Nestmate Recognition in the Large Carpenter Bee, *Xylocopa virginica*

by

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Abstract

Many species of social insects have the ability to recognize their nestmates. In bees, sociality is maintained by bees that recognize which individuals should be helped and which should be harmed in order to maximize fitness (either inclusive or individual) (Hamilton 1964; Lin and Michener 1972). Since female bees generally lay eggs in a single nest, it is highly likely that bees found cohabitating in the same nest are siblings. According to the kin selection hypothesis, individuals should cooperate and avoid aggression with same sex nestmates (Hamilton 1964). However, in opposite sex pairs that are likely kin, aggression should increase among nestmates as an expression of inbreeding avoidance (Lihoreau et al. 2007). Female bees often guard nest entrances, recognizing and excluding foreign conspecific females that threaten to steal nest resources (Breed and Page 1991). Conversely, males that aggressively guard territories should avoid aggression towards other males that are likely kin (Shellman-Reeve and Gamboa 1984). In order to test whether Xylocopa virginica can distinguish nestmates from non-nestmates, circle tube testing arenas were used. Measures of aggression, cooperation and tolerance were evaluated to determine the presence of nestmate recognition in this species.

The results of this study indicate that male and female X. virginica have the ability to distinguish nestmates from non-nestmates. Individuals in same sex pairs demonstrated increased pushing, biting, and C-posturing when faced with non-nestmates. Males in same sex pairs also attempted to pass (unsuccessfully) non-nestmates more often than nestmates, suggesting that this behaviour may be an expression of dominance in males. Increased cooperation exemplified by successful passes was not observed among nestmates. However, increased tolerance in the form of head-to-head touching
was observed for nestmates in female same sex and opposite sex pairs. These results supported the kin selection hypothesis. Moreover, increased tolerance among opposite sex non-nestmates suggested that *X. virginica* do not demonstrate inbreeding avoidance among nestmates.

The second part of this study was conducted to establish the presence and extent of drifting, or travelling to different nests, in a *Xylocopa virginica* population. Drifting in flying Hymenoptera is reported to be the result of navigation error and guard bees erroneously admitting novel individuals into the nest (Michener 1966). Since bees in this study were individually marked and captured at nest entrances, the locations where individuals were caught allowed me to determine where and how often bees travelled from nest to nest. If bees were captured near their home nests, changing nests may have been deliberate or explained by navigational error. However, if bees were found in nests further away from their homes, this provides stronger evidence that flying towards a novel nest may have been deliberate.

Female bees are often faithful to their own nests (Kasuya 1981) and no drifting was expected in female *X. virginica* because they raise brood and contribute to nest maintenance activities. Contrary to females, males were not expected to remain faithful to a single nest. Results showed that many more females drifted than expected and that they were most often recaptured in a single nest, either their home nest or a novel nest. There were some females that were never caught in the same nest twice. In addition, females drifted to further nests when population density was low (in 2007), suggesting they seek out and claim nesting spaces when they are available. Males, as expected, showed the opposite pattern and most males drifted from nest to nest, never recaptured in the same
location. This pattern indicates that males may be nesting wherever space is available, or nesting in benches nearest to their territories. This study reveals that both female and male *X. virginica* are capable of nestmate recognition and use this ability in a dynamic environment, where nest membership is not as stable as once thought.
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I would like to dedicate this thesis to my Dad who passed away in 2007. It was our shared love for animals that inspired me to take on a Master’s in animal behaviour. Without him, I likely would not have managed to put together the functional bee traps that were critical to the success of this study. Wherever you are Dad, thank you, and I hope I made you proud.

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"The richness I achieve comes from Nature, the source of my inspiration."

-Claude Monet
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Chapter 1: Nestmate Recognition in *X. virginica* Females and Males

Introduction

*Social Recognition in Insects*

Social recognition is widespread in many animals, including insects. On its most basic level, recognition is the ability of an individual to assign other individuals it encounters into functional categories. Upon encountering another individual, an animal that recognizes chooses appropriate behaviours based on the relationship between the two and its relevance to the recognizer’s fitness. In other words, recognition itself cannot be measured, but the behaviours performed by animals in response to each other can be measured as an assay of recognition.

Recognition functions in a variety of contexts in insects. An animal must be able to distinguish conspecifies from other species to choose matings that will result in viable offspring. This also includes the ability to tell opposite-sex kin from non-kin, as inbreeding often leads to less than healthy young (Fletcher and Michener 1987). For example, female non-dispersing cockroaches (*Blatella germanica*) avoid mating with both familiar individuals and kin (Lihoreau et al. 2007), suggesting that they shun matings with any male that could potentially be a relative. Once progeny arrive, individuals must recognize their own offspring so that parental resources are not misdirected, leading to fitness costs (Fletcher and Michener 1987). It is evident that most animals, including insects, possess recognition ability at this level, allowing them to produce and raise young to the next generation. However, social, or group-living insects often have more advanced recognition capabilities. These include distinguishing group members from non-group members and distinguishing kin from non-kin. In a study of the
wasp *Polistes fuscatus*, females discriminated (by remaining in closer proximity to) nestmate sisters from non-kin, non-nestmates, but did not discriminate between nestmate sisters and non-nestmate nieces. In other words, these wasps can recognize based on familiarity, but kinship also plays a role in the recognition system of this species (Gamboa et al. 1987b). Other species of insects are capable of individual recognition, including *Ropalidia marginata* workers, who recognize their queen despite the fact that she is morphologically and behaviourally similar (docile) to other bees in the nest (Lamba et al. 2007).

There are at least two individuals involved in the expression of recognition, a cue-bearer, the individual that expresses a phenotype specific to group members, and the cue-responder or perceiver (Gamboa et al. 1986b; Fletcher and Michener 1987). The perceiving insect has an array of behaviours to perform in response to a cue-bearer. If the conspecific is familiar, the perceiver should react with tolerance or cooperation. Tolerance occurs when an individual allows another to remain in close physical proximity where aggression is a potential risk. For example, male *Polistes fuscatus* wasps in triads tolerate nestmates more than non-nestmates by remaining closer to familiar wasps for a longer period of time (Shellman-Reeve and Gamboa 1984). Cooperation occurs when the perceiver or recipient acts in a way that may benefit the other. For instance, passing, or allowing another bee to continue in its direction of travel by moving to accommodate it. However, tolerance or cooperation is insufficient evidence for recognition because acceptance may indicate that the species under investigation cannot discriminate familiar from unfamiliar individuals and behaves indifferently to all conspecifics (Michener 1974). This is true in the halictid bee *Lasioglossum*
hemichalceum, which demonstrates successful passing in 98% of all encounters with nestmates and non-nestmates (Kukuk 1992).

Aggressive behaviour in response to a conspecific indicates that an individual recognizes another as an adversary or as unfamiliar, and behaves in a way that will deter the novel individual. Aggression is often a more reliable indicator of recognition than tolerance or cooperation, because it tends to be costly, potentially resulting in harm to individuals who engage in it. As a result, aggression may be expressed less ubiquitously than tolerance when individuals are evaluating familiarity. Increased aggression is often described as a reliable indicator of recognition of unfamiliar individuals, as many animals are intolerant of those that do not belong in a particular area or group. This is true in females of the stingless bee, Trigona fulviventris, that bite and grapple with unfamiliar non-nestmates earlier than nestmates in behavioural assays (Buchwald and Breed 2005). However, most studies of recognition cite increased tolerance and cooperation with familiar or ‘acceptable’ individuals (such as kin), coupled with increased aggressive behaviour towards novel individuals as joint indicators of recognition. In a study of the wasp Polybia paulista, individuals from different nests were introduced into nest entrances. The results of this experiment revealed significantly increased acceptance of nestmates into the nest and aggression (chasing, biting and stinging) and eventual rejection from the nest towards all wasps from different colonies (Kudo et al. 2007). A study of unrelated females of the halictid bee, Lasioglossum zephyrum in artificial colonies revealed analogous results: admittance of familiar nestmate bees into the nest and rejection of non-nestmates (Bell et al. 1974). Similarly, Melipona quadrifasciata and
M. rufiventris were significantly more tolerant of nestmates and more aggressive towards non-nestmates when placed in a tube with blocked ends (Breed and Page 1991).

Social insects (especially bees) are ideal for the study of recognition systems in social groups. The reason for this is that bees often live in clearly defined social groups contained in nests, allowing investigators to easily recognize group members. Also, bees usually nest with closely related individuals, setting the stage for kin selection as the motivation for individuals to help nestmates. Nestmate recognition is defined as the ‘ability to distinguish nestmates from non-nestmates’ (Breed 2003) and the term is often used interchangeably with kin recognition. Whether or not nestmate bees are kin requires genetic analysis and is often difficult (and expensive) to determine, nevertheless, nestmate recognition still functions when nest-dwellers are not relatives (Breed 2003).

**Mechanisms of Social Recognition in Hymenoptera**

Recognition cues often result from a combination of genetic and environmental influences, depending on the species under study (Fletcher and Michener 1987; Lehmann and Perrin 2002). Genetically, a specific odour phenotype in kin groups may facilitate recognition when kin share a nest (Lehmann and Perrin 2002). Male L. zephyrum, when exposed to conspecific females, reject mating with females closely related to the first female they encountered, suggesting that they detect and use a pheromonal cue to avoid inbreeding (Smith 1983). Sometimes it is difficult to tease apart whether the odour cues are entirely genetic (pheromones), or whether environmental influences alter the scent of cuticular hydrocarbons because similar diet (Barrows et al. 1975; Buczkowski and Silverman 2006) and common nesting substrate (Batra 1968 as cited in Michener 1969)
have been shown to affect odour cues and play a role in nestmate recognition. In *Melipona panamica*, female guard bees readily accept increasingly related bees, as well as those raised separately, but in the same colony environment as the guard (Inoue et al. 1999). Also, both genes and nest substrate play a role in wasp odour and recognition in *Polistes fuscatus* (Gamboa et al. 1986a). There is plenty of evidence that social insects learn the scent of their nestmates (who are most often kin) and apply this information to the conspecifics they encounter, or in other words, that perceivers match phenotypes (Lacy and Sherman 1983) to a mental template to determine familiarity, and behave accordingly. In some cases, familiarity takes precedence over kinship. In a study of *Exoneura robusta*, female bees were given a choice between establishing new groups with unknown kin or with previous nestmates. Relatedness in the new nests remained constant (although slightly lower) suggesting that *E. robusta* cannot or do not discriminate between unfamiliar kin and nestmates and may prefer nesting with unrelated but familiar individuals (Bull and Adams 2000).

Phenotype matching can also be based on visual cues. In a study of *P. fuscatus*, a wasp with highly variable facial markings, individuals with altered facial colouration were attacked when returned to their nest of origin (Tibbetts 2002). However, when visual cues are removed (under red light not visible to bees), *L. zephyrum* females still eject unrelated intruders from their nests (Bell 1974). Regardless of how recognition cues are generated, the perception of these cues by individual bees allows them to carry out the various functions of nestmate recognition in Hymenoptera.
Functions of Recognition in Social Groups

Previous studies of nestmate recognition in Hymenoptera have been female-biased, as females live in groups while males generally disperse. In bees and wasps with multi-female nests, each individual must benefit from being a part of the group. Kin selection (Hamilton 1964) requires that individuals recognize whom to help in order to maximize their inclusive fitness, or familial shared genes passed on to the next generation (Hamilton 1972). If nestmates are kin, an individual who indiscriminately helps another individual that wanders into the nest (likely to be non-kin), significantly reduces its own fitness (Gamboa et al. 1987b). In mutualistic relationships, helping another who does not reciprocate is costly to the helper. Consequently, nestmate recognition is essential for colony cohesion and defense (Ryan et al. 1984; Breed and Page 1991).

Colony cohesion in social Hymenoptera is often established by a dominant female, who is most often larger than the other females occupying the nest. In most social bees, large bees (often queens) attain dominance by pushing other bees with their heads (Arneson and Wcislo 2003). In H. ligatus, the highest levels of female aggression are correlated with a large size difference between the bees in an encounter (Pabalan et al. 2000). L. malachurum females show the same pattern in which larger females are more successful in aggressive confrontations (Zobel and Paxton 2007). However, this phenomenon is not present in all bees. In L. (Chilalictus) platycephalum, L. (Ctenonomia) sp. (McConnell-Garner and Kukuk 1997), L. (Dialictus) figueresi (Wcislo 1997), Bombus atratus (Cameron and Jost 1998), and Panurgus calcaratus, (Paxton et al. 1999) body size is unrelated to aggression.

Another factor known to influence female dominance behaviour is ovarian development. During temporary periods with excess females in the colony in B.
bifarious (Foster et al. 2004) and B. atratus (Cameron and Jost 1998), aggression is positively linked with increased ovarian development. The same effect is observed in H. ligatus, where large queens with enlarged ovaries are more aggressive than smaller bees with less pronounced ovarian development (Pabalan et al. 2000). Also, L. figueresi females with higher ovarian development are most often the initiators of aggression, while those with less developed ovaries are first to withdraw (Weislo 1997). There are many exceptions to this trend, mostly in communal bees, or unrelated females that raise brood independently in a shared nest (Michener 1974). In L. (Chilalictus) platycephalum, L. (Ctenonomia) sp. (McConnell-Garner and Kukuk 1997) and P. calcaratus, degree of ovarian development is unrelated to aggressive behaviour (Paxton et al. 1999).

