 $^{\circ}\text{C}$ for 15 min; 32 cycles of 94 $^{\circ}\text{C}$ for 30 s, 60 $^{\circ}\text{C}$ for 90 s, 72 $^{\circ}\text{C}$ for 60 s, and, finally, 60 $^{\circ}\text{C}$ for 30 min. After PCR, we added 40 μL of sterile H_2O . Samples were sent to the Advanced Analysis Centre Genomic facility at the University of Guelph for fragment analysis. We used GeneMarker 1.91 software (SoftGenetics, State College, PA, USA; Holland and Parson 2011) for the identification of alleles. For the final analysis, we excluded all samples in which amplification failed in more than three (out of 14) loci.

If an individual was homozygote, its sex was determined as male, but if it was heterozygous in at least one locus, it was determined to be female. All heterozygotes were heterozygous in at least three loci. Moreover, we confirmed this method of sex determination on 59 adult females and 9 adult males (including 8 males which were used for testing microsatellite loci, but genotypes were not included in the final dataset).

We successfully analyzed 201 individuals (141 offspring, 59 adult females, and 1 adult male). For further analyses of within-nest relatedness, we used 13 two-female (26 adult females and 76 offspring) nests and 9 solitary nests (9 adult females and 30 offspring) collected in May in Kelaat Mgouna. Results of relatedness in three-female nests, four-female nests and in different locations or parts of the season are presented in [Supplementary Materials](#) in the section *Relatedness in interesting nests*.

We used two methods of calculations for the analysis of in-nest relatedness: 1) Colony (Jones and Wang 2010) and 2) Kingroup (Kononov et al. 2004) software. For Kingroup software, there is supposed $r = 0$ for unrelated individuals, $r = 0.25$ for half-sisters, $r = 0.5$ for mother–daughter association, and $r = 0.75$ for full sisters. However, due to a limited number of loci, real coefficients fluctuate around these values (therefore empiric data often show slightly

negative relatedness ($r = 0.01$ – 0.1) for unrelated individuals and occasional outliers can be present (e.g., $r = 1$ for full sisters). Kingroup does not calculate the correct asymmetric coefficients between males and females in haplodiploids and calculates the average value of female–male and male–female relatedness. Therefore, to obtain correct coefficients of female–male relationships, we multiplied the original values by 2/3. After this correction, relatedness coefficients were very close to the expected 0.5 in mother–son dyads which were assigned by Colony. If the results of Colony and Kingroup disagreed, we visually inspected genotypes and chose the more probable scenario. In two-female nests, we tested which female is the mother of the offspring and the relatedness between adult females. In solitary nests, we tested maternity of offspring (if the present female is the mother of the offspring). Additional details for relatedness calculations are available in the [Supplementary Table S4](#).

Demographic data

Number of brood cells

For each nest, we counted the number of brood cells provisioned from dissected nests.

Brood cell mortality

For calculations of brood cell mortality, we included all nests containing brood or parasites (active brood nests, full brood nests, and damaged nests without stage identified). *Gasteruption* wasps often destroyed multiple brood cells, and the exact number was difficult to identify. Therefore, the number of brood cells damaged by *Gasteruption* was assumed as three, if there were not clear distinctions of the number of brood cells (Mikát and Rehan 2022). We calculated the proportion of brood cells destroyed by natural enemies.

Number of live brood

The proportion of live brood was calculated by subtracting the proportion of brood cell destroyed from 1. The number of live brood was obtained by multiplying the number of brood cells provisioned (corrected to nests stage) and the proportion of live brood.

Probability of death of social primary in two-female nests

The social primary is most probably the principal forager in two-female nests (Mikát and Rehan 2022), similarly as in most carpenter bees, where the reproductively dominant female also usually dominates foraging (Hogendoorn and Velthuis 1995, 1999; Schwarz et al. 2011; Mikát et al. 2021). As foraging behavior is highly risky, we suppose that the probability of death of the foraging female is much higher than the probability of death of the non-foraging female. Non-foraging female should have unworn wings and we suppose that if a female with totally unworn wings occurs in solitary nests, it should be a false solitary nest—originally a two-female nest where the social primary died. Therefore, we calculated the probability of death of social primary from the proportion of solitary nests with a lone adult female with totally unworn wings. The probability of death of social primary should be equivalent to the probability of nest inheritance of a social secondary.

