



Knowing who's who: nestmate recognition in the facultatively social carpenter bee, *Xylocopa virginica*

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ARTICLE INFO

Article history:

Received 12 February 2009
Initial acceptance 8 June 2009
Final acceptance 16 October 2009
Available online 4 January 2010
MS. number: A09-00091R

Keywords:

carpenter bee
circle tube
facultative sociality
nestmate recognition
Xylocopa virginica

When animals live in groups, the ability to discriminate group members from nonmembers allows individuals to adjust their behaviour in ways that enhance both individual and group fitness. In social insects, especially bees, this kind of recognition has mainly been studied with reference to colony-based social interactions, but they also interact in other kinds of group contexts. In the facultatively social carpenter bee, *Xylocopa virginica*, individuals of both sexes interact repeatedly, in such activities as cooperative brood raising by females, territorial competition by males, and matings near nest entrances. In light of these varied interactions, in which the ability to discriminate familiar from unfamiliar individuals might be advantageous, the aim of our study was to determine whether *X. virginica* adults are capable of nestmate recognition. Nestmates were defined operationally as bees that were caught at the same nest entrance after spending the night together, whereas non-nestmates were from different nests. We used circle tube assays of male–male, female–female and male–female dyads to compare rates of aggressive (pushing, biting and C-postures) and tolerant/cooperative (head-to-head touching and passing) behaviour in nestmates versus non-nestmates. In general, aggression occurred sooner and more frequently among non-nestmates than among nestmates, whereas tolerance was more common among nestmates. This indicates that male and female bees can recognize familiar individuals of both sexes and adjust their behaviour accordingly. Since tested individuals may have been nestmates for less than 24 h, this further suggests that both female and male bees may learn the identities of their nestmates quickly. © 2009 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

When animals live in groups, the ability to discriminate members of the group from nonmembers confers the opportunity for individuals to adjust their behaviour in ways that enhance both individual and group fitness. Nestmate recognition involves at least two individuals, a cue-bearer and a cue-responder or perceiver (Gamboa et al. 1986; Fletcher & Michener 1987), and is recognized by human observers when cue-responders behave differently towards group members versus nonmembers. In many social contexts, such as cooperative care of juveniles, individuals are expected to favour group members over nonmembers, and in these situations, a perceiver should respond to a familiar cue-bearer with tolerance or cooperation and to an unfamiliar individual with aggression. On the other hand, in social contexts in which group members may be discriminated against, such as inbreeding

avoidance, a perceiver should respond more favourably to an unfamiliar individual than to a familiar one.

In social insects, the most important type of group is a colony, an assemblage of adults and juveniles, usually related, that live together, often in a particular structure called a nest (Michener 1974). In social Hymenoptera, most adult colony members are closely related females whose social interactions take place mainly in the context of the colony. Consequently most research on nestmate recognition in social bees and wasps has focused on the ability of females to discriminate between colony members and nonmembers, or more subtly, on the ability to distinguish among nestmates based on their degree of genetic relatedness (Greenberg 1979; Gamboa et al. 1987b). Numerous studies of social bees and wasps have demonstrated the ability of nest guards to accept foragers returning to their home nests with food provisions, and to repel intruders, including nest usurpers, pollen robbers and kleptoparasites that lay eggs on brood provisions (Michener 1966, 1974; Bell & Hawkins 1974; Gamboa et al. 1987a; Breed & Page 1991; Inoue et al. 1999; Steen 2000; Moritz & Neumann 2004).

However, the social interactions of social insects are not limited to those experienced by adult females in the context of colonies. Social insects also live in other kinds of groups, or experience other

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kinds of repeated social interactions in which the ability to recognize and discriminate between familiar versus unfamiliar individuals could be advantageous. For instance, in some species of bees, males congregate in sleeping clusters (Alcock 1998) or guard contiguous territories (Barrows 1983). In the latter case, territorial males that can recognize familiar males that defend territories nearby may be able to reduce costly aggressive interactions with known competitors, but remain more vigilant or aggressive with novel individuals, a phenomenon known as the 'dear enemy' effect (Gordon 1989). Another potential function of nestmate or group-mate recognition is inbreeding avoidance. One mechanism by which inbreeding could be avoided would be for both males and females to discriminate against nestmates as potential mates, since nestmates would often be kin. The haplodiploid sex determination system of bees results in heightened sensitivity to deleterious inbreeding effects (Zayed & Packer 2001), so both females and males may benefit by avoiding mating with group members that are likely to be relatives.

Previous studies of nestmate recognition in bees have largely focused on social (communal and eusocial) species and have tested the twin predictions that adult females should show tolerant or cooperative behaviour towards nestmates and intolerant or aggressive behaviour towards non-nestmates. In general, both these predictions have been supported in eusocial bees (Breed 1983, 2003), while only the former prediction is supported in communal species (Kukuk & Crozier 1990; Kukuk 1992). Interspecific comparisons suggest that semisocial and eusocial bees (both types show reproductive division of labour between queen-like and worker-like individuals) generally show the strongest discrimination, being both more cooperative with familiar individuals and more aggressive and intolerant with unfamiliar individuals (Pabalan et al. 2000; Packer 2006). In contrast, communal bees (in which females have equivalent reproductive status) are quite tolerant of unfamiliar individuals (Kukuk 1992; Paxton et al. 1999; Packer 2006). That nestmate discrimination appears to be strongest in obligately social species illustrates its important role in maintaining colony cohesiveness and in helping colony members to interact and cooperate efficiently, but nestmate recognition also occurs in solitary bees, which live in family groups, but not in colonies (as defined by Michener 1974). Two studies in primarily solitary bees, *Manuelia postica* (Flores-Prado et al. 2008) and *Lasioglossum figueresi* (Wcislo 1997), have found experimental evidence for nestmate recognition by adult females. Although the function of nestmate recognition per se in the nesting biology of these solitary species is not clear, the phylogenetic position of *M. postica* suggests that nestmate recognition preceded the evolution of eusociality in the family Apidae (Flores-Prado et al. 2008), whereas the phylogenetic position of *L. figueresi* illustrates that nestmate recognition is a trait that can be retained in evolutionary reversions from sociality to solitary behaviour (Wcislo 1997).