Whether or not there is a large, reproductive female dominating a group, colony females defend their nests at the entrances, preserving colony cohesion. Nest defense, or guarding, benefits a colony by minimizing predation (Bell and Hawkins 1974) and preventing intrusions by conspecific intruders who may remove pollen (Steen 2000), brood or nesting materials (Breed and Page 1991). Effective guarding requires the ability to recognize nestmates (Breed and Page 1991), admitting them into the nest while excluding potential trespassers through aggressive behaviour. Guarding through effective nestmate recognition has been reported for Apis mellifera (Moritz and Neumann 2004), Melipona panamica (Inoue et al. 1999), Bombus occidentalis (Gamboa et al. 1987a), and the halictids Lasioglossum zephyrum (Bell and Hawkins 1974; Barrows et al. 1975; Breed et al. 1978), L. malachurum (Michener 1974) and L. versatum (Michener 1966).

All of these species also show increased cooperation with nestmates. In addition, females of species that do not guard at nest entrances recognize and react aggressively towards
non-nestmates and cooperatively towards nestmates. These include the bees
_Lasioglossum figueresi_ (Wcislo 1997), _Melipona quadrispilota, M. rufiventris_ (Breed and Page 1991), _Trigona fulviventris_ (Buchwald and Breed 2005), and the wasps, _Polybia paulista_ (Kudo et al. 2007) and _Polistes fuscatus_ (Gamboa et al. 1986b). Nestmate recognition is also present in social insects that defend territories away from their nests, such as termites and ants (Breed 2003).

However, some bee and wasp females do not demonstrate increased cooperation with nestmates and increased aggression with non-nestmates, suggesting a lack of nestmate recognition. _Lasioglossum imitatum_ can be transferred to any conspecific nest without aggression from the resident bees (Michener 1974) while _L. hemichalceum_ not only refrain from aggression towards non-nestmates, but also cooperate (successfully pass) indiscriminately (Kukuk 1992). _Lasioglossum erythrurum_ females feed nestmates and non-nestmates equally through trophallaxis (Kukuk and Crozier 1990). Moreover, _Andrena scotica_ and _P. calcaratus_ do not differ in cooperative or aggressive behaviour between nestmates and non-nestmates (Paxton et al. 1999). Although nestmate recognition is beneficial in a variety of contexts, not all bees and wasps have this ability.

**Implications for Sociality**

There are many benefits to group-living, or sociality (Alexander 1974), in animals. Being social can offer an individual shared shelter space, protection from predators and parasites, access to local resources known to the group and contact with potential mates. For sociality to be maintained by selection, there needs to be some benefit for every individual living in a group (Alexander 1974). In social insects, kin
selection, or preferentially helping closely related kin (altruism; Hamilton 1964) is used to explain the existence of sociality. There may be a substantial fitness cost to the altruist for aiding others in the group, but this cost is recouped by the benefit to kin, who propagate shared genes into the next generation (Hamilton 1964). Another possible mechanism maintaining sociality in insects is mutualism, in which every individual in a group receives a benefit from living with others regardless of relatedness to other group members (Lin and Michener 1972). Mutualism is maintained by selection for direct fitness, where every individual has increased reproductive success from some aspect of group-living (Lin and Michener 1972). In either of these cases, outsiders may make their way into a colony or nest and reap the benefits of assistance from group members without making an individual contribution (social parasitism) (Kudo et al. 2007), thus lowering the overall benefit to colony-dwellers. If it is costly to admit social parasites, recognition of group members must exist to maintain group cohesion and preserve the advantages of living together. Nestmate recognition is indispensable and functions in the same way whether sociality is maintained through altruism or mutualism.

**Inbreeding Avoidance**

Nestmate recognition is also relevant to studies of inbreeding avoidance, or preferentially mating with non-nestmates under the assumptions that nestmates are kin and that inbreeding negatively impacts fitness (Lihorcau et al. 2007). In these assays, male and female pairs and triads with varying levels of familiarity are permitted to associate with the opposite sex. Researchers record behavioural differences to determine nestmate recognition and inbreeding avoidance. Increased aggression, avoidance and
fewer mating attempts with nestmates strongly suggests the presence of inbreeding avoidance. Inbreeding avoidance is present in males of *L. zephyrum*, who habituate to the odour of females and do not attempt to mate with those with a familiar scent (Barrows et al. 1975; Greenburg 1982; Smith 1983). Furthermore, in *L. zephyrum*, once males attempt to mate with model females, those females become less attractive to other males, suggesting males leave behind a chemical anti-aphrodisiac that subsequent males recognize (Kukuk 1985). In the wasp *P. fuscatus*, male-female nestmate pairs had higher tolerance values, but lower mating frequency than non-nestmate pairs (Gamboa et al. 1986b). A complication in some studies of inbreeding avoidance is that depending on the experimental design, it is difficult to determine which sex is recognizing the other and which is behaving in a way that discourages mating (Gamboa et al. 1986b). For this reason, both males and females in inter-sex pairs require careful behavioural observation in assays of nestmate recognition.

**Males, Territoriality and Mate Competition**

Except for studies of inbreeding avoidance, male social insects are generally excluded from studies of nestmate recognition, because they disperse from their natal nests. Consequently, the single species in which male-male nestmate recognition has been explored is *Polistes fuscatus*. In the absence of their natal nests, males in nestmate pairs are more tolerant (spending more time in close proximity) of each other than wasps in non-nestmate pairs (Shellman-Reeve and Gamboa 1984). However, more research is required in order to determine the adaptive function of this behaviour in other bees and wasps.
Another context in which recognition has not been addressed is in Hymenoptera with territorial males. Territory defense is comparable to nest defense in that individuals defending a territory must recognize familiar individuals versus intruders (Breed 2003). In some carpenter bees, a lek-like or female defense mating system (Barthell et al. 2006) leads to males guarding territories that are right next to nesting sites. Territorial males must be able to distinguish females from males, to allow the females passage into the territory. Furthermore, in order to prevent constant and costly aggression, territory-holders should recognize those familiar males who defend territories nearby and reduce aggression towards them. This phenomenon is called the ‘dear enemy’ effect (Gordon 1989) as individuals are less aggressive to known competitors (enemies) than novel individuals. Aggression and territory defense has also been linked to size. In X. virginica, larger males are more successful at aggressively defending their territories, thus securing the areas with the most females (Barthell and Baird 2004; Barthell et al. 2006). This is also true of the burrowing bee, Amegilla dawsoni (Alcock 1997).

**Social Recognition in Xylocopa virginica**

*Xylocopa virginica* is a large carpenter bee (approximately two centimetres long), endemic to the eastern half of North America. They are social bees, as more than one female occupies a nest, but, the nature of this sociality is still uncertain. There is some suggestion of a division of labour, as one female often appears to be the most worn, on the mandibles (from chewing tunnels) and wings (from foraging flights) due to an imbalance of working among nest-dwellers (Gerling and Hermann 1978). A division of labour suggests primitive eusociality (Michener 1974), but *X. virginica* is not obligately
social, as lone females can raise progeny just as effectively as others who share a nest (Gerling and Hermann 1978). On the other hand, whether one female monopolizes most of the reproduction in a colony is still unknown because relatedness assays have never been conducted on this species. If females share reproduction equally, each constructing, provisioning and laying in her respective brood cells, *X. virginica* may be communal (Gerling et al. 1989). As a result of this confusing evidence, details about colony social organization, such as dominance relationships, caste relationships and genetic relatedness are still a mystery.

*Xylocopa virginica* offers a unique opportunity to study nestmate recognition among both sexes. Male and female siblings overwinter together in their natal nests (Gerling and Hermann 1978; Gerling et al. 1989). In the spring, males emerge first, aggressively defending territories near nesting sites (Gerling and Hermann 1978; Barrows 1983; Gerling et al. 1989; Barthell and Baird 2004). Unlike most Hymenoptera, these males have repeated contact with each other, and during the mating season, they encounter other males more often than females (Barrows 1983).

Approximately two to three weeks after male emergence, females can be observed flying outside the nest (Gerling and Hermann 1978; Gerling et al. 1989). During this time, males continue to defend territories near the females’ nesting substrates and attempt to mate with females that return home from trips outside the nest (Barrows 1983; Barthell and Baird 2004). Given that males defend territories near nesting substrates containing multiple nests (potentially including their natal nests), they are likely to encounter their nestmates (likely sisters) while choosing a mate.
After the short period of male and female overlap, males disappear, while females remain active for another six to eight weeks (Gerling and Hermann 1978; Gerling et al. 1989). Most female *X. virginica* live in nests of two or more bees who are likely sisters (Gerling and Hermann 1978; Gerling et al. 1989). Generally, one of the females forages, builds and reproduces, while the others guard the nest (Gerling and Hermann 1978; Gerling et al. 1989). Although presently unknown in *X. virginica*, pollen robbery by non-nestmate conspecifics is common in other xylocopines including *X. aeratus* (Steen 2000) and *X. pubescens* (Hogendoorn and Velthuis 1993).

**Objectives and Hypotheses**

This study consists of two parts. In the first, I wish to determine the presence and function of nestmate recognition in *Xylocopa virginica*. In the second, I wish to examine whether nestmates are members of a stable group or a unit consisting of constantly changing members. To do this, I will examine the nest fidelity of the individuals in my study population.

In order to establish whether *X. virginica* exhibit nestmate recognition, I will observe interactions of paired *X. virginica*. If bees alter their behaviour while with nestmates versus non-nestmates, in female-female pairs, male-male pairs, and male-female pairs I can conclude that the bees could discriminate based on familiarity. After establishing the existence of nestmate recognition in *X. virginica*, I will ascertain if these behaviours were consistent with kin selection/mutualism, in which bees are less aggressive and more cooperative towards nestmates. The presence of inbreeding avoidance was present in male-female nestmate pairs will also be addressed. Finally, I
will examine other factors known to influence bee behaviour, such as body size and ovarian development difference between two bees in an encounter, to determine if these factors contribute to the behaviour observed in the nestmate recognition assays. If these behaviours are not correlated with body size and ovarian development, I can conclude they were expressed as a display of nestmate recognition (assuming no other unknown confounds).

Due to the variable life histories of female and male _X. virginica_, my predictions varied according to the sexes of the individuals in the behavioural assay. Since females live together in nests, I predicted that female _X. virginica_ would demonstrate recognition, by being less aggressive towards nestmate females and more aggressive towards non-nestmate females (potential pollen robbers) (Figure 1.1). Equally, I predicted that they would show more cooperation in nestmate pairs and less cooperation in non-nestmate pairs (Figure 1.1). Given that _X. virginica_ males are territorial, I predicted that they have the ability to recognize nestmate males (most likely brothers) and would show less aggression towards familiar bees and more aggression towards unfamiliar bees. As males do not live together in a nest and generally do not cooperate in bee colonies (Michener 1974), I did not expect an overt increase in cooperation among nestmate males, but I did expect increased tolerance in male-male nestmate pairs (Figure 1.1). Also, I predicted that bees in male-female pairs would demonstrate more aggression in nestmate pairs (where inbreeding is costly) and less aggression in non-nestmate pairs. Moreover, males and females spend the night in shared nests; accordingly, I predicted that aggression in inter-sex nestmate pairs will not be as severe as in same-sex pairs (Figure 1.1).
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**Figure 1.1:** Hypotheses concerning nestmate recognition in female-female, male-male and male-female pairs of *X. virginica.*
Methods

Study Site
This study took place in St. Catharines, Ontario, Canada (43° 11' N 79° 14' W) in a courtyard on the Brock University campus (Figure 1.2). The site contained seven cedar benches each constructed from 12 wooden boards (5.1 cm x 121.9 cm) with concrete legs, situated on a concrete pathway and surrounded by a grass lawn. The study population consisted of *X. virginica* that used the benches as nest substrates.

Period of Study
Behavioural observations were conducted from 19 April-23 June 2006 and 9 May-25 June 2007, corresponding with the period of *Xylocopa* flight activity in Niagara. In total, 193 males and 165 females in the population were captured and marked in 2006. In 2007, 93 males and 126 females were captured and marked. Behavioural data for male-male and female-female pairs were collected in 2006 and 2007. Data for male-female pairs were collected in 2007. The females collected and killed for ovarian development analyses were tested and collected near the end of the 2007 season to avoid any major population losses between the first and second seasons of observations.