Fitness benefits from nest inheritance

We assumed that the social secondary which inherited a nest has the same reproductive capacity as a solitary female, as is typical for facultatively social Hymenoptera (Smith et al. 2009; Rehan et

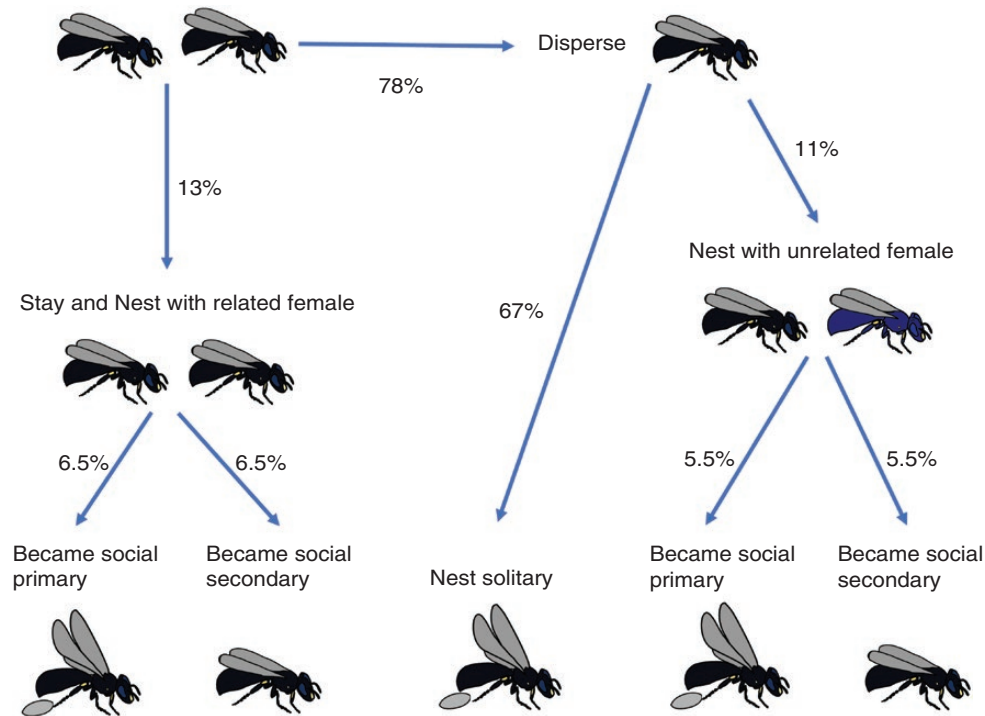


Figure 1

Possible routes of females of *Ceratina albosticta*. Solid arrows show reproductive strategies of females. Numbers next to arrows shows the proportion of females in population with this strategy. Females inhabiting nests with more than two females are not shown in diagram. Reproductive females shown with an egg under their abdomens and foraging females have wings risen. Relatedness of females is shown by color of bee with black females related and blue females unrelated.

al. 2014). Therefore, we use the fitness of a solitary female also for direct fitness benefits of a social secondary which inherits the nest. This result is very similar to average fitness benefits of the two nests in which we detected nest inheritance (see [Supplementary Materials](#), section fitness in benefits false solitary nests).

RESULTS

Mating frequency

We analyzed mating frequency in 15 nests where there were at least two female offspring ($N = 5$ solitary, 8 two-female, 1 three-female, and 1 four female nests). The average relatedness calculated by Kingroup between female offspring in the same nest was 0.60 (range 0.10–1.00). This result shows that single mating is prevalent, as the expected relatedness of single mating is 0.75. Colony analyses showed that the mother was singly mated in 80% of nests (12/15) and multiple mated in 20% of nests (3/15). The proportion of multiple-mated females was 20% (1/5) in solitary nests and 25% (2/8) in two-female nests. From nests with multiple mated females, in one case, the mother was double mated and in two cases there were three patriline. The average relatedness between female offspring in single-mated nests was 0.76 (range 0.41–1.00, $N = 12$ nests, 29 individuals). The average relatedness between female offspring in multiple mated nests was 0.34 (range 0.10–0.74, $N = 3$ nests, 8 individuals).

Maternity in solitary nests

Relatedness between the adult female and all offspring in solitary nests was 0.40 on average ($N = 30$, range -0.08 – 0.71 , $SD = 0.20$).