Nestmate recognition has not yet been shown in facultatively social bees, in which some females nest solitarily while others nest socially, or in male bees, which usually live solitary lives but may also sometimes spend time in groups. The large carpenter bee, *Xylocopa virginica*, which is endemic to the eastern half of North America, offers a unique opportunity to study nestmate recognition in a species in which females are facultatively social, males are territorial, and members of both sexes have many opportunities for repeated social encounters with nestmates and non-nestmates of both sexes (Gerling & Hermann 1978; Barrows 1983; Barthell & Baird 2004; Barthell et al. 2006). Several aspects of the reproductive behaviour of *X. virginica* suggest that nestmate discrimination would be advantageous for both females and males. In our study population in southern Ontario, about 10% of females nest solitarily, but most nest socially, in groups of two to five. In social nests, brood

provisioning and reproduction are dominated by one or two dominant females in each group, with subordinate females contributing little or nothing to reproduction, some remaining more or less inactive in the nest throughout the season (M. H. Richards, unpublished data). Although co-nesting females may often be sisters that overwintered in their natal nest, colony membership is more fluid than is usually expected in primitively social bees, because females may move from nest to nest during the breeding season (M. Peso & M. H. Richards, unpublished data), and foragers sometimes bring pollen provisions to more than one nest (C. Course & M. H. Richards, unpublished data). However, females that attempt to enter new nests are often aggressively excluded by the residents, which implies some sort of discrimination between familiar versus unfamiliar individuals. Second, subordinate social females that do not forage probably consume pollen and nectar brought to the nest by the dominant females, and it is not clear why their presence is tolerated by the dominant females, even if they are kin. Certainly, dominant females should prevent additional, non-contributing subordinates from joining their colonies. Third, adult males vigorously defend contiguous territories surrounding the nests, then return to the nests at night, often to nests they have slept in previously (M. Peso & M. H. Richards, unpublished data). During these overnights, males and females have opportunities to interact with both familiar and unfamiliar individuals of both sexes. In short, both females and males experience daily and repeated interactions with nestmates and non-nestmates of both sexes, some of which are likely to be kin and many of which are not, both inside and outside of nests.

The major objective of our study was to determine experimentally whether nestmate recognition occurs in *Xylocopa virginica*, based on observations of nestmates and non-nestmates interacting in circle tube arenas, which have been used in many investigations of social recognition in bees (McConnell-Garner & Kukuk 1997; Wcislo 1997; Pabalan et al. 2000; Arneson & Wcislo 2003; Packer et al. 2003; Packer 2006). A second objective was to determine whether female–female, male–male and male–female pairs would show similar patterns of discrimination. Studies of nestmate recognition in social insects cite increased tolerance and cooperation with familiar or acceptable individuals coupled with increased aggressive behaviour towards unfamiliar or unacceptable individuals as joint indicators of nestmate or group-member recognition (Bell et al. 1974; Breed & Page 1991). Consistent with this, we predicted that in same-sex pairs, nestmates would show greater tolerance or cooperation whereas non-nestmates would show more aggression. We predicted that in male–female pairs, the opposite pattern would occur, as both sexes would be expected to discriminate against familiar individuals as potential mates.

METHODS

Study Population

The following summary describes the social behaviour and nesting phenology of *X. virginica* in the Niagara region of southern Ontario. The timing of major events in Niagara differs in several aspects from previously described, more southerly populations (Gerling & Hermann 1978; Barrows 1983; Barthell & Baird 2004; Barthell et al. 2006). Most importantly, the bees observed here were all overwintered adults flying at a time of year when brood were being provisioned or reared and were still several weeks from emerging as adults.

Since at least 2001, a population of 200–300 adult *X. virginica* has nested in cedar benches in a sheltered courtyard of the Brock University campus, in St Catharines, Ontario, Canada (43°11' N, 79°14' W). Adult males and females overwinter together in their

natal nests. In spring, some adults disperse to other locations, but many remain in our study aggregation. Males establish and aggressively defend territories (the cedar benches) for several weeks in May and June. During this period, they encounter other males more often than they do females (Barrows 1983; Barthell & Baird 2004). Females emerge from their nests several days to weeks after the males, to feed themselves and then to forage for brood provisions. Each year most females reoccupy old nests, so at the beginning of the breeding season, social nests consist of multi-female associations that are probably composed of sisters that overwintered together. A minority of females nest solitarily. Solitary females are often found in new nests or reoccupying abandoned burrows.