Bee Capture and Care
*X. virginica* were collected via a device that will be referred to as a 'cup trap'. A cup trap consists of a clear plastic drinking cup (Polar brand™) with a 1-2 cm hole drilled through the cup's bottom. Parafilm™ was stretched over the open end of the cup and
Figure 1.2: a) Scale diagram of Courtyard site showing location of all benches and 1 meter area surrounding them. b) Expanded view of courtyard and surrounding buildings. Box indicates path along which benches are located. Figure courtesy of S. Prager.
affixed with clear tape for extra security. Tiny holes were punched in the plastic wrap for additional ventilation. Two strips of Velcro (the ‘fuzzy’ side) were adhered to the base of the cup on either side of the drilled hole and the opposing two strips were adhered to the nesting substrate on either side of the *X. virginica* nest entrance. The cup trap was then adhered to the entrance hole and aligned with the cup’s drilled hole. This way, when bees exited their nests, they were confined to the cup trap (Figure 1.3). Nests with entrances on the bottom of the bench boards were fitted with traps, but this was not possible for nests with entrances on the sided of the boards. Traps were placed over nest entrances in the morning before the onset of flight activity and were kept in place until a bee was captured. Upon bee capture, traps were replaced with an empty one. This process continued until the end of the day, when all traps were removed.

After capture, unmarked bees were chilled on ice in order to anesthetise them. While inactive, the bee’s head width and intertегular width (distance between the wing bases) were measured using an eyepiece micrometer (Figure 1.4). Afterwards, the bee was painted with Testor’s enamel in a unique colour combination on its thorax and abdomen for individual identification (Figure 1.5).

Bees captured in cup traps were considered to be ‘nestmates’ if they were caught at the same nest entrance on the same day and ‘non-nestmates’ if they were trapped at different nests. Individuals to be used for behavioural testing were kept overnight in small plastic cages, and were supplied with a mixture of bee pollen, honey and water as nourishment. Cardboard partitions were placed between the cages so that bees could not see or interact with each other.
Figure 1.3: Bench with ‘cup traps’ adhered over *X. virginica* nest entrance holes.
Figure 1.4: Head width (blue) and intertegular width (red) measurements of *X. virginica*. Photo courtesy of Kaitlin Falkauskas.
Figure 1.5: Painted bees recovering from cold anaesthesia sitting on a picnic table.
**Behavioural Testing**

All behavioural testing was performed outdoors within 24 hours of capture. Bees were tested in male-male pairs, female-female pairs, and male-female pairs, as nestmates and non-nestmates. The order of the test conditions for each bee (nestmate or non-nestmate) was randomly chosen. Weather conditions were always fair on testing days, with the sun shining and a temperature over 15°C. Testing was always done outdoors, in the shade and on the grass a few metres from the benches where the *X. virginica* nest. The investigator remained far enough away (over 50 cm) from the tube as to not have the bees react to her presence. In addition, trials were conducted when other bees in the population were observed to be active, between 1000 and 1600h, 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). 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 (Figure 1.6). According to the guidelines established by Packer (2006), the outer tube's diameter was approximately double the head width of an individual *Xylocopa virginica*, and its length was 40 times the average *X. virginica* body length, approximately 80 cm long (Packer 2006). I chose circle tubes for this investigation because they allow for recognition assays to be carried out in natural light (Wcislo 1997), where *X. virginica* males encounter females and other males. In addition, encounters both within and between sexes occur in natural light at *X. virginica* nest entrances. I could not open up nests to observe females as doing so would quickly destroy my study population. Fortunately, testing female same-sex, male same-sex, and opposite sex pairs in the same manner allowed for comparison of males and females in all pair types.
a) 80 cm plastic tubing

Figure 1.6: A circle tube is created by putting the connector tube inside of one end of the 80 cm plastic tubing. Then the tubing is bent and joined end to end by inserting the connector tube into the other end of the tubing, forming a circle.
The bees were placed in plastic 50 mL eppendorf tubes on ice prior to testing, causing them to become inactive. Before the trial began, two bees were simultaneously placed in opposite ends of the circle tube to remove any 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. Behaviours and their times of occurrence were recorded with the aid of a handheld stopwatch.

Trials were planned beforehand so that bees were tested twice on the same day, once in the nestmate condition and once in the non-nestmate condition. Bees never participated in two trials in a row. The bees were chilled between trials and released back into the population after two bouts of testing. If both of the bees did not move enough to see or interact with one another for half (600s) of a trial, that trial was removed from the data analysis.

**Behaviours Analyzed**

A total of 22 behaviours were observed during this study and categorized in an ethogram (Appendix A, Table A1). However, only nine behaviours are presented in the body of this thesis. Behaviours that did not involve interactions between bees are not presented here because I did not consider these to be relevant as indicators of nestmate
recognition. Some interactive behaviours, such as push with abdomen (Appendix A, Table A1), were very infrequent and were not correlated with any behaviours of known biological significance. For these reasons, they were also left out of the primary analysis.

The behaviours listed below were categorized into event and state behaviours (Richardson 1994). Event behaviours are those which occurred for a very brief period of time while state behaviours are those which are maintained for more than a few seconds. Event behaviours, due to their short durations, were counted and analyzed as frequencies, or the total number of occurrences of the behaviour in a trial. State behaviours were analyzed as time spent performing a behaviour, and recorded as the total duration of the behaviour in a trial. Some behaviours were recorded as both event and state behaviours as they could be of short or long duration. In order to ensure accuracy of the analysis, these behaviours were reported in terms of frequency and time spent. Latency, or the time from the start of the trial until the behaviour was performed by either bee, was recorded for all behaviours. If a behaviour did not occur in a trial, the latency value was recorded as 1200s (the total duration of the trial).

**Aggressive Behaviours**

**Push** (event and state): One bee applies force to another with its head. This behaviour has been previously described as ‘nudging’ (Steen 2000; Arneson and 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 and Wcislo 2003). Here, I have described this behaviour as pushing because *X. virginica* does not touch the head of the other
bee and back away, but maintains head contact and continues to apply pressure. Oftentimes the instigating bee would push the other a fair distance around the tube before stopping. Latency, frequency and time spent measures are presented for this behaviour.

**Bite (event):** 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 all previous studies of bee behaviour in halictids (Bell and Hawkins 1974; Breed and Page 1991; McConnell-Garner and Kukuk 1997; Arneson and Wcislo 2003; Packer et al. 2003), andrenids (Paxton et al. 1999), a meliponine (Buchwald and Breed 2005) and one xylocopine, *X. aeratus* (Steen 2000). 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 are more difficult to observe than actual bites. Latency and frequency measures are presented for this behaviour.

**C-posture (event):** 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 (Breed et al. 1978; Kukuk 1992; McConnell-Garner and Kukuk 1997; Wcislo 1997; Pabalan et al. 2000; Arneson and Wcislo 2003; Packer et al. 2003). Both males and females exhibit C-posturing in *X. virginica*. Latency and frequency measures are presented for this behaviour.

**Sting (event):** A female bee curls her abdomen under her head and thorax, extending her sting and directing it at the other bee. Males cannot perform this behaviour as they
do not have stings. Stinging behaviour is one of the most violent acts in a bee’s behavioural repertoire (McConnell-Garner and Kukuk 1997; Copren and Geard 2005). During stinging, venom is injected into the bee being stung and the result can be lethal, although death from stinging was not observed in this study. Latency and frequency measures are presented for this behaviour.

**Unsuccessful pass attempt** (event): Involves one bee putting its head above or below another bee, attempting to force the other to turn over for a venter-to-venter pass. This behaviour has been reported for *X. aeratus* as a ‘complicated pass’ (Steen 2000) and was often coupled with other aggressive behaviours such as biting and stinging. Latency and frequency measures are presented for this behaviour.

**Cooperative and Tolerant Behaviours**

**Pass** (event): 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 (Paxton et al. 1999). Since passing requires action by both bees, it is considered to be cooperative (Kukuk 1992; McConnell-Garner and Kukuk 1997) or ‘an act of social tolerance’ (Arneson and Weislo 2003; Packer et al. 2003). Passing has been examined in *X. aeratus* observation nests and quantified as a cooperative behaviour or ‘simple’ pass that is not associated with aggression (Steen 2000). Latency and frequency measures are presented for this behaviour.
**Head-to-head touch** (state): 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. I have called this behaviour cooperative as both bees accept close physical contact within biting or stinging distance of the other. To remain in this position, especially for a prolonged time period, implies that the bees are (at the very least) tolerant of each other’s presence. Latency and time spent measures are presented for this behaviour.

**Equivocal Behaviours**

Behaviours were designated as ‘equivocal’ when the literature presented conflicting motivations for the same described behaviour, or when there was no clear association between the equivocal behaviours and others of known behavioural significance. The equivocal behaviours described below are interactions between two bees that occurred relatively frequently during the course of this study.

**Antennate** (event): Both bees move their antennae so as to touch the face of the other.

Antennation has various conflicting explanations in bee behaviour literature. In some insects, antennation is described as ‘smelling’ behaviour, and is considered cooperative because nestmates antennate more (Copren and Geard 2005). However, in *L. zephyrum*, a halictid, antennation occurred immediately before aggression (Bell and Hawkins 1974). In *Xylocopa aeratus*, antennating occurred prior to most interactions in observation nests (Steen 2000). Latency and frequency measures are presented for this behaviour.
Follow backing bee (event and state): Forward movement by a bee towards another bee that is walking backwards. Like following a walking bee, this behaviour was recorded when bees were no more than 45° apart in the circle tube. In some bee species, this behaviour is believed to be related to how queens control workers (Wcislo 1997). In L. zephyrum, queens have been observed to back away from the nest entrance while a worker follows to where the cells are constructed. In H. ligatus, following a backing bee is believed to be an act of acquiescence by a subordinate bee (Pabalan et al. 2000). However, in an investigation of fourteen other halictid species in Chile, following a backing bee was only correlated with avoidance behaviours (Packer 2006) suggesting this behaviour is neither cooperative nor aggressive. Latency, frequency and time spent measures are presented for this behaviour.

Follow walking bee (event and state): Forward movement by a bee towards another walking bee. This behaviour was recorded when bees were no more than 45° apart in the circle tube, ensuring that the follower could see the bee it was following. This behaviour has not been recorded in any previous studies. Latency, frequency and time spent measures are presented for this behaviour.

Collection of Females and Analysis of Ovarian Development

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 the -20°C freezer to be euthanized. The next day they were put in redistilled ethanol for preservation, until they were dissected to determine the level of ovarian development (Figure 1.7). Fully
Figure 1.7: Representation of ovarian development scoring in *Xylocopa virginica*. a) Undeveloped ovaries. Ovarian development (OD) score = 0. b) Partially developed ovaries. OD score = 1.25. c) Fully developed ovaries. The largest egg on the left side would be scored as a fully-developed egg. OD = 2.5.
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. Ovarian development score in the population ranged from 0 to 2.5.

**Data Analysis**

Behavioural data were examined in a number of ways. Latency, or the time from the start of the trial until the first occurrence of the behaviour of interest (by either bee) was analyzed for each pair. Latency was recorded for all behaviours, both event and state. To determine whether a behaviour was performed significantly earlier in nestmate vs. non-nestmate trials and the effect of the sexes of the bees in a pair, a 2-way ANOVA was used, examining the effects on behaviour 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 behaviours per pair, or the total number of times a behaviour occurred, was also analyzed. Frequencies were recorded for all event behaviours. Akin to the latency data, in order to detect significant differences in behaviour between the nestmate and non-nestmate condition in all pairings a 2-way ANOVA was employed, examining condition, sex and the interaction term condition by sex. Since these distributions were non-normal, the ANOVA was performed on ranked data. Bonferroni post-hoc tests were used to determine significant pair-wise differences in both the frequency and latency models.
To describe differences in the behaviour of individual bees towards nestmates vs. non-nestmates, the frequency of an event behaviour in a nestmate pair, minus the frequency of the same behaviour in a non-nestmate pair was calculated, generating a single difference value for each bee. If bees behaved no differently in the nestmate and non-nestmate conditions, the mean difference would be zero. If the mean difference per pairing was positive, the behaviour occurred more with nestmates whereas a negative mean difference would imply that the behaviour occurred more in non-nestmates.

The time spent performing a behaviour was also analyzed for individuals performing state 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. Distributions of behavioural differences per individual (both frequency and time spent) were tested for normality. Where the distribution of a particular behavioural parameter was normal, a Student’s t test was used to determine whether behaviour differences were significantly different from zero. Where the distribution was not normal, a Signed rank test was used.

To determine whether body size or ovarian development might influence behavioural interactions, Spearman correlations were completed. For male-male and female-female pairs, the intertlegular width difference between bees in a pair and biting frequency difference were analyzed. For female same-sex pairs, push frequency difference was also compared to the intertlegular width difference as females demonstrate pushing in many dominance interactions (Pabalan et al. 2000).

In order to determine the effect (if any) of ovarian development on aggression in female-female pairs, an analysis of ovarian development score difference and bite
frequency difference between bees in a pair was conducted using a Spearman rank correlation. The same correlation was repeated with push frequency difference instead of bite frequency difference as the measure of aggression.

All data analyses were conducted using SAS 9.1. ANOVA analyses were accomplished using PROC GLM at a significance level of $p=0.05$. For the general linear models, if the overall model significance was $p \leq 0.05$ then partial $F$ values with $p$ values less than 0.10 are also reported. Reporting these values provides a clearer and more complete representation of which factors contribute to the significance of the model.
Results

Section 1: Aggressive Behaviour

Males should demonstrate more aggression than females because males are territorial and demonstrate aggression as part of their daily activity. In same-sex pairs, aggression should increase among unfamiliar individuals and decrease with familiar individuals. In opposite-sex pairs, aggression should increase among (potential sibling) nestmates. Familiar opposite-sex bees should repel each other with aggressive behaviour as mating between them would be detrimental.