Table 1

Relatedness coefficients obtained from Kingroup between adult females in two-female nests

	Related females	Unrelated females	All nests
N nests	7	6	13
Average relatedness	0.73	-0.06	0.37
Range	0.51–0.93	-0.19–0.18	-0.19–0.93
SD	0.14	0.15	0.43

The average relatedness between the adult female and female offspring was 0.39 ($N = 15$, range -0.06 – 0.71 , $SD = 0.22$), and the average relatedness between adult female and male offspring was 0.40 ($N = 15$, range -0.08 – 0.57 , $SD = 0.18$).

Colony-assigned maternity of all offspring to the adult female present in the nest in 77% ($N = 9$ nests) of solitary nests examined. According to Kingroup, relatedness between female and offspring was 0.48 on average (range 0.21–0.70, $SD = 0.12$) in these nests. Another two nests had more complicated scenarios. Both Colony and Kingroup confirmed that in one of the solitary nests, no offspring ($N = 4$) were assigned to the adult female that was present in nest, and in a second nest, the two offspring at the bottom of the nest were not assigned to present female, but the three offspring in the upper nest toward the entrance were assigned to her. For more detailed results and relatedness coefficients in these nests, see [Supplementary Materials](#) (section Maternity in solitary nests).

Relatedness between adult females in two-female nests

We analyzed 13 two female nests. Kingroup software showed pronouncedly bimodal pattern of relatedness between social primary and social secondary. In six nests, the relatedness coefficient between adult females was very small (-0.06 on average; Table 1). Analysis in Colony software confirmed that females with low relatedness coefficients are unrelated (Supplementary Table S3). In seven nests, the relatedness coefficient between adult females was very high (0.73 on average; Table 1). Analysis in Colony software and Kingroup showed support that these females are most probably full sisters ($r = 0.75$) and not mother and daughter ($r = 0.5$; see Supplementary Table S3). Thus, seven nests were categorized as related and six unrelated in terms of social females in these two-female nests.

Maternity in two-female nests

Combining results of Colony, Kingroup and manual inspection of genotypes, we assigned 74/76 of offspring to one of the adult females in each of the nests, 1/76 of offspring was most probably not the offspring of any of the females present, and 1/76 of offspring was not reliably assigned (Supplementary Table S4). All studied nests ($N = 13$) had offspring produced by only one of the present females (social primary), we did not find any reliable evidence for the reproduction of the social secondary. In all nests with related females ($N = 7$), we assigned all offspring to the social primary in six nests. In one nest, we assigned all but one offspring to the social primary, and for one offspring, we were unable to make a reliable assignment. In nests with unrelated females ($N = 6$), we assigned all offspring to the social primary in five nests. In one nest, three offspring are assigned to one mother, and one offspring is not assigned to either of the two adult females. For relatedness coefficients between adult females and offspring, see Table 2. For a comparison between different methods of offspring assignment, see Supplementary Table S4.

Table 2
Relatedness between female and offspring in two-female nests

	Related females		Unrelated females		All nests	
	Social primary	Social secondary	Social primary	Social secondary	Social primary	Social secondary
All offspring						
<i>N</i> nests	7	7	6	6	13	13
<i>N</i> offspring	42	42	34	34	76	76
Average	0.51	0.37	0.48	-0.06	0.51	0.14
Range	0.42–0.67	0.10–0.57	0.05–0.52	-0.27–0.12	0.05–0.67	-0.27–0.57
SD	0.05	0.10	0.11	0.15	0.08	0.25
Female offspring						
<i>N</i> nests	6	6	6	6	12	12
<i>N</i> offspring	11	11	14	14	25	25
Average	0.51	0.41	0.51	-0.04	0.51	0.16
Range	0.42–0.67	0.33–0.57	0.05–0.67	-0.27–0.44	0.05–0.67	-0.27–0.57
SD	0.08	0.06	0.16	0.19	0.12	0.25
Male offspring						
<i>N</i> nests	7	7	5	5	12	12
<i>N</i> offspring	31	31	20	20	51	51
Average	0.51	0.36	0.47	-0.07	0.51	0.14
Range	0.43–0.55	0.10–0.51	0.32–0.52	-0.24–0.18	0.32–0.55	-0.24–0.51
SD	0.04	0.11	0.04	0.13	0.057	0.26

Demographic data for fitness calculations

Number of brood cells provisioned and mortality

The number of brood cells provisioned was on average 2.24 in solitary nests, 9.00 in two-female social nests with related females, and 7.16 in social nests with unrelated females (Table 3). Brood cell mortality was 23% in solitary nests, 15% in two-female nests with related females, and 5% in two-female nests with unrelated females (Tables 3, Supplementary Table S5). The total number of live brood we obtained from the number of brood cells minus brood cell mortality presented above and it was on average 1.71 in solitary nests, 7.65 in social nests with related females, and 6.77 in social nests with unrelated females.