During a 'mating season' that lasts only a few weeks in May to early June, males attempt to intercept and mate with females as they leave their nest entrances or when they return. Since male territories may contain multiple nests, potentially including males' natal nests, mating encounters between siblings are possible. Males often enter nests to spend the night, although some evidently overwinter on nearby flowers and shrubs. After males disappear from the aggregation, females remain active, foraging and bringing provisions to nests from May to early or mid-July. As many as 90% of nests are social, containing two to five overwintered females. In most social nests, only one or two females provision brood on any particular day, but observations of marked females show that multiple females bring pollen into nests over the course of the summer, and that individual females sometimes bring pollen to more than one nest (C. Course & M. H. Richards, unpublished data). Both females and males move from nest to nest. Occasional violent fights between females suggest attempts at nest usurpation, although pollen robbery has not been observed.

Bee Capture and Care

Bees were collected using 'cup traps'. A cup trap consisted of a clear plastic drinking cup with a 1–2 cm hole drilled through the bottom and parafilm stretched over the open end of the cup. Tiny holes were punched in the plastic wrap for additional ventilation. Strips of velcro were used to adhere the cup traps over the nest entrance holes, confining bees when they exited their nests. Traps were set in the morning before flight activity began and were kept in place until a bee was captured. Upon capturing a bee, the trap was immediately replaced with an empty one, so nests were monitored continuously until the end of the day.

When captured, unmarked bees were placed individually in 50 ml plastic tubes and chilled on ice for anaesthesia. While inactive, the bee's head width and intertegular width (distance between the wing bases) were measured using an eyepiece micrometer. Afterwards, the bee was painted with Testor's enamel in a unique colour combination on its thorax and abdomen for individual identification. Chilling the bees was necessary in order to keep them immobilized until their paint marks dried, as otherwise they attempted to remove the wet paint with their mouthparts and legs. Note that *X. virginica* nesting at Brock University regularly experience chilling temperatures below 5 °C (and sometimes below 0 °C) during April and May, and below 10 °C in June.

Individuals to be used for behavioural testing were kept overnight in small plastic cages in the laboratory, and were supplied with a mixture of bee pollen, honey and water ad libitum. Card-board partitions were placed between the cages so that bees could not see or interact with each other. Bees were considered to be nestmates if they were caught at the same nest entrance on the same day, and they were considered to be non-nestmates if they were trapped at different nests on the same day. Since traps were put in place before daily activity began and kept in place

continuously, all bees captured at the same nest entrance must have spent the night inside the same nest. Patterns and rates of recapture of marked bees will be presented elsewhere (M. Peso & M. H. Richards, unpublished data).

Behavioural Testing

Behavioural observations were conducted from 19 April to 23 June 2006 and from 9 May to 25 June 2007, corresponding with the period of *X. virginica* flight activity in Niagara. At this time of year, all adult bees in the population were overwintered adults, and the vast majority would have been brood of the previous year. In 2006 and 2007, we found no worn females in spring, so all of the bees tested here were breeding for the first time. Most bees were tested both as nestmates and non-nestmates in male–male pairs (nestmates: $N = 29$ pairs, non-nestmates: $N = 28$), female–female pairs (nestmates: $N = 40$, non-nestmates: $N = 49$) and male–female pairs (nestmates: $N = 23$, non-nestmates: $N = 22$); some bees were tested only once because of escapes, nonactivity in one assay, or the onset of rain. All behavioural testing was performed outdoors within 5 m of the benches containing their nests within 24 h of capture, and only when other bees in the population were observed to be active, between 1000 and 1600 hours, to ensure that the tested bees were observed during their regular activity periods.

Observations were conducted by placing bees in circle tubes (Breed et al. 1978), which provide a neutral arena in which two bees can be observed without the influence of other individuals or the nest of origin (Pabalan et al. 2000). The tubes also approximate the nest tunnels in which the bees spend much of their time (Gerling & Hermann 1978) and allow recognition assays to be carried out in natural light (Wcislo 1997). The clear plastic tubing used to create the circle tube testing arena had an inner diameter of 1.6 cm and an inner connector tube with an outer diameter of 1.6 cm. According to the guidelines established by Packer (2006), the outer tube diameter was approximately double the head width of an individual *X. virginica*, and its length was 40 times the average *X. virginica* body length (2 cm), approximately 80 cm long (Packer 2006). A new piece of tubing was used for each dyad.

Prior to testing, bees were placed in plastic 50 ml tubes on ice, causing them to become inactive. Before the trial, two bees were simultaneously placed in opposite ends of the circle tube to prevent behavioural effects resulting from the order in which bees were placed in the tube (Wcislo 1997). The pair was placed in the tube facing in opposite directions and the tube was bent and closed with the connector tube, forming a circle. Bees warmed up and became active in their respective ends of the tube. If one bee warmed more quickly than the other, it was confined to its end by pinching the circle tube until the other bee became active. Trials were started when both bees became animate. All behaviours were recorded for both bees for 1200 s. If bees were inactive for 10 min, they were allowed to walk out of the tube and fly away freely.