Pushing

Overall, non-nestmates pushed more often than nestmates and males pushed the most, exhibiting the most aggression. Latency was scored from the start of the trial until the behaviour of interest was performed by either bee. Pushing occurred earlier in male-male pairs than in other pairings (partial F=10.89, d.f. =2, p<0.0001, Figure 1.8). Overall, non-nestmates of all sexes pushed earlier than nestmates (partial F=4.35, d.f. =1, p=0.038, Figure 1.8). Male-male pairs also pushed more often than female same sex pairs, but not opposite sex pairs (partial F=9.29, d.f. =2, p=0.0001, Figure 1.9). Individual push frequency difference, calculated as the frequency of an individual’s pushing in a nestmate pair minus the frequency in a non-nestmate pair, also followed this pattern. Individual females tended to push same-sex nestmates more than non-nestmates, but not significantly so (Figure 1.10). Individual difference in time spent pushing, calculated in the same way as individual frequency difference, demonstrated a pattern
Figure 1.8: Mean latency (± standard error) to push in all pairings. The overall 2-way ANOVA model was significant (F= 4.98, d.f. =5, 185, p=0.003). Male-male pairs pushed significantly earlier than other pairings.

Figure 1.9: Mean number (± standard error) of pushes per trial in all pairings. The overall 2-way ANOVA model was significant, (F=4.70, d.f. =5, 185, p=0.0005). Male-male pairs pushed significantly more than other pairings.
Figure 1.10: Mean number of pushes per individual for all pairings. The mean difference values between the nestmate and non-nestmate conditions were analyzed statistically. Individuals in female-female pairs push less in nestmate pairs (Signed rank test, S=-142, p=0.061, n=73).

Figure 1.11: Mean time spent pushing per individual for all pairings. The mean difference values between the nestmate and non-nestmate conditions were analyzed statistically. Individuals in female-female pairs push for significantly less time in nestmate pairs (Student’s t test, t=-1.39, p=0.050, n=73).
identical to that of the individual frequency data. Females in same sex pairs pushed their non-nestmates significantly longer than their nestmates (Figure 1.11).

**Biting**

Biting largely followed the same pattern as pushing behaviour, with increased aggression in non-nestmate pairs. Bees in all sex pairings bit nestmates significantly later (partial F=9.12, d.f. =1, p=0.003, Figure 1.12) and bit nestmates significantly less than non-nestmates (partial F=9.18, d.f. =1, p=0.003, Figure 1.13). In addition, individual bees bit their nestmates less often in all cases, showing nearly significant differences in males in same sex and males in opposite sex pairs (Figure 1.14).

**C-posturing**

C-postures, on average, occurred near the end of trials in both same sex and inter sex pairings. Female-female and male-female pairs demonstrated C-posture threats significantly earlier than male-male pairs (partial F=7.49, d.f. =2, p=0.0007, Figure 1.15). This aggressive behaviour arose significantly earlier in non-nestmate pairs of all sexes (partial F=3.64, d.f. =1, p=0.058, Figure 1.15). Overall, nestmate bees demonstrated C-postures significantly less than non-nestmates (partial F= 4.17, d.f. =1, p=0.043, Figure 1.16) and female-female pairs C-postured more than male same sex pairs, but did not differ from male-female pairs (partial F=8.50, d.f. =2, p=0.003, Figure 1.16). C-postures per individual did not vary between the nestmate and non-nestmate condition (Figure 1.17).
**Stinging**

Mean latency to stinging fell near the end of a trial. Females in same sex and intersex pairs showed no differences in latency to sting between nestmates and non-nestmates (Figure 1.18). Sting frequency corresponded with the pattern seen in sting latency. Stinging was a very low frequency behaviour relative to the other behaviours studied here. Also, sting frequency per pair did not differ between the nestmate and non-nestmate pairs in female same-sex and inter-sex pairs (Figure 1.19). There was no difference in individual sting frequency between female same sex and inter sex dyads (Figure 1.20). For both pair and individual frequency analyses, there was a trend towards stinging nestmates less than non-nestmates (Figure 1.19, Figure 1.20).

**Unsuccessful pass attempts**

In male-male trials, attempts to pass occurred significantly earlier than in male-female trials, but not female-female trials (partial F=5.78, d.f. =2, p=0.004, Figure 1.21). Also in male-male trials, passing occurred significantly earlier in non-nestmate pairs (partial F=2.83, d.f. =2, p=0.061, Figure 1.21). Otherwise, there was no difference in latency or frequency of unsuccessful pass attempts between all pairings of nestmates and non-nestmates (Figure 1.21, Figure 1.22). Individual bees presented no difference in unsuccessful pass attempts between nestmates and non-nestmates (Figure 1.23).
Figure 1.12: Mean latency (± standard error) to bite per trial in all pairings. The overall 2-way ANOVA model was significant (F=2.49, d.f. =5, 185, p=0.033).

Figure 1.13: Mean number (± standard error) of bites per trial in all pairings. The overall 2-way ANOVA model was significant (F=2.56, d.f. =5, 185, p=0.029).
Figure 1.14: Mean number of bites per individual for all pairings. The mean difference values between the nestmate and non-nestmate conditions were analyzed statistically. Individuals in male-male pairs (Signed rank test, \( S=-34, p=0.078, n=33 \)) and males in male-female pairs (Signed rank test, \( S=-11.5, p=0.078, n=21 \)) tended to bite less when with nestmates.
Figure 1.15: Mean latency (± standard error) to C-posture per trial in all pairings. The overall 2-way ANOVA model was significant ($F=3.89$, d.f. = 5, 185, $p=0.002$). C-posturing was not observed in male-male nestmate pairs, and all latencies were recorded as 1200s.

Figure 1.16: Mean number (± standard error) of C-postures per trial in all pairings. C-posturing was not observed in male-male nestmate pairs. The overall 2-way ANOVA model was significant ($F=4.39$, d.f. = 5, 185, $p=0.0008$).
Figure 1.17: Mean number of C-postures per individual for all pairings. The mean difference values between the nestmate and non-nestmate conditions were analyzed statistically. All tests were non-significant.
**Figure 1.18:** Mean latency (± standard error) to sting. The overall 2-way ANOVA model was not significant (F=0.64, d.f. =3, 129, n.s.).

**Figure 1.19:** Mean number (± standard error) of stings per trial. The overall 2-way ANOVA model was not significant (F=2.12, d.f. =1, 86, n.s.).
Figure 1.20: Mean number of stings per individual for all pairings. The mean difference values between the nestmate and non-nestmate conditions were analyzed statistically. All tests were non-significant.
Figure 1.21: Mean latency (± standard error) to pass attempt. The overall 2-way ANOVA model was significant (F=3.45, d.f. =5, 185, p=0.005).

Figure 1.22: Mean number (± standard error) of unsuccessful pass attempts per trial. The overall 2-way ANOVA model was not significant (F=1.48, d.f =5, 185, n.s.).
Figure 1.23: Mean number of unsuccessful pass attempts per individual for all pairings. The mean difference values between the nestmate and non-nestmate conditions were analyzed statistically. All tests were non-significant.
Section 2: Cooperative or Tolerant Behaviours

Increased cooperation expressed through passing was expected among female nestmates, as they share nesting space and are required to pass regularly. Cooperating bees both must act in order to achieve a common purpose, such as travelling in opposite directions during a pass. On the other hand, increased tolerance was not expected among male same-sex pairs, as cooperation is not regularly expressed in male same sex and opposite sex pairs. Increased tolerance, expressed by head-to-head touching, is expected among familiar bees.

Passing

Male and female same-sex pairs passed significantly earlier than inter-sex pairs (partial F=6.15, d.f. =2, p=0.003, Figure 1.24). However, there was no difference in latency to pass between nestmates and non-nestmates in all pairings (Figure 1.24). In other words, there is no difference in latency to cooperation between familiar and unfamiliar bees. Male-male pairs passed significantly more than male-female pairs, but not more than female-female pairs (partial F=7.55, d.f. =2, p=0.0007, Figure 1.25). There was no difference in passing nestmates and non-nestmates in all pairings (Figure 1.25). Individual males in same-sex pairs passed their nestmates significantly less often than their non-nestmates (Figure 1.26). Individuals in all other pairings passed nestmates and non-nestmates with the same frequency (Figure 1.26).
**Figure 1.24:** Mean latency (± standard error) to successful pass. The overall 2-way ANOVA model was significant ($F=3.02$, d.f=5, 185, $p=0.012$).

**Figure 1.25:** Mean frequency (± standard error) of successful passes per trial. The overall 2-way ANOVA model was significant ($F=3.70$, d.f=5, 185, $p=0.003$).
Figure 1.26: Mean number of passes per individual for all pairings. The mean difference values between the nestmate and non-nestmate conditions were analyzed statistically. Individuals in male-male pairs pass non-nestmates significantly more (Student’s t test, $t=-1.97$, $p=0.058$, $n=33$).
**Head-to-head touching**

In both same sex and opposite sex pairings, tolerance expressed through head-to-head touching occurred earlier nestmate pairs versus non-nestmate pairs (partial F=6.69, d.f.=1, p=0.011, Figure 1.27). Males in same-sex pairs also head-to-head touch earlier than opposite-sex pairs, but not female same-sex pairs (partial F=2.90, d.f. =2, p=0.063, Figure 1.27). On the other hand, individuals in male-male pairs and both males and females in inter-sex pairs, head-to-head touched nestmates significantly longer than non-nestmates (Figure 1.28).

**Section 3: Equivocal Behaviours**

*Antennating*

Antennation latency and frequency did not differ between nestmates and non-nestmates in all pairings (Figures 1.29 and 1.30). Individuals in all pairings showed no difference in antennation frequency between nestmates and non-nestmates (Figure 1.31).

*Following a backing bee*

There is no difference in latency and frequency of following a backing bee between nestmates and non-nestmates in all pairings (Figures 1.32 and 1.33). Moreover, individual bees do not follow their nestmates more often or longer than their non-nestmates in all pairings (Figures 1.34 and 1.35).
Figure 1.27: Mean latency (± standard error) to head-to-head touch. The overall 2-way ANOVA model was significant (F=1.53, d.f. =5, 185, p=0.031).

Figure 1.28: Mean time spent head-to-head touching per individual for all pairings. The mean difference values between the nestmate and non-nestmate conditions were analyzed statistically. Individuals in male-male pairs (Signed rank test, S=116, p=0.03, n=33), males in male-female pairs (Student’s t test, t=0.57, p=0.04, n=21) and females in male-female pairs (Signed rank test, S=82, p=0.005, n=22) head-to-head touch nestmates significantly more.
Figure 1.29: Mean latency (± standard error) to antennate. The overall 2-way ANOVA model was not significant, \((F= 1.12, \text{ d.f.} =5, 185, \text{n.s.})\).

Figure 1.30: Mean number (± standard error) of antennates per trial in all pairings. The overall 2-way ANOVA model was not significant, \((F= 2.15, \text{ d.f.} =5, 185, \text{n.s.})\).
Figure 1.31: Mean number of antennates per individual in all pairings. The mean difference values between the nestmate and non-nestmate conditions were analyzed statistically. All tests were non-significant.
Figure 1.32: Mean latency (± standard error) to following a backing bee. The overall 2-way ANOVA model was not significant, (F= 0.73, d.f. =5, 185, n.s.).

Figure 1.33: Mean number (± standard error) of follows of a backing bee per trial in all pairings. The overall 2-way ANOVA model was not significant, (F=0.54, d.f. =5, 185, n.s.).
Figure 1.34: Mean number of follows of a backing bee per individual in all pairings. The mean difference values between the nestmate and non-nestmate conditions were analyzed statistically. All differences are non-significant.

Figure 1.35: Mean time spent following a backing bee per individual in all pairings. The mean difference values between the nestmate and non-nestmate conditions were analyzed statistically. All differences are non-significant.
Following a walking bee

Latency and frequency of following a walking bee was not significantly different between nestmate and non-nestmate pairs in all pairings (Figures 1.36 and 1.37).

Concordant with following a backing bee, individual bees do not follow their nestmates more often or longer than their non-nestmates in all pairings (Figures 1.38 and 1.39).

Section 4: Other factors that may influence behaviour

Size difference between bees in a pair

Previous data concerning bees in male-male and female-female pairings have shown that the larger bee in the pair tends to be the aggressor. To determine whether such a relationship was evident for this population, frequency of aggressive behaviours (biting, pushing) was correlated with size (intertegular width). In male and female same-sex pairs, biting frequency difference (number of pushes by bee one minus the number of pushes by bee two) was not correlated with intertегular width difference (intertegular width of bee one minus intertегular width of bee two) (males: Spearman rank correlation, $\rho = -0.14$, $p=0.40$, $n=37$, Figure 1.40, females: Spearman rank correlation, $\rho = 0.15$, $p=0.41$, $n=31$, Figure 1.41). Pushing was also correlated with size in females because pushing is a common behaviour used to attain dominance and large bees are often dominant. In this population, there was no relationship between push frequency difference and size difference in female-female pairs (Spearman rank correlation, $\rho=0.21$, $p=0.26$, $n=31$, Figure 1.42).
Figure 1.36: Mean latency (± standard error) to follow a walking bee. The overall 2-way ANOVA model was not significant, (F=1.00, d.f. =5, 185, n.s.).