Nest inheritance

We supposed that females who did not forage should have totally unworn wings (wing wear score = 0). The proportion of these females in solitary nests was 5.47% (4/73). Therefore, we suppose that the proportion of false solitary nests in solitary nests is 5.47%. Solitary nests had a proportion in the population of 80.43%. Therefore, the proportion of false solitary nests from all nests should be 4.40% ($= 0.8043 \times 0.0547$).

The proportion of two-female nests in the beginning of the nesting season should be the sum of the proportion of detected two-female nests (14.49%, 20/138) and false solitary nests (4.40%), therefore 18.89%. The probability of nest inheritance is calculated as the proportion of false solitary nests (4.40%) from the proportion of two-female nests in the beginning of nesting season (18.89%), therefore is 23.32% ($0.044/0.1889$).

Model for fitness calculations

Inclusive fitness has two components: direct fitness from own reproduction, and indirect fitness from reproduction of related individuals. Indirect fitness is the result of reproductive success of related individuals. Social behavior strongly influences not only reproduction of the focal female but also reproduction of

Table 3
Number of brood cells provisioned and brood cell mortality in solitary and two-female nests

	Solitary nest	Two-female nests: related	Two-female nest: unrelated
Number of brood cells provisioned			
<i>N</i> Nests	111	7	6
Average	2.24	9.00	7.16
Range	1–9	3–13	4–10
SD	1.67	3.41	2.31
Brood cell mortality			
<i>N</i> Brood cells	226	60	37
% Dead brood	23.45	15	5.4
Average number of offspring survived	1.71	7.65	6.77

her social mate—as we showed above, only one female in a social nest reproduces. If two full sisters nest together, the consequence are offspring produced in social nests (direct fitness for social primary, indirect fitness for social secondary), but also loss of indirect fitness as this prevents the full sister for solitary nesting.

The indirect fitness component is easily measurable in social nests with related females, where both sisters are in the same nest. However, sisters also reproduce in situations when they disperse and reproduce elsewhere. In our study system, each female has three main options: nest solitary, nest with a related female and nest with unrelated female (Figure 1). Nest with related females is composed by females which originated from same nest, solitary nests and two-female nests with unrelated females are the consequence of dispersal of at least some females. We can imagine that in beginning of the nesting season, we have two full sisters, which can nest together (social nest with related females), or separately (solitary nests or form social nest with unrelated females). We estimated inclusive fitness of each female from two main components: 1) direct fitness as a result of direct reproduction 2) indirect fitness as result of reproduction of full sister (regardless of if this sister is present in the same or other nest). Additionally, we added possible direct fitness benefits from nest inheritance.

The direct fitness of female of each strategy was calculated from the number of offspring and relatedness to them (see below). Indirect fitness for social secondaries in nests with related females was calculated based on the number of offspring of the social primary multiplied by the relatedness of these offspring to the social secondary. For correct fitness calculation, we need to include indirect fitness benefits from full-sister nesting elsewhere. This fitness benefit is relevant for solitary females, social primaries, and also social secondaries from nests with unrelated females. If a full sister disperses, she can nest solitary (86% of cases), become social primary in nests with unrelated females (7%) or become social secondary in nests with unrelated females (7%). The number of offspring, which this female produce differs according to the situation, 1.71 for solitary nests, 6.77 if becomes a social primary, and 0 if becomes a social secondary in nests with unrelated females. Therefore, she most probably produces 1.95 offspring ($0.86 \times 1.71 + 0.07 \times 6.77 + 0.07 \times 0$). The relatedness of the focal female to offspring of a full sister is 0.37. Therefore, indirect fitness benefits are $1.95 \times 0.37 = 0.72$.

Fitness benefits of different strategies

Solitary female

Direct fitness benefits equate to the number of live offspring (1.71) multiplied by relatedness to these offspring (0.4) and therefore equal 0.68. Indirect fitness benefits from sister nesting elsewhere is 0.72. The final inclusive fitness benefit is thus $0.68 + 0.72$ or 1.40.