Bees were tested twice on the same day, once in the nestmate condition and once in the non-nestmate condition, in random order. Bees never participated in two consecutive trials. Bees were chilled between trials and most were released back into the population by disconnecting the ends of the circle tube and letting the bees crawl out and fly away after two bouts of testing. In 2007, bees tested in female–female pairs were tested on the day of capture. After testing, the bees were placed in individual plastic tubes and into a –20 °C freezer to be euthanized. The next day they were preserved in redistilled ethanol and stored until they were dissected to determine the level of ovarian development. Fully developed oocytes (characterized by their large size and nearly uniform sausage shape) were recorded as having a score of one,

three-quarter sized oocytes were scored as 0.75, half-sized oocytes as 0.5, and quarter-sized oocytes as 0.25. Total ovarian score was determined by taking the sum of the oocyte scores present in all ovarioles and ranged from 0 to 2.5.

Behaviours Analysed

We present results for interactive behaviours often recorded in circle tube assays of primitively social bees, including aggressive behaviours (push, bite, C-posture) and tolerant or cooperative behaviours (pass, head-to-head touch); noninteractive behaviours, such as grooming or walking, were also recorded (Peso 2008), but are not included here. Behaviours were quantified as appropriate in terms of the frequency of events, the time spent performing each behaviour, or latency, the time from the start of the trial until either bee performed the behaviour. If a behaviour did not occur in a trial, the latency value was recorded as 1200 s (the total duration of the trial). The following is a description of each interactive behaviour.

Push

One bee applies force to another with its head. This behaviour has been previously described as 'nudging' (Steen 2000; Arneson & Wcislo 2003) and 'lunging' or head-butting (Packer et al. 2003). In all cases, pushing is described as a display of aggression and may be used to assert dominance (Pabalan et al. 2000; Arneson & Wcislo 2003). Here, we have described this behaviour as pushing, because *X. virginica* does not touch the head of the other bee and then back away, but maintains head contact and continues to apply pressure. The instigating bee would often push the other a fair distance around the tube before stopping.

Bite

A bee opens its mandibles and closes them on a body part of the other bee. Biting was classified as an aggressive behaviour in accordance with previous studies of *Xylocopa aeratus* (Steen 2000), as well as several communal or primitively eusocial halictids (Bell & Hawkins 1974; McConnell-Garner & Kukuk 1997; Packer et al. 2003) and andrenids (Paxton et al. 1999). In some of these studies, 'lunges' or attempts to bite were recorded as aggressive behaviour, but they were omitted here as biting is a more conservative estimate of aggression and rapid bite attempts were more difficult to observe than actual bites.

C-posture

A bee curls its abdomen under its head and thorax to form a 'C' with its body. In this position, both the mandibles and sting are pointed directly at the other bee. Like biting, C-posturing is classified as an aggressive threat display in studies of halictids (Kukuk 1992; McConnell-Garner & Kukuk 1997). Both males and females show C-posturing in *X. virginica*.

Pass

Walking bees meet and manoeuvre to accommodate each other while travelling in opposite directions in the circle tube. Passing always occurs venter to venter, and requires that one bee turn over so that the other can walk over it. In this position, a bee is vulnerable to the other's mandibles and sting (Arneson & Wcislo 2003; Packer et al. 2003). Since passing requires action by both bees, it is considered to be cooperative (Steen 2000) or 'an act of social tolerance' (Breed et al. 1978).

Head-to-head touch

One bee touches its head to the head of the other and both remain stationary in this position. This behaviour has not been

previously reported in bee behaviour literature. We interpret this behaviour as tolerant or cooperative as both bees accept close physical contact within biting or stinging distance of the other for a prolonged period.

Data Analysis

Behavioural data were examined in terms of latency to first instance, frequency, and duration, as appropriate for each behaviour. We used two-way ANOVA to examine the influence on latency of testing condition (nestmate or non-nestmate), sex of interactants (female–female, male–male or male–female pairing), and the statistical interaction between condition and sex. The total frequency of a behaviour per pair was analysed, with the same two-way ANOVA model, but on ranked frequencies, since their distributions were non-normal. Bonferroni post hoc tests were used to determine significant pairwise differences in both the frequency and latency models.

The time spent performing a behaviour was analysed for individuals performing prolonged behaviours by subtracting the total duration of the behaviour expressed by a particular bee in the non-nestmate condition from the duration expressed by the same bee in the nestmate condition. On average, these differences would be zero if bees' behaviour was similar with nestmates and non-nestmates. Since the majority of these differences were non-normally distributed, we used signed rank tests to determine whether behaviour differences were significantly different from zero.

ANOVA analyses were accomplished using PROC GLM at a significance level of $P = 0.05$. For the general linear models, if the significance of the overall two-way model (comparing nestmates versus non-nestmates and the three pair types) was $P \leq 0.05$, then partial F values with P values less than 0.10 are also reported. The effects of body size or ovarian development on aggressive behaviour were examined using Spearman rank correlations relating differences between bees in their physical traits to differences in aggression. All statistical analyses were conducted using SAS 9.1 (SAS Institute, Chicago, IL, U.S.A.).

Ethical Note

All work was conducted in accordance with regulations and guidelines established by the Canadian Council for Animal Care and the Brock University Animal Care Committee. No licenses or permits were required for this research.

RESULTS

Aggressive Behaviour (Pushing, Biting and C-Postures)

Overall, non-nestmates pushed earlier (partial $F_{1,185} = 4.43$, $P = 0.037$) and more frequently (partial $F_{1,185} = 4.35$, $P = 0.038$) than nestmates (Fig. 1). Pushing occurred earlier in male–male pairs than in other pairings (partial $F_{2,185} = 7.85$, $P < 0.0005$). Male–male pairs pushed more frequently than female–female pairs, with male–female pairs in between (partial $F_{2,185} = 9.29$, $P = 0.0001$).