Figure 1.37: Mean frequency (± standard error) of following a walking bee per trial. The overall 2-way ANOVA model was not significant, (F=1.24, d.f. =5, 185, n.s.).
**Figure 1.38:** Mean number of follows of a walking bee per individual in all pairings. The mean difference values between the nestmate and non-nestmate conditions were analyzed statistically. All tests were non-significant.

**Figure 1.39:** Mean time spent following a walking bee per individual in all pairings. The mean difference values between the nestmate and non-nestmate conditions were analyzed statistically. All tests were non-significant.
Figure 1.40: Bite frequency difference vs. intertegular width difference in male bees. When both the x and y coordinates have the same sign, the larger bee bites more in that trial. The outlier is the result of a trial where a bee bit the other bee much more than usual. (Spearman rank correlation, \( \rho = -0.14, p=0.40, n=37 \)).
Figure 1.41: Bite frequency difference vs. intertegular width difference in female-female pairs. When both the x and y coordinates have the same sign, the larger bee bites more in that trial. (Spearman rank correlation, \( \rho = 0.15, p=0.41, n=31 \)).

Figure 1.42: Push frequency difference vs. intertegular width difference in female-female pairs. When both the x and y coordinates have the same sign, the larger bee pushes more in that trial. (Spearman rank correlation, \( \rho = 0.21, p=0.26, n=31 \)).
**Ovarian development difference between bees in a pair**

Previous studies examining aggression in bees have revealed that females with more developed ovaries tend to be the aggressor in a pair. However, no significant relationship was found between aggression as biting (Spearman rank correlation, $\rho=-0.21$, $p=0.27$, $n=29$, Figure 1.43) or as pushing (Spearman rank correlation, $\rho=-0.14$, $p=0.47$, $n=29$, Figure 1.44) and size in female *X. virginica*. 
Figure 1.43: Bite frequency difference vs. ovarian development score difference. When both the x and y coordinates have the same sign, the bee with more developed ovaries bites more in that trial. (Spearman rank correlation, \( \rho = -0.21, \ p = 0.27, \ n=29 \)).

Figure 1.44: Push frequency difference vs. ovarian development score difference. When both the x and y coordinates have the same sign, the bee with more developed ovaries pushes more in that trial. (Spearman rank correlation, \( \rho = -0.14, \ p=0.47, \ n=29 \)).
Discussion

**Summary of Nestmate Recognition in X. virginica**

Both male and female *Xylocopa virginica* alter their behaviour when with a familiar bee, demonstrating that they have the ability to discriminate nestmates from non-nestmates (Table 1.1). I predicted that individuals in same sex pairs would increase aggression when with non-nestmates (Table 1.1), and indeed, more pushing, biting, C-posturing and unsuccessful pass attempts were observed when non-nestmates occupied the testing arena. Conversely, I predicted increased cooperation and tolerance in same sex nestmate pairs. Although overt cooperation was not expressed through passing, tolerance in male-male and male-female pairs was evident with increased head-to-head touching between nestmates. These results supported the kin selection hypothesis (Figure 1.1) as nestmates are potential kin and inclusive fitness is maximized by avoiding aggression with relatives. Increased tolerance was not expected in opposite sex pairs because as potential relatives, they should be avoiding potential inbreeding (Figure 1.1). However, this result is also consistent with the kin selection hypothesis (Figure 1.1).

Circle tubes have been used in many investigations of nestmate recognition (Breed et al. 1978; Kukuk and Crozier 1990; Kukuk 1992; Paxton et al. 1999; Buchwald and Breed 2005). In addition, this method has been used to predict social organization, both in inter and intra species comparisons, especially in studies where nests cannot be destroyed and nests that are difficult to locate (McConnell-Garner and Kukuk 1997; Weislo 1997; Pabalan et al. 2000; Arneson and Weislo 2003; Packer et al. 2003; Packer 2006). Using circle tubes allows for the observation of two bees without the influence of other individuals and the nest of origin (Pabalan et al. 2000). In other words, circle tubes are neutral arenas for the bees in the assay. Also, the tube approximates the tunnels in
Table 1.1: Summary of results ($p \leq 0.05$). NM= nestmate pair and NN= non-nestmate pair.

<table>
<thead>
<tr>
<th>Category</th>
<th>Behaviour</th>
<th>Pairing</th>
<th>Prediction</th>
<th>Significant difference between nestmates and non-nestmates?</th>
<th>Prediction correct?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggressive</td>
<td>Push</td>
<td>Same sex</td>
<td>NM &lt; NN</td>
<td>$\delta-\delta$, $\varphi-\varphi$</td>
<td>✓</td>
</tr>
<tr>
<td>Aggressive</td>
<td>Bite</td>
<td>Same sex</td>
<td>NM &lt; NN</td>
<td>$\delta-\delta$, $\varphi-\varphi$</td>
<td>✓</td>
</tr>
<tr>
<td>Aggressive</td>
<td>C-posture</td>
<td>Same sex</td>
<td>NM &lt; NN</td>
<td>$\varphi-\varphi$</td>
<td>✓</td>
</tr>
<tr>
<td>Aggressive</td>
<td>Sting</td>
<td>Same sex</td>
<td>NM &lt; NN</td>
<td>None</td>
<td>✗</td>
</tr>
<tr>
<td>Aggressive</td>
<td>Unsuccessful pass</td>
<td>Same sex</td>
<td>NM &lt; NN</td>
<td>$\delta-\delta$</td>
<td>✓</td>
</tr>
<tr>
<td>Cooperative</td>
<td>Pass</td>
<td>Same sex</td>
<td>NM &gt; NN</td>
<td>$\delta-\delta$</td>
<td>✗</td>
</tr>
<tr>
<td>Tolerant</td>
<td>Head-to-head touch</td>
<td>Same sex</td>
<td>NM &gt; NN</td>
<td>$\delta-\delta$, $\varphi-\varphi$</td>
<td>✓</td>
</tr>
<tr>
<td>Equivocal</td>
<td>Antennate</td>
<td>Same sex</td>
<td>?</td>
<td>None</td>
<td>N/A</td>
</tr>
<tr>
<td>Equivocal</td>
<td>Follow walking bee</td>
<td>Same sex</td>
<td>?</td>
<td>None</td>
<td>N/A</td>
</tr>
<tr>
<td>Equivocal</td>
<td>Follow backing bee</td>
<td>Same sex</td>
<td>?</td>
<td>None</td>
<td>N/A</td>
</tr>
</tbody>
</table>
which the bees spend much of their time (Pabalan et al. 2000).

After examining behaviour in circle tubes, nestmate recognition becomes evident when bees change their behaviour from nestmate pairs to non-nestmate pairs, as is true in many circumstances in this study. According to Breed, recognition may be cryptic or present only in certain contexts. Therefore, a positive result indicates that the species under investigation possesses a functioning recognition system (Breed 2003). Moreover, there is no correlation between size and aggression in males or females, and no correlation between ovarian development and aggression. This suggests that all the behaviours observed here are results of the level of familiarity between the individuals involved and not affected by the reproductive status or size of the interacting pair.

**Female-female recognition**

For female same-sex pairs, aggressive behaviour increased in non-nestmate pairs. Increased aggression among non-nestmate *X. virginica* females is consistent with guarding behaviour, as guarding bees need to distinguish non-nestmates from nestmates in order to prevent entry to nest intruders. This is the first evidence that *X. virginica* females have the ability to distinguish foreign bees and have the capacity to guard. In *X. aeratus*, a bee that demonstrates guarding, antennation occurs prior to most adult interactions (Steen 2000). This is also true of *Melipona panamica* in encounters between guards and bees attempting to enter the nest (Inoue et al. 1999). *X. virginica* also antennate frequently and given that antennation does not differ between nestmates and non-nestmates, antennation may function as a way to identify individuals by scent.

Although it is difficult to directly observe guarding in nests tunnelled into wood, I have observed pollen-carrying females in this population occasionally entering the
wrong nest and retreating shortly thereafter, still laden with pollen. Also, pushing occurs most often in female same-sex pairs, suggesting that this is their primary means of aggression. However, there were no significant differences observed between nestmates and non-nestmates in terms of stinging. A reason why this may have occurred is that stinging occurs at low frequencies and more tests may be required to detect a difference. This problem has been circumvented in other studies by incorporating stinging into an aggression score (Foster et al. 2004). However, since stinging is highly correlated with other aggressive behaviours (Appendix A, Table A4) and I wanted to illustrate which behaviours bees perform in the presence of non-nestmates more clearly, I kept stinging separate from other aggressive behaviours. Due to the low frequency of stinging, and the fact that it occurs near the end of a trial, it may be a last resort behaviour.

Conversely, increased cooperation was not observed in female-female nestmate pairs. This result was unexpected as female bees that show social tendencies generally show increased cooperation with nestmates, by passing them more often (Packer et al. 2003). This is not true in _X. virginica_, who do not increase passing with familiar bees as primitively eusocial bees do. Also unlike _X. virginica_, communal bees show indiscriminate cooperation (Kukuk and Crozier 1990; Kukuk 1992), suggesting that _X. virginica_ is not communal either (Gerling et al. 1989). However, decreased aggression towards nestmates supports kin-selection (Hamilton 1964) or mutualism (Lin and Michener 1972) as the adaptive function of nestmate recognition. It appears that _X. virginica_ females and males overwinter as siblings; however, travel from nest to nest is exceedingly common (to be addressed in Chapter 2) and this likely decreases relatedness among nestmates. Since the genetic relationship between nestmates is unknown, I cannot
accept kin selection over mutualism (or vice versa) as to how sociality increases \( X. \) virginica fitness.

**Male-male recognition**

Like females, males are also more aggressive towards same-sex non-nestmates. Among all pairings, they showed the most aggression, as expected because of the overt aggression involved in territoriality. Decreased aggression towards nestmates (familiar bees) indicates that male \( X. \) virginica possess the recognition ability required for the 'dear enemy' effect (Gordon 1989). This ability allows males to reduce aggression towards familiar males defending territories nearby, and direct it at unfamiliar, potential territory usurpers. Recognizing territory-neighbours is highly beneficial as males can hold territories for up to two weeks (Gerling and Hermann 1978) and constant attacking of nearby males would be physically costly. However, this hypothesis needs to be tested with males holding territories by observing aggressive behaviour between neighbours and non-neighbours. I did not anticipate males to cooperate more with male nestmates than non-nestmates as overt cooperation is not a part of their life history. Furthermore, since males overnight in nests together, I expected them to be more tolerant of their nestmates than their non-nestmates. In male \( P. \) fuscatus pairs, they demonstrate increased tolerance towards nestmate males by spending more time in close proximity to nestmates than non-nestmates (Shellman-Reeve and Gamboa 1984). The same is true for \( X. \) virginica male pairs, with nestmates touching heads more than non-nestmates. This behaviour suggests a high level of tolerance towards nestmates because touching males are vulnerable to
attack, as a bee can easily transition from a head-to-head touch to a push, bite or pass attempt.

A behaviour that warrants some extra attention in male-male pairs is passing. In females, passing is considered to be a cooperative behaviour (Kukuk 1992; McConnell-Garner and Kukuk 1997; Paxton et al. 1999; Arneson and Wcislo 2003; Packer et al. 2003). In males, on the other hand, successful passes occur significantly more in non-nestmate pairs. Since increased passing occurs with non-nestmates, coinciding with increased aggression, this behaviour may be an assertion of dominance. In female L. zephyrum, passes did not occur right away, but after a series of encounters where fighting occurred (Breed et al. 1978), also suggesting dominance. The bee who is trying to pass puts its head above the other’s and continues to move forwards. This action prevents the other from doing anything aside from accepting the pass or backing up. An examination of passing in male dyads illustrates that behaviours may have different motivations and meanings depending on the sex of the bees being examined.

Male-female recognition

In opposite-sex pairs, I expected increased aggression in nestmate pairs for the reason that related individuals would avoid inbreeding, but such was not the case. There are two possible explanations for this result. The first is that males cannot discriminate female nestmates from non-nestmates and vice versa. In colonies of Apis mellifera, female guards admit drones into colonies more often than they admit other workers, suggesting that guards may recognize foreign females more readily than foreign males (Moritz and Neumann 2004). The second explanation for this apparent lack of
recognition is that both females and males in my study population frequently move from nest to nest (to be discussed in the next chapter). Consequently, females and males sharing a nest may not be related, so increased aggression or inbreeding avoidance behaviour would not be necessary. However, the patterns of aggression and cooperation in male-female pairs do reflect those of same-sex pairs: increased tolerance (head-to-head touching) among nestmates and increased aggression among non-nestmates. This difference in behaviour between nestmate and non-nestmate same-sex pairs suggests that both males and females can distinguish opposite sex nestmates from non-nestmates.