Social primary in nests with related females

Direct fitness benefits equate to the number of live offspring (7.65) multiplied by relatedness to these offspring (0.51) and therefore equal 3.90. There are no indirect fitness benefits and therefore the final fitness is 3.90.

Social secondary in nests with related females

There is no direct fitness from reproduction in the presence of a social primary. There is potential direct fitness benefits in case of nest inheritance. Fitness from nest inheritance was the probability of nest inheritance (0.23) multiplied by the number of brood produced after nest inheritance (1.71) and relatedness to this brood (0.4), therefore equaling 0.16. Indirect fitness benefits are the number of offspring in a nest (7.65) multiplied by her relatedness to these offspring (0.37), and therefore equal 2.83. Final inclusive fitness is $2.83 + 0.16$ totaling 2.99.

Social primary in nests with unrelated females

The number of live offspring (6.77) multiplied by their relatedness to these offspring (0.48) equals 3.25. Indirect fitness benefits from sister nesting elsewhere as above calculated equals 0.72. Taken together, the final inclusive fitness benefit is $3.25 + 0.72$, which is 3.97.

Social secondary in nests with unrelated females

There is no direct fitness from reproduction in the presence of a social primary, but there are potential direct fitness benefits in the case of nest inheritance. Fitness from nest inheritance was the probability of nest inheritance (0.23) multiplied by the number of brood produced after nest inheritance (1.71) and relatedness to these broods (0.4), therefore totaling 0.16. Indirect fitness benefits from sister nesting elsewhere is 0.72. Therefore, the final inclusive fitness benefit of this strategy is 0.88.

Fitness calculations

The final inclusive fitness benefits were 1.40 for solitary females. In comparison with solitary females, social primaries had much higher fitness benefits in nests with related females (3.90) also in nests with unrelated females (3.97). Social secondaries had high fitness benefits in nests with related females (2.99), but smaller inclusive fitness in nests with unrelated females (0.88).

Fitness calculations for social females regardless their relatedness

For the calculation of fitness averaged across social primary and secondary females, we combined data from nests with related and unrelated females in a ratio 7:6, as this was empirical ratio that we found in the population. The fitness benefits of an average social primary was 3.94; the fitness benefit of an average social secondary was 1.94. Therefore, regardless of relatedness, a random social secondary could have fitness benefits higher than a solitary female (1.40).

DISCUSSION

Identification of key factors responsible for forming social groups is still under intensive scientific discussion (Riehl 2013; Liao et al. 2015; Gadagkar 2016; Quiñones and Pen 2017; Ohkubo et al. 2018; González-Forero and Peña 2021). Helping behavior can evolve in extreme situations based on direct or only indirect fitness benefits (García-Ruiz et al. 2022). However, in simple insect societies (Reeve and Keller 2001; Leadbeater et al. 2011) as well as cooperative breeding vertebrates (Riehl 2013; Josi et al. 2021) reproductive subordinates often benefit from a combination of direct and indirect fitness benefits. We studied demographic parameters and within-nest relatedness in the facultative social small carpenter bee, *Ceratina albosticta*. In social nests, we found variability in relatedness between adult females, but reproduction was fully dominated by one of them. Social primaries always have high inclusive fitness in comparison with solitary females, mainly because of the high nest productivity of social nests. As social secondaries do not reproduce, their fitness is highly dependent on their relatedness to the social primary, because they can benefit from inclusive fitness only in situations when they are related. Social secondaries have higher inclusive fitness in comparison with solitary females only when she was related to the social primary. We show that direct fitness benefits play only a marginal role for social secondaries in *C. albosticta* and do not influence if social nesting is beneficial or not.

Ceratina albosticta are unique in the combination of a low proportion of social colonies with the main role of indirect fitness benefits of social nesting for the social secondary. High indirect fitness benefits for the social secondary are caused by the large increase in nest productivity of social nests. Small proportions of social colonies in a population are typical for *Ceratina* and *Xylocopa* bees, but per capita productivity usually does not increase and often decreases with the number of adult females (Prager 2014; Rehan et al. 2014; Mikát et al. 2022).