Biting largely followed the same pattern as pushing behaviour, with more aggression in non-nestmate pairs (Fig. 2). Bees in all sex pairings bit non-nestmates significantly sooner (partial $F_{1,185} = 9.12$, $P = 0.003$) and less often (partial $F_{1,185} = 9.18$, $P = 0.003$) than they did nestmates. The difference in biting rate between paired females was not correlated with the size difference between them (Spearman rank correlation: $r_s = 0.20$, $N = 28$, $P = 0.31$) or with the difference in their rates of ovarian development ($r_s = -0.22$, $N = 28$, $P = 0.27$).

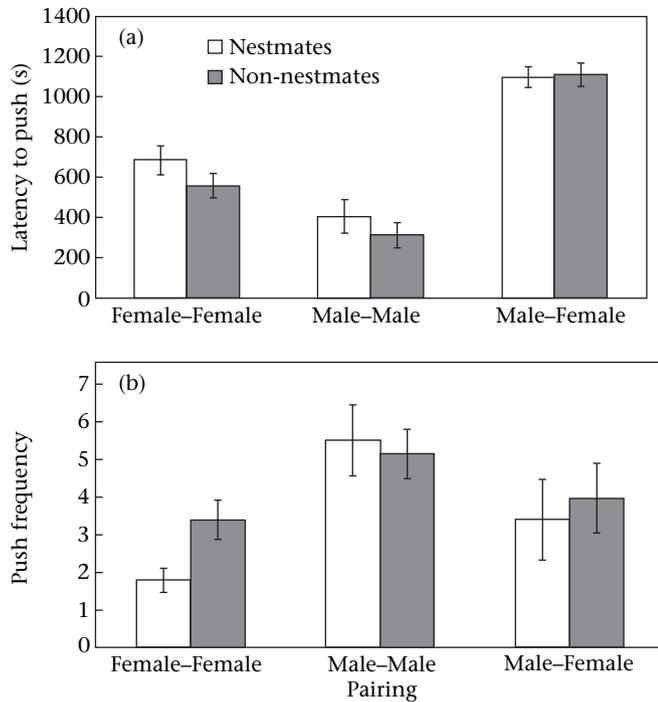


Figure 1. Differences in aggression among nestmate and non-nestmate carpenter bees, as indicated by the push behaviour. (a) Mean latency to the first push in each trial (two-way ANOVA for effects of nestmate versus non-nestmate and pairing type: $F_{5,185} = 4.98$, $P = 0.003$). (b) Mean push frequency per trial (two-way ANOVA: $F_{5,185} = 4.70$, $P = 0.0005$). Error bars represent ± 1 SE.

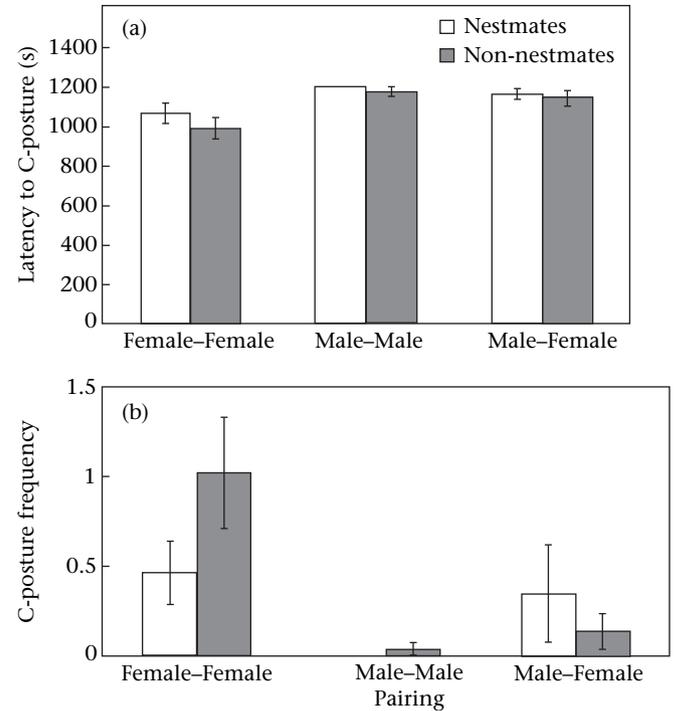


Figure 3. Differences in aggression among nestmate and non-nestmate carpenter bees, as indicated by the C-posture behaviour. (a) Mean latency to the first C-posture per trial (two-way ANOVA for effects of nestmate versus non-nestmate and pairing type: $F_{5,185} = 3.89$, $P = 0.002$). C-posturing was not observed in male-male nestmate pairs, and all latencies were recorded as 1200 s. (b) Mean frequency of C-postures per trial (two-way ANOVA: $F_{5,185} = 4.39$, $P = 0.0008$). Error bars represent ± 1 SE.