**Further research**

There are many questions about recognition behaviour in *Xylocopa virginica* that warrant investigation. Recognition ability may vary depending on the number of nests nearby, with individuals in dense aggregations demonstrating more accurate recognition ability than those living in more widely distributed nests. The colonial Cliff swallow that nests in highly dense aggregations is more adept at offspring recognition than the Bank swallow, whose nests are more spread out (Beecher 1988).

Another factor that may influence recognition ability is the number of females living in a nest. In *Megalopta genalis*, females with fewer nestmates were first to initiate aggression in a pair (Arneson and Wcislo 2003). It is possible that females who encounter more females on a regular basis are more tolerant than those living alone. If a lone female encounters any other bee inside the nest, the novel bee is more likely to be an intruder, requiring less-specific recognition ability.
More investigation can also be done to determine the relationships among females in a nest. In order to determine the presence of kin selection in these bees, relatedness of nestmates needs to be determined. Also, the pattern of nest inheritance needs to be investigated. It is possible that daughters inherit nests. In Georgia, *Xylocopa virginica* females generally live two years, thus creating nests with two generations, a mother and her daughters (Gerling and Hermann 1978). However, in Niagara, the colder weather likely negatively affects the bees’ survival as few individual females (the mothers) are seen to survive longer than one season. Moreover, if the mother bees survive the winter, they are often found dead early in the season, leaving the nest open for inheritance by one of the daughters. If females inhabiting the nest are unrelated, females may guard for a chance to inherit the nest if the primary forager does not survive.

Another factor to examine in terms of aggression between nestmate bees is age. In the multi-female nests of the wasp *P. colobopterus*, older females are more aggressive to the younger females (Platt et al. 2004). *Xylocopa virginica* in Georgia live in nests with both one and two-year old females, with the older female doing most of the foraging and construction (Gerling et al. 1989). The older female may attain dominance through aggression towards younger bees as dominance is attained through increased aggression towards the other bees in the nest (Weislo 1997; Pabalan et al. 2000; Arneson and Weislo 2003).
Chapter 2: Nest Fidelity in *Xylocopa virginica*

**Introduction**

*Nest Fidelity in Bees and Wasps*

Given the ability to discriminate nestmates from non-nestmates and the fact that bees are normally attached to their home nests (Kasuya 1981), accidentally ending up in the ‘wrong’ nest seems unlikely. However, ‘drifting’ does occur in many species of Hymenoptera. Drifting occurs when an individual bee or wasp moves into a nest other than the one it is primarily associated with (Michener 1966). In order for a winged hymenopteran to drift, there are two distinct events that must occur. First, the bee or wasp has to stray from its course and try to gain admittance into a nest other than its own. Second, nest guards must allow the individual to enter and remain in the nest (Paar et al. 2002; Moritz and Neumann 2004). In other words, drifting suggests that both navigation and nestmate discrimination are error prone.

*Navigation and Drift*

Bees and wasps have complex and effective navigation systems, using orientation flights to commit the terrain around their nests to memory (Kasuya 1981). Therefore, winged Hymenoptera are unlikely to travel to a foreign nest when attempting to fly home. If a navigation error is made, upon entering the ‘wrong’ nest, bees most often fly back out in seconds. For example, promptly exiting a nest other than its home nest has been reported for *Lasioglossum versatum* (Michener 1966). However, drifting is often analyzed in artificial circumstances devoid of distinctive landmarks (Kasuya 1981; Neumann et al. 2001; Birmingham et al. 2004; Birmingham and Winston 2004), increasing rates of navigation errors, and therefore artificially inflating the rate of drift.
For example, in a commercial tomato greenhouse, up to 34.8% of *Bombus occidentalis* and *B. impatiens* workers were observed drifting (Birmingham and Winston 2004). Also, other navigation cues, such as orientation of the flight entrance towards the sun are known to affect the number of drifted individuals in *Apis mellifera* (Moritz and Neumann 1996).

There are very few studies in which drifting has been examined in the field. In a study of *Megachile rotundata* released in the field, 32.3% and 51.8% of bees in two field sites were observed drifting (Goerzen et al. 1995). An examination of a natural population of the wasp *Polistes canadensis* revealed that 56% of females drifted to a location other than their home nest (Sumner et al. 2007). A similar level of drift was observed between two nests of *Polistes jadwigae* situated 150 cm apart, where 52.2% of workers drifted at least once (Tsuchida and Ito 1987). These high levels of drift suggest that bees and wasps may not be lost when entering other nests, but have some other reason for doing so. Furthermore, if individuals are making navigational errors we would expect that they would drift into nests nearer to their home nests more often than nests located further away (Sumner et al. 2007).

Guarding, Nestmate Recognition and Drift

After flying to a novel nest, a bee must be accepted into the nest in order to successfully drift. In order to get into the colony, foreign individuals often have to pass guards that keep non-nestmate bees outside the nest. In studies of nestmate recognition in bees and other Hymenoptera, recognition ability is often evaluated by scoring admittance of nestmates into the nest and rejection of non-nestmates (Bell 1974; Bell et al. 1974;
Bell and Hawkins 1974; Tibbetts 2002; Moritz and Neumann 2004). In a study of *Lasioglossum versatum*, a species where guard bees block the nest entrance with their abdomens, a high level of drift was observed, suggesting failure of guards to discriminate nestmates from non-nestmates (Michener 1966). However, *Apis mellifera* guards, known to have effective nestmate recognition abilities, can allow 50-90% forager drift if landmarks used for navigation are removed (although drift is generally very low) (Paar et al. 2002). It is unlikely that a temporary loss of nestmate recognition ability accounts for the potentially high admittance of foreign bees into a colony. Another explanation for these fluctuating levels of drift is that under some conditions, it may be advantageous to admit foreign individuals into the nest.

**Benefits and Costs of Drifting**

Allowing foreign bees into an established colony may sometimes be beneficial for nest members as well as drifted bees. In *Apis mellifera*, ‘lost’ bees offer guards food in exchange for passage into the nest (Moritz and Neumann 1996). A more lasting benefit of admitting a foreign female into a nest is that she could aid in nest activities (Neumann et al. 2001). For example, *P. canadensis* wasps behave like workers in all of the nests they visit, helping increase fitness in many colonies (Sumner et al. 2007). This is also true for *P. chinensis antennalis* females, who are reported to behave ‘as if they were genuine workers’ in the nests they drift into (Kasuya 1981). Also, the drifting individual may benefit from entering a new nest by increasing its chance at reproduction as a potential egg layer (Sumner et al. 2007).
Drifting may also be costly to a colony. Drifting females may be pollen or nectar robbers (Paar et al. 2002), potential nest usurpers (Vinson and Frankie 2000) looking to extract valuable resources from the colony, or social parasites, laying eggs in novel nests but contributing nothing to colony maintenance (Neumann et al. 2001; Birmingham et al. 2004). Drifting Bombus occidentalis workers probably are social parasites as they have more mature oocytes than resident workers (Birmingham et al. 2004). Moreover, in L. malachurum 'alien' workers (unrelated to the queen) produce gynes in their new nests (Paxton et al. 2002). In more extreme cases, such as in the Cape honeybee (A. m. capensis), drifted workers develop into 'pseudoqueens' (primary egg layers) in host colonies of A. m. scutellata, resulting in a fitness loss to host bees that expend energy raising unrelated individuals (Neumann et al. 2001).

**Males and Drifting**

Very few studies have examined drifting in male Hymenoptera and those that have concern drones in Apis mellifera. Admitting drones into a colony is costly as they feed on colony provisions, and the presence of drifted drones results in decreased male production in the colony (Moritz and Neumann 1996). Drones drift more frequently than workers (Moritz and Neumann 1996; Moritz and Neumann 2004), suggesting that guards admit foreign drones into nests more often than foreign workers. A DNA fingerprinting study of drifted drones indicates that the band-sharing coefficients between queens and drifted drones are high (Moritz and Neumann 1996). Consequently, this genetic similarity may result in confusion of nestmate recognition cues by the guards, who allow these foreign drones to enter the nest (Moritz and Neumann 1996). Moreover, females may
have different nestmate recognition systems for drones and workers and may discriminate female nestmates from non-nestmates more effectively (Moritz and Neumann 2004). However, these studies were conducted in apiaries and more work needs to be done to determine the causes of male drift in natural populations.

Drifting in Xylocopa virginica

*Xylocopa virginica* live in either single or multi-foundress nests burrowed into wood. Since nest substrate is generally limited and nests are energetically costly to build, nests are often reused and expanded year after year. Consequently, nest space is highly valuable and it may be advantageous for a bee to leave its own nest in search of available nest space to raise brood, as single and multi-foundress nests are equally productive (Gerling and Hermann 1978). Also, due to limited substrate, nests tend to be in close proximity to each other, increasing the chance of accidentally flying into the wrong nest. However, entering a nest in an entirely different location than the home nest suggests that females are deliberately choosing new nests to move into.

In the previous chapter, I showed that female *Xylocopa virginica* can distinguish nestmates from non-nestmates. Female *X. virginica* likely use this ability to guard nests, admitting nestmates and preventing non-nestmates from entering the nest. The existence of guarding behaviour suggests that there is a cost associated with allowing non-nestmate females into the nest. However, guarding is not error proof, and finding many females in nests other than their own would suggest that *X. virginica* females make poor guards. Alternatively, new females may be admitted into nests so that they may contribute to nest activities, resulting in an overall benefit to nest inhabitants (Sumner et al. 2007).
*Xylocopa virginica* males, on the other hand, defend territories adjacent to nest substrates (Barrows et al. 1975; Barthell and Baird 2004). Defending territories near their natal nests may increase a male’s chance of mating with a relative, so drifting may be beneficial for males that may spend the night in a nest near to their territories. In addition, since males have to compete for territories, they may have to settle with any territory they can win and overnight in the nearest nest they can gain admittance to. Given this competition, males may drift at random to wherever they can gain a territory.

**Objectives and Hypotheses**

In this study I examined the frequency of drifting by both females and males in a natural population of *X. virginica* over two breeding seasons. Since females must provision and lay brood that are confined to one nest, I predicted that female *X. virginica* would not drift, or drift very little. On the other hand, consistent with observations of males in *A. mellifera*, I predicted that males would drift more often than females. Also, I believed that *X. virginica* drifting is a result of navigational error and therefore predicted that more bees (both males and females) would be found drifting into nearby nests than nests further away.


Methods

Data collection

These data were collected in 2006 and 2007 during the study presented in Chapter one. The *Xylocopa virginica* population studied here resided in nests burrowed into cedar benches (Figure 1.1) with varying numbers of nests. Bees were captured using 'cup traps'. Traps were placed over the nest entrances in the morning prior to the onset of daily flight activity in order to catch bees that spent the night in the same nest. Once a trap was removed, it was replaced with a new trap so as to catch any bees that emerged later in the day. When captured, bees were chilled, marked uniquely on the abdomen and thorax with paint, and released near the bench where they had been trapped. Some bees were tested behaviourally before being released back into the population (Chapter 1). After marking or testing, bees were released at the location of testing, adjacent to the benches the bees reside in after testing is complete. Neither the testing nor the release seemed to affect them as they returned to regular behaviour, females often returning immediately to the nest they were captured from, and males returning to territory-guarding. Moreover, the number of behaviourally tested bees that were not seen again, that did not drift, and that did drift were not significantly different from the number observed in bees that were not tested behaviourally (Figure 2.1). When the bees were found in the traps, the date, location and time of each capture was recorded.

A recapture was defined as any occasion in which the bee was caught after the initial capture, when the bee was measured and marked. Only bees that were recaptured at least once were included in this analysis. The location of initial capture was compared to subsequent locations of captures to determine the existence of drifting in the *X.*
Figure 2.1: a) The fate of female bees recaptured after behavioural testing vs. untested females on their first recapture. There is no difference in outcomes between tested bees and non-tested bees ($\chi^2 = 3.43$, d.f. = 2, n.s.). b) The fate of male bees recaptured after behavioural testing vs. non-tested males on their first recapture. There is no difference in outcomes between tested bees and non-tested bees ($\chi^2 = 4.29$, d.f. = 2, n.s.).
virginica population (Figure 1.2, Chapter 1). Bees that could not be identified with absolute certainty by their abdomen and thorax markings were not included to ensure accuracy of the data.

The number of nests with single foundresses was determined by examining the number of females that emerged from each nest throughout the course of the study. If a single female exited each nest throughout the study period, the nest was designated a single foundress nest. By definition, these nests did not have any drifters as no other bees were ever observed there. If more than one female exited a nest during the study period, the nest was designated as a multi-foundress nest. The number of times a bee was found in its 'home' nest (the nest it was first found in) versus any other nest was examined. A bee that was found in another nest in the same bench as its home nest, represented nearby drift. This type of drift was predicted to be most likely as a bee may veer slightly off course and accidentally end up in a neighbouring nest. Finding a bee in another bench entirely represented a more extreme version of drifting, as a bee found at another bench has either made a grave error in navigation, or has deliberately left its nest for another.