On the other hand, taxa with increasing per capita productivity from one to two females, such as allodapine bees, usually have higher proportion of social nests in population (Chenoweth and Schwarz 2007; Bernauer et al. 2021; Jeanne et al. 2022). High fitness benefits of social secondary bring into question why social nesting is present only in a minority of nests. It is possible that the fitness of social nesting varies between seasons, as is reported for *Xylocopa sulcatipes* (Stark 1992). Another explanation can be that the habitat where we studied *C. albosticta* (human-managed rose plantation) had artificially increased population density and also the density of nesting opportunities. Therefore, this species may be adapted to different ecological conditions than is present nowadays, and the observed pattern may have no adaptive benefit. Possibly, there is a problem with forming groups of related females—as we showed above, social nesting is beneficial for a social secondary only if females are related. We suppose that social nests usually originate from females that overwintered together in the same twig and later reused hibernaculum for a new nest. *C. albosticta* females can probably overwinter solitarily but also in groups with other conspecific individuals (Daly 1983; Mikát and Rehan 2022). Group or related females can form only in situations when these females overwinter together. Moreover, in the late phase of the nesting season, adults of *Ceratina* bees frequently exchange between nests (Mikát et al. 2017, 2020, 2021), therefore, individuals which overwinter together can also be unrelated. Joining of individual from another nest cannot be beneficial for a female which becomes a social secondary. This is in contrast in cooperative breeding vertebrates (Clutton-Brock

2007) and also some social wasps (Fanelli et al. 2005; Leadbeater et al. 2011), where unrelated helpers often have substantially high direct fitness benefits.

Two-female nests had generally higher fitness benefits in comparison to solitary nests. We sampled only few nests with more than two adult females, therefore, it is difficult to evaluate their fitness. However, we suppose that increased colony sizes with greater than two adult females are not beneficial, as per-capita productivity strongly decreases from two-female to three- and four-female nests (Mikát and Rehan 2022), and relatedness between females in three- and four-female nest is generally low (see Supplementary Materials, section relatedness in interesting nests). This is not surprising as unimodal relationship between per capita productivity and colony size is known also from several allodapine bees (Bull and Schwarz 2001; Bernauer et al. 2021).

Relatedness between adult females

We found diversity in relatedness between adult females in social nests. In almost half of two-female nests females were surely unrelated. In other nests, adult females were related and likely full sisters. The presence of full sisters and not half sisters is a consequence of prevailing single-mating in this species, which is typical for simple social Hymenoptera and increases the indirect fitness benefits for social secondaries (Hughes et al. 2008). However, it is interesting that together with strongly prevailing single mating (possibly favored by kin selection), there is also the presence of nests with cooperative brood rearing among unrelated females. In May, we did not detect any nests in which social females were mother and daughter associations. As nests share females from the same generation, social nests can be classified as semisocial (Wilson 1971; Michener 1974). However, there is the possibility that mother-daughter associations emerge later in the nesting season after females of first brood reach adulthood. One two-female social nest collected in September probably contained a mother and daughter association where the mother reproduced and probably also foraged and the daughter probably served as a guard (see Supplementary Materials).

Reproductive division of labor

Reproductive dominance is easily established in eusocial species with discrete castes, where some females are apparently subfertile or even sterile (Crespi and Yanega 1995; Beekman and Oldroyd 2019). However, in simple societies, the establishment of social hierarchies is more difficult as differences between reproductive dominant and subordinate females are weak or even missing. In simple societies, reproductive dominance is typically dependent on behavioral interactions between adult females (Bridge and Field 2007; Lucas et al. 2011; Saleh et al. 2022). *C. albosticta* is a species without apparent castes and without reproductive dominance hierarchies based on body size based (Mikát and Rehan 2022). Similarly, absolute reproductive skew was detected in a facultatively social collectid bee, *Amphyleus morosus*, which also do not have morphological differentiation between social primary and secondary females (Hearn et al. 2022). Reproductive skew models suppose non-complete skew if unrelated females nests together (Dunn and Richards 2003). Interestingly, we detected absolute reproductive skew also in nests with unrelated females, where the social secondary has no inclusive fitness benefits. There can be two reasons for unrelated social secondaries to adopt a reproductive subordinate role: possible benefits from nest inheritance and the inability perform kin recognition (see below).