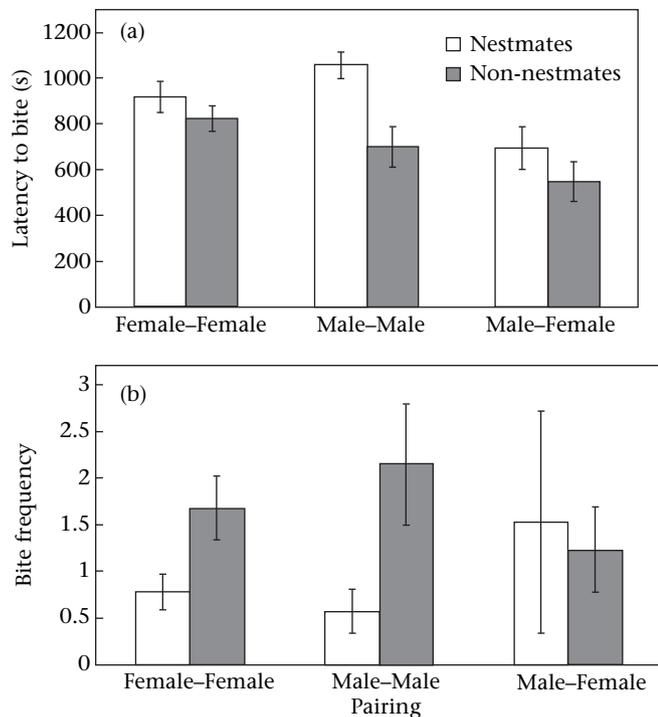


Figure 2. Differences in aggression among nestmate and non-nestmate carpenter bees as indicated by the bite behaviour. (a) Mean latency to the first bite in each trial (two-way ANOVA for effects of nestmate versus non-nestmate and pairing type: $F_{5,185} = 2.49$, $P = 0.033$). (b) Mean bite frequency per trial (two-way ANOVA: $F_{5,185} = 2.56$, $P = 0.029$). Error bars represent ± 1 SE.

C-postures, on average, occurred near the ends of trials in both same-sex and opposite-sex pairings, including male-male non-nestmate pairs (Fig. 3), despite their lack of stings. This aggressive behaviour occurred earlier (partial $F_{1,185} = 3.64$, $P = 0.058$) and more frequently (partial $F_{1,185} = 4.17$, $P = 0.043$) in non-nestmate pairs of all sexes. Female-female and male-female pairs demonstrated C-posture threats significantly earlier than male-male pairs (partial $F_{2,185} = 7.49$, $P = 0.0007$). Female-female pairs C-postured more often than male-male pairs, but not more often than male-female pairs (partial $F_{2,185} = 8.50$, $P = 0.003$).

Cooperative/Tolerant Behaviour (Passing and Head-to-Head Touching)

There was no consistent difference in latency to pass (Fig. 4) between nestmates and non-nestmates ($F_{1,185} = 0.33$, NS), although same-sex pairs passed significantly sooner than opposite-sex pairs (partial $F_{2,185} = 6.15$, $P = 0.003$). There was also no overall difference between nestmates and non-nestmates in the frequency of passing ($F_{1,185} = 1.71$, $P = 0.19$) but same-sex pairs passed significantly more often than male-female pairs (partial $F_{2,185} = 7.68$, $P = 0.0006$). An almost significant interaction between condition and pair type ($F_{5,185} = 2.75$, $P = 0.067$) suggested that, in male-male pairs, passing was slightly more frequent in non-nestmates than in nestmates, a trend opposite that in the other pair types.

In both same-sex and opposite-sex pairings, tolerance expressed through head-to-head touching occurred earlier in nestmates than in non-nestmates (partial $F_{1,185} = 6.69$, $P = 0.011$; Fig. 5). In general, individual bees spent more time head-to-head touching with nestmates than they did with non-nestmates (Table 1). The difference in behaviour between nestmate and

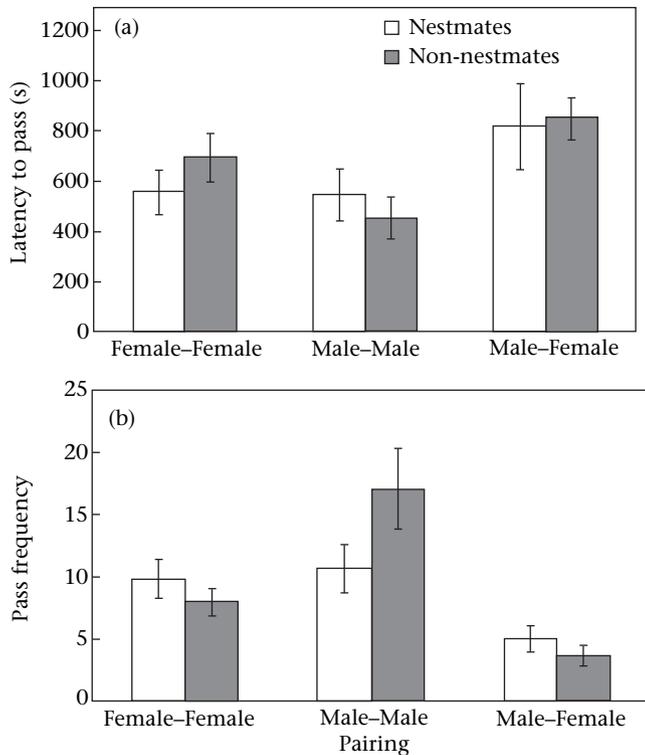


Figure 4. Differences in tolerance/cooperation among nestmate and non-nestmate carpenter bees as indicated by the pass behaviour. (a) Mean latency to the first successful pass in each trial (two-way ANOVA for the effects of nestmate versus non-nestmate and pairing type: $F_{5,185} = 3.02$, $P = 0.012$). (b) Mean frequency of passes per trial (two-way ANOVA: $F_{5,185} = 3.70$, $P = 0.003$). Error bars represent ± 1 SE.

non-nestmate conditions was especially marked in male–female pairs, which also took longer to initiate head-to-head touching than other pairs (partial $F_{2,185} = 2.90$, $P = 0.063$). The difference was due to the fact that male–female nestmate pairs spent considerably more time in this behaviour than did same-sex nestmate pairs (ANOVA: $F_{2,134} = 4.83$, $P = 0.010$).