**Statistical Analysis**

The distributions of overall recapture frequencies in 2006 and 2007 were compared using Komolgorov-Smirnov tests to determine whether they differed significantly between years. The numbers of drifters (bees recaptured in at least one nest other than their home nests) and non-drifters (bees recaptured only in their home nests) were compared by year and sex using Chi-square tests. Also, the locations of first recapture, in the home nest, another nest in the same bench or a nest in another bench
were compared using Chi-square tests. Lastly, the overall drifting strategy or the number of recaptures at each location was compared between the sexes using a Chi-square analysis. P-values less than 0.05 were considered significant.

Results

Populations and Overall Drift in 2006 and 2007

In total, 165 females and 193 males were captured and marked in 2006, while 126 females and 93 males were captured and marked in 2007. Any uncertain observations were removed from the analysis, leaving 117 females and 106 males for 2006, and 119 females and 81 males for 2007. The majority of nests in 2006 and 2007 were multi-foundress nests. In 2006, a single female exited only 10 of 48 nests, suggesting these nests had a single foundress. In 2007, a single female exited 9 nests of 50 throughout the study period.

The distributions of recaptures did not differ between 2006 and 2007 for females or males (Figure 2.2). Therefore, the data for both years were combined for most of the analyses. In 2006, only 53 of 117 (45.3%) females were recaptured at the study site (Figure 2.2 a). Of these 53, 27 were only recaptured at their home nest, while the other 26 drifted to another nest on at least one occasion. In 2007, 65 of 159 (40.9%) females were recaptured at least once (Figure 2.2 a). Of these 65, 19 were non-drifters and 46 were drifters. More females drifted in 2007 than 2006 ($\chi^2 = 4.96$, d.f. =1, p=0.026, Figure 2.3 a).
Figure 2.2: a) Numbers of recaptures for female bees in 2006 and 2007. There is no difference between the 2006 and 2007 distributions (Komolgorov-Smirnov, KSa=0.74, d.f. =1, n.s.). b) Numbers of recaptures for male bees in 2006 and 2007. There is no difference between the 2006 and 2007 distributions (Komolgorov-Smirnov, KSa=1.01, d.f. =1, n.s.). The population density was higher in 2006 than 2007 as suggested by the greater number of marked bees in that year.
As for males, 47 of 106 (44.3%) were recaptured at least once in 2006; 12 of the 47 did not drift and were only found in their home nests (Figure 2.2 b). In 2007, 42 of 81 (51.9%) of males were recaptured once or more (Figure 2.2 b). Only 6 of the 42 recaptured males in 2007 did not drift. There was no significant difference in the number of male drifters vs. non-drifters in 2006 and 2007 ($\chi^2 = 2.36$, d.f. =1, n.s., Figure 2.2 b). Overall, males drifted more than females in both 2006 ($\chi^2 = 5.53$, d.f. =1, $p=0.018$, Figure 2.2) and in 2007 ($\chi^2 = 3.77$, d.f. =1, $p=0.052$, Figure 2.2).

Locations of Recapture
Since recapture rates did not differ between 2006 and 2007, and locations of first recapture do not differ from year to year in both sexes (females: $\chi^2 = 4.03$, d.f. =2, n.s., males: $\chi^2 = 4.03$, d.f. =2, n.s., Figure 2.4), I compared observed locations of first recapture to the expected values for each sex. I expected that females would be philopatric and therefore found in home nests 100% of the time. However, females were recaptured in foreign nests significantly more than expected ($\chi^2 = 70.94$, d.f. =2, $p<0.0001$). I expected that males would drift more or less randomly and so be first recaptured in home nests, nearby nests, and nests in other benches equally often. Male location of first recapture did not differ significantly from random ($\chi^2 = 4.55$, d.f. =2, n.s.). Of the bees that drifted, both females ($\chi^2 = 0.69$, d.f. =1, n.s.) and males ($\chi^2 = 0.39$, d.f. =1, n.s.) drifted to nearby nests (nests in the same bench) or further nests (in another bench), no different from the expectation that drift is random. Overall, the locations of first recaptures in home nests, nearby nests and nests in another bench were not significantly different between females and males ($\chi^2 = 0.05$, d.f. =2, n.s., Figure 2.5). Furthermore, drifting strategy, or whether an individual bee stays predominantly in the
same nest, half or more than half the time in the same nest, less than half the time in the same nest or is always recaptured at a different nest was significantly different between males and females ($\chi^2 = 30.60$, d.f. =3, p<0.0001, Figure 2.6). Females were generally more consistent in terms of nest choice, whereas males were more likely to move from nest to nest.
Figure 2.3: a) Number of females recaptured at the home nest vs. those that drifted in 2006 and 2007. b) Number of males recaptured at the home nest vs. those that drifted in 2006 and 2007.
Figure 2.4: a) Number of female bees at location of first recapture in 2006 and 2007. b) Number of male bees at the location of first recapture in 2006 and 2007.
Figure 2.5: Location of first recapture for males and females (2006 and 2007 data combined).
Figure 2.6: Recapture location patterns for individual bees for both females and males (2006 and 2007 data combined). Recapture location patterns are significantly different between female and male bees.
Discussion

*Drifting by female X. virginica*

Overall, female *X. virginica* drifted significantly more than expected based on the hypothesis that females are entirely faithful to their nests. The number of *X. virginica* females observed to drift (nearly half of recaptured females) is similar to other studies of natural populations of bees and wasps, such as *Megachile rotundata* (Goerzen et al. 1995), *Polistes canadensis* (Sumner et al. 2007) and *Polistes jadwigae* (Tsuchida and Ito 1987). Given this high rate of drift, and the fact that nestmate recognition is present in females of this species, the amount of drift is not likely the result of navigation errors coupled with nestmate recognition errors.

In 2006 and 2007, females were first recaptured in nests in other benches just as often as in nests near their home nest. This suggests that females were not simply lost as navigational errors should result in their being more likely to mistakenly enter a nest near to their home than one farther away. In addition, the overall bee population in my study site was much lower in 2007 than 2006, suggesting that there was more available nest space in that year. As a result, drifting females may have been looking for new nesting space. *Centris bicornata*, another species of bee that reuses conspecific nests tunnelled into wood, competes intensely for nest space (Vinson and Frankie 2000). With fewer bees occupying (or guarding) the nests, drifting *X. virginica* females may have more success in usurping established nests when population densities are low. When examining patterns of recapture, females were found most often in the same nest all the time or in the same nest more than half the time. This indicates that females either stay in their natal nests, or move permanently into another, supporting the idea that drifting females are looking for new homes. On the other hand, this pattern of recapture where females are
most often found in a single nest but occasionally in another nest, suggests that females may be visiting other nests to rob pollen. Furthermore, females in other *Xylocopa* species, such as *X. aeratus* (Steen 2000) and *X. pubescens* (Hogendoorn and Velthuis 1993), are known to rob pollen from other nests. Since just as many females stay home as leave infrequently, some females appear to be pollen robbers while others are not and this suggests one reason why guarding might be important.

**Drifting in male X. virginica**

Concordant with studies of drone drifting in *Apis mellifera*, male *X. virginica* drift significantly more than females (Moritz and Neumann 1996; Moritz and Neumann 2004) suggesting that males are admitted into novel nests more often than females. For *A. mellifera*, researchers suggested that guarding females have a different recognition system for novel males and females (Moritz and Neumann 2004). My studies of interactions between opposite sex nestmate and non-nestmate pairs of *X. virginica* (Chapter 1) also suggest a different recognition system for males and females, because in opposite sex non-nestmate pairs, aggression remained at the same level as in nestmate pairs. To effectively eject foreign individuals, guard bees increase aggression towards non-nestmates (Bell and Hawkins 1974; Michener 1974; Gamboa et al. 1987a; Moritz and Neumann 2004). However, in *X. virginica*, females do not increase aggression towards novel males, whereas they exhibit increased tolerance towards male nestmates, indicating they can discriminate male nestmates from non nestmates. This result suggests that, as in *Apis mellifera*, *X. virginica* females may possess different recognition systems for males and females.
Males drift significantly more than females in terms of overall drift, location of first recapture and overall recapture pattern. In terms of first recapture, there is no clear pattern showing males drifting to nearby nests in the same bench versus nests in other benches. Like females, if males were getting lost en route to their home nests, they would more likely be found in nearby nests than in another bench. This does not appear to be the case, as males drift at random, caught equally as often in nearby nests and nests in another bench. In terms of overall recapture pattern, males are most often found drifting from nest to nest on a regular basis. An explanation for the pattern of first recaptures and overall recaptures is that males occupy nest space overnight nearest to their territories, and may be moving into nests near the best territories they can win. In order to investigate this hypothesis, the proximity of a male's territory to the nest he overnights in must be determined. However, the factors that determine territory quality for Xylocopa virginica are not known, and whether males drift toward nests that contain superior territories also needs to be investigated.

Further Studies

One of the questions that still remains unanswered is whether female Xylocopa virginica drift into nests as social parasites. In Bombus occidentalis, drifting females have higher ovarian development than nest residents, suggesting that they enter novel nests to have their own offspring, using colony resources in order to raise them (Birmingham et al. 2004). In this study, drifting females were not killed and dissected in order to determine ovarian development as damage to the study population would have adversely affected the results presented in the first chapter of this study. If drifting X.
virginica females have higher ovarian development than resident females, drifers may be social parasites.

Furthermore, if nesting space is the limiting factor for individual reproduction in X. virginica females, as it is in C. bicornata, drifting females may simply be looking for nesting space, drifting until they find a suitable location. In the ant Aphaenogaster araneoides, colonies relocate to another nest when they become too large (McGlynn et al. 2004). This may also be true in female X. virginica who potentially drift in order to find a larger nest that can accommodate an extra individual, not necessarily to become social parasites.
Overall Conclusions

In the first chapter of this thesis I examined the nestmate recognition abilities of female and male *Xylocopa virginica* in same sex and opposite sex pairs. In same sex pairs, both females and males were more aggressive towards unfamiliar bees and more tolerant towards those they had shared a nest with at least on the day they were captured. In opposite sex pairs, there was no evidence of inbreeding avoidance. However, opposite sex nestmates were more tolerant (head-to-head touching more) than non-nestmates, indicating that they do recognize each other.

Chapter Two revealed that *X. virginica* move from nest to nest frequently, making nest membership unstable. In order for drift to occur, an individual must attempt to enter a nest other than its home nest and then must be accepted by the residents of that nest. Females drift much less than males, with most females being recaptured in one or two nests overall. Since females can distinguish nestmates from non-nestmates, the fact that they can successfully enter another nest indicates either that they are accepted into the nest for some adaptive reason (such as guarding in exchange for possible nest inheritance) or that recognition is error-prone. There are some females that are never caught in the same nest twice, suggesting that these females may be better than others at avoiding detection by nest residents.

Males, on the other hand, seem to drift at random. Since drifting is so prevalent among male *X. virginica*, nestmate males and females are unlikely to be kin. This explains why behaviour in opposite-sex *X. virginica* pairs did not suggest the presence of inbreeding avoidance. Furthermore, the ability of males to move regularly from nest to nest suggests that females do not actively prevent males from entering. Simultaneously,
females recognize and show increased tolerance towards familiar males. Given that females readily accept males into their nests, doing so may be beneficial to resident females or at least is probably not maladaptive.