Nest inheritance

The possibility of nest inheritance can be an important potential benefit for social secondaries (Leadbeater et al. 2011). We detected two situations, where offspring in the bottom of nests were not assigned to the adult female which was present in the nest, but upper offspring were clearly assigned. Therefore, nests where the social primary died can be inherited by another female, which may use it to continue their reproduction, and therefore, the possibility of nest inheritance can play a role in the fitness of social secondaries in this species. It is possible that we underestimated the frequency of nest inheritance, as we sampled nests in the beginning of the nesting season. However, we suppose that nest inheritance probably plays a relatively small role, as benefits from nest inheritance for a social secondary are much smaller than benefits from indirect fitness. The reason can be a relatively high probability of adult female survival until adulthood of her immature offspring, which is caused by the short developmental time of *Ceratina* bees (between 30 and 45 days; Rehan and Richards 2010; Rehan et al. 2010). Generally, nest inheritance plays an important role in situations where the probability of nest inheritance is high and/or benefits of inheriting a nest is high. Nest inheritance plays an important role in Stenogastrine wasps, where the social primary often does not survive until the adulthood of immature offspring due to long juvenile development times (Landi et al. 2003; Bolton et al. 2006). Inheritance-based benefits can be high in situations where the nest or territory itself is valuable as evidenced in cooperative breeding mammals (Marino et al. 2012), birds (Cockburn 1998; Pen and Weissing 2000; Riehl 2013; Kingma 2017), large carpenter bees (Stark 1992; Ostwald et al. 2021), and in situations when adult females also inherit a group of helpers, such as in Polistinae or Stenogastrinae wasps (Field et al. 1998; Leadbeater et al. 2011). None of this is true in the case of *C. albosticta*, because the availability of nest opportunities seems not to be a limiting factor, as a lot of available twigs were present in the studied location (Mikát and Rehan 2022), and if the social primary dies in a two-female nests, the social secondary became a solitary reproductive female.

Possible influence of recognition

There is the question of why a social secondary that is unrelated to the social primary stays in the nest with such reduced fitness benefits. Our results show that adopting the role of social secondary is strongly beneficial if the social secondary is related to the social primary, but strongly disadvantageous if the social secondary is unrelated to the primary in comparison with solitary nesting. We might predict that a social secondary unrelated to the social primary should perform direct reproduction and, therefore, have some direct fitness benefits (Reeve and Keller 2001; Leadbeater et al. 2011). However, the reproductive skew was the same in nests with related and also unrelated females. Thus, social secondaries do not reproduce in the presence of primaries. A possible explanation for this pattern is that a social secondary does not know if it is related or unrelated to the social primary, as true kin recognition is missing in many social insects, and recognition is usually based on nestmateness, not relatedness (Hogendoorn and Leys 1993; Breed 2014; Vickruck and Richards 2017). If the social secondary is unable to assess her relatedness to the social primary, this reproductive strategy may persist if the fitness of the average social secondary is greater than or equal to that of a solitary female. We found that at random, the average fitness of a social secondary is higher than that of a solitary female. If the social secondary is

unable to assess relatedness to the social primary, it is, on average, more beneficial to adopt the role of social secondary than to perform solitary nesting. Therefore, we suggest that for understanding the evolution of sociality, it is relevant to know which strategy is beneficial but also to assess if a focal female has ability to distinguish kin from non-kin.

CONCLUSIONS

Ceratina albosticta lacks morphological castes, as is usual in simple insect societies. However, although true castes are missing, we detected absolute reproductive skew in studied nests. Reproductive dominance of one female occurred in all nests regardless of the variability in within-nest relatedness between adult females. Indirect fitness is a key fitness benefit of reproductive subordinates, and therefore, within-nest relatedness strongly influence their benefit. We showed that reproductive monopolization is possible in a simple society although this situation is strongly disadvantageous for social secondaries in some nest—in nests where unrelated females nests together. Nests with different relatedness situations had the same reproductive skew. As these different relatedness situations lead to strongly different fitness benefits for social subordinates, there remains a question as to how reproduction division of labor is controlled—it can be caused by coercion of the reproductive dominant or by the subordinate's inability to distinguish between different relatedness situations. Therefore, future studies of kin and nestmate discrimination as well as within-nest behavior are a critical next step and will be most useful for understanding how social hierarchies are established in simple societies.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>.

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CONFLICT OF INTEREST

We declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Michael Mikát (Conceptualization [Equal], Formal analysis [Lead], Funding acquisition [Equal], Investigation [Lead], Visualization [Lead], Writing – original draft [Lead]), and Sandra Rehan (Conceptualization [Equal], Formal analysis [Supporting], Funding acquisition [Equal], Investigation [Equal], Project administration [Lead], Resources [Lead], Supervision [Lead], Writing – review & editing [Lead])

DATA AVAILABILITY

Analyses reported in this article can be reproduced using the data provided by Mikát and Rehan (2023).

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