DISCUSSION

Both male and female *X. virginica* altered their behaviour when interacting with nestmates and non-nestmates. We predicted that non-nestmates would be more aggressive, and indeed, more

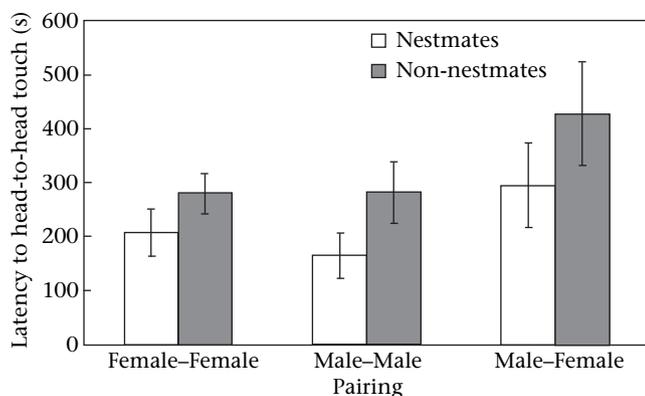


Figure 5. Differences in tolerance/cooperation among nestmate and non-nestmate carpenter bees as indicated by the head-to-head touching behaviour. Mean \pm SE latency to the first head-to-head touch in each trial (two-way ANOVA: $F_{5,185} = 1.53$, $P = 0.031$).

Table 1

Differences in cooperative/tolerant behaviour for individual carpenter bees in nestmate and non-nestmate conditions as shown by mean difference of time spent head-to-head touching per individual

Pair type	Sex of individual (N)	Mean time spent head-to-head touching (s)		Signed-ranks test
		Nestmates	Non-nestmates	
F–F	F (68)	139.5	121.2	$t_5=35.5$, NS
M–M	M (29)	136.7	132.5	$t_5=78$, NS
F–M	F (18)	262.8	80.1	$t_5=47$, $P < 0.005$
F–M	M (19)	262.8	108.2	$t_5=54$, $P = 0.029$

F = female; M = male. Signed-ranks test was performed on individual differences in time spent head-to-head touching when bees were tested in the nestmate and non-nestmate conditions. Note that the difference between males and females in M–F pairs reflects the fact that some partners of either sex were excluded because they were not tested twice.

pushing, biting, and C-posturing were observed when non-nestmates occupied the testing arena. We also made the converse prediction, that nestmates would be more tolerant or cooperative. This prediction was supported by the observation that individuals spent more time in head-to-head touching when paired with nestmates than when paired with non-nestmates. Passing was the only behaviour in which we found no differences between nestmates and non-nestmates. We also found no correlations between size and aggression in either sex, and in females, no correlation between ovarian development and aggression. These patterns suggest that adult *X. virginica* of both sexes can and do discriminate between nestmates and non-nestmates, and that in the circle tube assays, bees were responding to the level of familiarity between interactants, rather than to differences in body size or reproductive status.

Given that *X. virginica* adults do move from nest to nest, at least in our population, it would appear that female nest fidelity in *X. virginica* is lower than that commonly observed in primitively social bees (Paxton et al. 2001, 2002; Richards et al. 2005; Soro et al. 2009); no comparable data are available for males. Bees that we classified as nestmates might have been together in a nest for less than 24 h preceding observations, and some of those classified as non-nestmates might have been previous nestmates. Therefore, our behavioural assay for differences between nestmates and non-nestmates was quite conservative. That bees were still able to discriminate between nestmates and non-nestmates indicates that they may be able to learn each other's identities rather quickly. Moreover, we kept the bees captive and separated for up to 24 h before observing them, so this means that bees can remember each other's identities at least for that long.

Colony social organization of *X. virginica* in southern Ontario is rather different from that in primitively eusocial bees. As in other social *Xylocopa* (Hogendoorn & Velthuis 1993), dominant *X. virginica* females both monopolize reproduction and provision most of the brood (Gerling & Hermann 1978). In our study population, subordinate females act as low-productivity auxiliary foragers or are completely unproductive, remaining inactive inside the nest (M. H. Richards, unpublished data). Social nests appear to produce no more brood than do solitary nests (M. H. Richards, unpublished data), so there is no obvious advantage to social nesting, and it is surprising that dominant females allow subordinates, especially those that do not work, to remain in the nest. One function of nestmate recognition in female *X. virginica* may be for dominants to prevent too many nonproductive females from joining colonies. Females that nest solitarily or in small associations (say, 2–3 females) may be those that are more successful in recognizing and excluding unfamiliar or undesirable nest associates. If females can remember the identities of overwintering nestmates (which are

likely to be siblings) for long periods, then dominant nest females might even be able to differentiate between related and unrelated subordinates, although not necessarily by means of direct kin recognition. Dominants might be more likely to allow familiar subordinates (which would tend to be related) but not unfamiliar subordinates (which would be less likely to be related) to join or remain in the nest, whereas subordinates might make more attempts to join the nests of familiar (related) dominants. Thus, nestmate recognition may both facilitate and prevent movements of individual females among colonies.

We found that male–male pairs showed behavioural patterns similar to those of female–female pairs. Males were more aggressive towards non-nestmates than towards nestmates. Male non-nestmates also showed the most aggression among all pairings, as expected because of the overt aggression involved in territoriality (Gerling & Hermann 1978; Barrows 1983). There may be inclusive fitness benefits to behaving less aggressively and more tolerantly to familiar males. Since siblings overwinter together, males have opportunities to learn the identities of their brothers before the breeding season begins in spring. During the breeding season, males that overwinter in the same nests may learn the identities of males defending territories nearby, which they would often encounter during the day. Learning the identities of brothers and neighbours could facilitate the ‘dear enemy’ effect (Gordon 1989), in which males reduce aggression towards familiar territory holders, and direct it at unfamiliar, potential territory usurpers. Recognizing territory neighbours could be highly advantageous as males can hold territories for up to 2 weeks (Barthell & Baird 2004; Barthell et al. 2006), and constantly attacking nearby males is physically and energetically costly. The only other social insect in which male–male nestmate recognition has been explored is *Polistes fuscatus*. Away from their natal nests, *P. fuscatus* males in nestmate pairs spend more time in close proximity than those in non-nestmate pairs, while males in triads remain closer to familiar individuals for longer periods (Shellman-Reeve & Gamboa 1984).

The behavioural patterns of male–female pairs reflected those of same-sex pairs: greater tolerance and less aggression among nestmates than non-nestmates. This suggests that nestmate recognition functions even in the context of interactions between the sexes. Although we had predicted recognition in male–female pairs, we had expected the opposite outcome, namely greater tolerance and less aggression towards non-nestmates that would be more suitable potential mates if there are deleterious consequences to inbreeding (Zayed & Packer 2001). Our observations suggest that male–female social interactions assayed in the circle tube context are not those relevant to inbreeding avoidance, rather they have more to do with intra-nest interactions, such as those that may occur at night. For instance, if circle tubes mimic the effect of being inside a nest tunnel, interactions between males and females might be related to whether females cooperate with and feed male ‘overnighters’, as it seems more likely that females would feed familiar rather than unfamiliar males. Alternatively, male–female interactions in the circle tubes might reflect efforts of females to prevent males from consuming resources; perhaps the head-to-head touching behaviour, which was much more common in male–female nestmate pairs, is a gentle block, more likely to be used on familiar males, with aggressive behaviour more likely to be directed at unfamiliar males.

A behaviour that warrants some extra attention is passing. Passing is usually considered to be a cooperative behaviour in circle tube assays of both solitary and social bees (reviewed in Packer 2006), because in a circle tube, there is only room for bees to pass one another if both turn sideways and each allows the other to crawl past. However, describing this behaviour as cooperative rather than tolerant, is somewhat problematic, because the term

‘cooperation’ as it is usually used in describing the behaviour of social insects, relates more to task allocation and division of labour, rather than to simple behaviours such as allowing other individuals to pass in very close proximity (Michener 1974). Moreover, the same passing behaviour that is interpreted as more ‘cooperative’ when expressed more frequently by nestmate females would seem to require a different interpretation when observed in males. We found no differences in passing between nestmates and non-nestmates, more passing in same-sex than in different-sex pairs, and slightly more passing by male non-nestmates, which were also more aggressive, than nestmates. Therefore, in *X. virginica* at least, passing does not seem to signify cooperation. A possible alternative interpretation is that passing is related to assertions of dominance, which might be more likely in same-sex interactions. In a pass, one bee puts its head above the other’s and continues to move forward, and this action prevents the other bee from doing anything aside from accepting the pass, blocking the pass, or backing up. The frequency of successful passing would be higher in dyads with one dominant bee that initiates a pass and one subordinate that acquiesces. In female *L. zephyrum*, passes did not occur right away, but after a series of encounters where fighting occurred (Kukuk & May 1991), also suggesting dominance.

CONCLUSIONS

Nestmate recognition has now been shown in two ‘largely solitary’ species, *Manuelia postica* (Flores-Prado et al. 2008) and *Lasioglossum (Dialictus) figueresi* (Wcislo 1997), and in male bees (this study). Clearly, the ability to recognize members of an individual’s own group can evolve outside the context of classical colony-based sociality (Michener 1974). *Xylocopa virginica* is unusual among commonly studied social bees in presenting simultaneous opportunities to study both female and male behaviour. In future studies of this species and other members of its genus, it would be useful to investigate whether kin recognition also occurs. Other interesting candidates for studies of nestmate recognition would be the ‘presocial’, twig-nesting carpenter bees in the genus *Ceratina*, many of which show extended associations between mothers and their offspring, and a few of which appear to have crossed the threshold from solitary to social behaviour (Rehan et al. 2009). Studies of nestmate recognition in bees like *Ceratina* would help to shed light on the ancestral functions of nestmate recognition and how these are co-opted during social evolution.

Acknowledgments

We thank S. Rehan, M. Frampton, S. Prager, C. Kerling, D. Szur-ozcki, and especially, K. Thur, for field assistance, as well as two anonymous referees and Mike Breed for their insights. Funding was provided by Brock University and a Natural Sciences and Engineering Research Council of Canada Discovery grant to M.H.R.

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