The combined results of this study suggest that nestmate recognition functions within and between sexes despite shifting nest membership. Whether female drifters are eluding detection and becoming social parasites is still unknown. If this is true, these females may be gaining direct fitness where nesting substrate is limited. The other possibility is that novel females enter a nest with mutual benefit to the residents and the new member. For example, a novel female may enter a nest with fewer female members and exchange some nest-maintenance activity (such as guarding or clearing debris) for an improved chance to inherit the nest. Further exploration of drifting and its effect on the fitness of nest residents is required in order to understand the complexity of interactions that occur in populations of *Xylocopa virginica* and many other flying Hymenoptera.
Literature Cited


## Appendix A

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Abbreviation</th>
<th>Definition</th>
<th>Event or State?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdomen pump</td>
<td>abpump</td>
<td>While stationary, the bee moves its abdomen up and down and in and out</td>
<td>State</td>
</tr>
<tr>
<td>Antennate</td>
<td>an</td>
<td>Both bees move their antennae so as to touch the face of the other</td>
<td>Event</td>
</tr>
<tr>
<td>Back</td>
<td>ba</td>
<td>Bee propels itself backwards with its legs without reversing</td>
<td>State</td>
</tr>
<tr>
<td>Bite</td>
<td>bi</td>
<td>Bee opens its mandibles and closes them on a body part of the other bee</td>
<td>Event</td>
</tr>
<tr>
<td>Bitee</td>
<td>bice</td>
<td>While stationary, the bee being bitten</td>
<td>Event</td>
</tr>
<tr>
<td>C-posture</td>
<td>cpost</td>
<td>Bee curls abdomen under head and thorax, forming a ‘C’ with her body</td>
<td>Event</td>
</tr>
<tr>
<td>Frontal encounter</td>
<td>fe</td>
<td>Bee becomes stationary within eyeshot of the other bee</td>
<td>State</td>
</tr>
<tr>
<td>Follow a backing bee</td>
<td>foba</td>
<td>Forward movement by bee towards another backing bee-at most 45° apart in circle tube</td>
<td>Event, State</td>
</tr>
<tr>
<td>Follow a walking bee</td>
<td>fowa</td>
<td>Forward movement by bee towards another walking bee-at most 45° apart in circle tube</td>
<td>Event, State</td>
</tr>
<tr>
<td>Groom</td>
<td>gr</td>
<td>The bee rubs its head, thorax and abdomen with its legs</td>
<td>Event, State</td>
</tr>
<tr>
<td>Head to abdomen touch</td>
<td>hta</td>
<td>One bee touches its head to the abdomen of the other and remains stationary</td>
<td>State</td>
</tr>
<tr>
<td>Head to head touch</td>
<td>hht</td>
<td>One bee touches its head to the head of the other and remains stationary</td>
<td>State</td>
</tr>
<tr>
<td>Pass</td>
<td>pa</td>
<td>Forwarding bees meet and manoeuvre to accommodate each other while trying to go in opposite directions in the circle tube</td>
<td>Event</td>
</tr>
<tr>
<td>Unsuccessful pass attempt</td>
<td>paa</td>
<td>Involves one bee putting its head above another bee, forcing the other to turn over for a venter-to-venter pass</td>
<td>Event</td>
</tr>
<tr>
<td>Passsee</td>
<td>pace</td>
<td>Bee in a pass attempt that is not allowing the pass</td>
<td>Event</td>
</tr>
<tr>
<td>Push</td>
<td>pu</td>
<td>One bee applies force to another with its head</td>
<td>Event, State</td>
</tr>
<tr>
<td>Pushee</td>
<td>puec</td>
<td>Bee being pushed</td>
<td>State</td>
</tr>
<tr>
<td>Push with abdomen</td>
<td>pwab</td>
<td>One bee applies force to another with its abdomen</td>
<td>Event</td>
</tr>
<tr>
<td>Reverse</td>
<td>re</td>
<td>Bee makes 180 degree turn</td>
<td>Event</td>
</tr>
<tr>
<td>Stop</td>
<td>x</td>
<td>Bee remains stationery</td>
<td>State</td>
</tr>
<tr>
<td>Sting</td>
<td>sting</td>
<td>Female bee curls abdomen under head and thorax (C-position), extending her sting and directing it at the other bee</td>
<td>Event</td>
</tr>
<tr>
<td>Walk</td>
<td>wa</td>
<td>Bee propels itself forward using its legs</td>
<td>State</td>
</tr>
</tbody>
</table>
Table A2: Male-male within-bee behavioural correlations. Top value = \( r^2 \), bottom value = \( p \). Significant correlations are in bold.

<table>
<thead>
<tr>
<th></th>
<th>Antennate</th>
<th>Bite</th>
<th>C-posture</th>
<th>Follow a backing bee</th>
<th>Follow a walking bee</th>
<th>Head-to-head touch</th>
<th>Pass</th>
<th>Unsuccessful pass attempt</th>
<th>Push</th>
</tr>
</thead>
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<tr>
<td>Antennate</td>
<td>1.00</td>
<td>0.23</td>
<td>0.00</td>
<td>0.06</td>
<td>-0.26</td>
<td>0.15</td>
<td>-0.35</td>
<td>-0.22</td>
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<tr>
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<td>0.08</td>
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<td>0.13</td>
<td>0.13</td>
<td>0.18</td>
<td>0.29</td>
</tr>
<tr>
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<td>1.00</td>
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<td>0.16</td>
<td>0.21</td>
<td>0.12</td>
<td>0.20</td>
<td>0.14</td>
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<tr>
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<td>0.23</td>
<td>0.34</td>
<td>0.55</td>
<td>0.28</td>
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<td></td>
<td>0.64</td>
<td>0.04</td>
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<td>0.01</td>
<td>&lt;.0001</td>
<td>0.04</td>
<td>&lt;.0001</td>
<td>0.12</td>
</tr>
<tr>
<td>Follow a walking bee</td>
<td>-0.26</td>
<td>0.31</td>
<td>0.16</td>
<td>0.34</td>
<td>0.40</td>
<td>0.53</td>
<td>0.62</td>
<td>0.64</td>
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<tr>
<td></td>
<td>0.05</td>
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<td>0.24</td>
<td>0.01</td>
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<td>Head-to-head touch</td>
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<td>0.13</td>
<td>0.21</td>
<td>0.55</td>
<td>0.40</td>
<td>0.35</td>
<td>0.43</td>
<td>0.48</td>
<td></td>
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<tr>
<td></td>
<td>0.25</td>
<td>0.32</td>
<td>0.11</td>
<td>&lt;.0001</td>
<td>0.00</td>
<td>1.00</td>
<td>0.01</td>
<td>0.0007</td>
<td>0.0001</td>
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<tr>
<td>Pass</td>
<td>-0.35</td>
<td>0.13</td>
<td>0.12</td>
<td>0.28</td>
<td>0.53</td>
<td>0.35</td>
<td>0.53</td>
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<td>0.01</td>
<td>1.00</td>
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<tr>
<td>Unsuccessful pass attempt</td>
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<td>0.20</td>
<td>0.32</td>
<td>0.62</td>
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<td>0.53</td>
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<td></td>
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<td>0.13</td>
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<td>0.00</td>
<td>&lt;.0001</td>
<td>1.00</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Push</td>
<td>0.02</td>
<td>0.29</td>
<td>0.14</td>
<td>0.21</td>
<td>0.64</td>
<td>0.48</td>
<td>0.27</td>
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<td>0.04</td>
<td>&lt;.0001</td>
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</table>
Table A3: Female-female within-bee behavioural correlations. Top value = $r^2$, bottom value = p. Significant correlations are in bold.

<table>
<thead>
<tr>
<th></th>
<th>Antennate</th>
<th>Bite</th>
<th>C-posture</th>
<th>Follow a backing bee</th>
<th>Follow a walking bee</th>
<th>Head-to-head touch</th>
<th>Pass</th>
<th>Unsuccessful pass attempt</th>
<th>Push</th>
<th>Sting</th>
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<td>-0.15</td>
<td>-0.18</td>
<td>-0.05</td>
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<td>Bite</td>
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<td>0.93</td>
<td>0.65</td>
<td>0.10</td>
<td>0.65</td>
<td>0.14</td>
<td>0.16</td>
<td>0.09</td>
<td>0.63</td>
<td>0.14</td>
</tr>
<tr>
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<td>0.09</td>
<td>0.26</td>
<td>0.44</td>
<td>0.09</td>
<td>0.03</td>
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<td>0.39</td>
<td>0.12</td>
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</tr>
<tr>
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<td>0.41</td>
<td>0.02</td>
<td>&lt;.0001</td>
<td>0.42</td>
<td>0.79</td>
<td>0.0003</td>
<td>0.0001</td>
<td>0.28</td>
</tr>
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<td>0.05</td>
<td>0.02</td>
<td>-0.13</td>
<td>-0.13</td>
<td>0.04</td>
<td>0.57</td>
</tr>
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<td>0.21</td>
<td>1.00</td>
<td>0.35</td>
<td>0.35</td>
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<td>0.31</td>
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<td>0.05</td>
<td>1.00</td>
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<td>0.02</td>
<td>0.35</td>
<td>0.18</td>
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<td>&lt;.0001</td>
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<td>-0.24</td>
</tr>
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<td>0.42</td>
<td>0.86</td>
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<td>0.09</td>
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<td>0.02</td>
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<td>1.00</td>
<td>&lt;.0001</td>
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<tr>
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<td>0.37</td>
<td>-0.13</td>
<td>0.31</td>
<td>0.67</td>
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<td>-0.04</td>
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</tr>
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<td>Push</td>
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<td>0.21</td>
<td>0.00</td>
<td>&lt;.0001</td>
<td>0.00</td>
<td>&lt;.0001</td>
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<td>&lt;.0001</td>
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<td>&lt;.0001</td>
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<td>-0.24</td>
<td>-0.19</td>
<td>-0.04</td>
<td>0.13</td>
<td>0.24</td>
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<tr>
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<td>0.57</td>
<td>0.17</td>
<td>0.17</td>
<td>-0.24</td>
<td>-0.19</td>
<td>-0.04</td>
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<td>0.24</td>
</tr>
<tr>
<td>Sting</td>
<td>0.14</td>
<td>0.28</td>
<td>&lt;.0001</td>
<td>0.11</td>
<td>0.32</td>
<td>0.02</td>
<td>0.07</td>
<td>0.68</td>
<td>0.24</td>
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</tr>
</tbody>
</table>
Table A4: Males in male-female pairs within-bee behavioural correlations. Top value = $r^2$, bottom value = $p$. Significant correlations are in bold.

<table>
<thead>
<tr>
<th></th>
<th>Antennate</th>
<th>Bite</th>
<th>C-posture</th>
<th>Follow a backing bee</th>
<th>Follow a walking bee</th>
<th>Head-to-head touch</th>
<th>Pass</th>
<th>Unsuccessful pass attempt</th>
<th>Push</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antennate</td>
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<td>0.16</td>
<td>0.60</td>
<td>0.32</td>
<td>0.15</td>
<td>0.32</td>
<td>0.28</td>
<td>0.22</td>
<td>0.11</td>
</tr>
<tr>
<td>Bite</td>
<td>0.21</td>
<td>-0.08</td>
<td>0.03</td>
<td>0.07</td>
<td>0.26</td>
<td>0.13</td>
<td>0.19</td>
<td>0.19</td>
<td>0.55</td>
</tr>
<tr>
<td>C-posture</td>
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<td>1.00</td>
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<td>0.07</td>
<td>0.02</td>
<td>0.03</td>
<td>-0.10</td>
<td>-0.03</td>
</tr>
<tr>
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<td>0.07</td>
<td>0.05</td>
<td>0.11</td>
<td>0.26</td>
<td>-0.07</td>
<td>0.15</td>
<td>0.23</td>
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</tr>
<tr>
<td>Follow a walking bee</td>
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<td>0.63</td>
<td>0.73</td>
<td>1.00</td>
<td>0.49</td>
<td>0.09</td>
<td>0.66</td>
<td>0.33</td>
<td>0.12</td>
</tr>
<tr>
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<td>0.32</td>
<td>0.13</td>
<td>0.02</td>
<td>0.26</td>
<td>0.17</td>
<td>0.44</td>
<td>0.57</td>
<td>0.31</td>
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</tr>
<tr>
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<td>0.03</td>
<td>0.39</td>
<td>0.88</td>
<td>0.09</td>
<td>0.27</td>
<td>1.00</td>
<td>0.56</td>
<td>0.47</td>
<td>0.46</td>
</tr>
<tr>
<td>Pass</td>
<td>0.28</td>
<td>0.19</td>
<td>0.03</td>
<td>-0.07</td>
<td>0.44</td>
<td>0.56</td>
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<td>0.48</td>
<td>0.31</td>
</tr>
<tr>
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<td>0.22</td>
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<td>0.66</td>
<td>0.00</td>
<td>&lt;.0001</td>
<td>1.00</td>
<td>0.0009</td>
<td>0.04</td>
</tr>
<tr>
<td>Unsuccessful pass attempt</td>
<td>0.22</td>
<td>0.19</td>
<td>-0.10</td>
<td>0.15</td>
<td>0.57</td>
<td>0.47</td>
<td>0.48</td>
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<tr>
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<td>0.00</td>
<td>1.00</td>
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<tr>
<td>Push</td>
<td>0.11</td>
<td>0.55</td>
<td>-0.03</td>
<td>0.23</td>
<td>0.31</td>
<td>0.46</td>
<td>0.31</td>
<td>0.52</td>
<td>1.00</td>
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<tr>
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<td>&lt;.0001</td>
<td>0.87</td>
<td>0.12</td>
<td>0.04</td>
<td>0.04</td>
<td>0.00</td>
<td>1.00</td>
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</tr>
</tbody>
</table>
Table A5: Females in male-female pairs within-bee behavioural correlations. Top value = $r^2$, bottom value = $p$. Significant correlations are in bold.

<table>
<thead>
<tr>
<th>Antennate</th>
<th>Bite</th>
<th>C-posture</th>
<th>Follow a backing bee</th>
<th>Follow a walking bee</th>
<th>Head-to-head touch</th>
<th>Pass</th>
<th>Unsuccessful pass attempt</th>
<th>Push</th>
<th>Sting</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.88</td>
<td>0.32</td>
<td>0.49</td>
<td>0.07</td>
<td>0.37</td>
<td>0.30</td>
<td>0.10</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>-0.02</td>
<td>0.05</td>
<td>0.05</td>
<td>0.32</td>
<td>-0.05</td>
<td>0.31</td>
<td>0.58</td>
<td>0.53</td>
<td>-0.12</td>
</tr>
<tr>
<td>Bite</td>
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<td>0.73</td>
<td>0.73</td>
<td>0.03</td>
<td>0.73</td>
<td>0.04</td>
<td>&lt;.0001</td>
<td>0.0002</td>
</tr>
<tr>
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<td>0.05</td>
<td>0.14</td>
<td>0.22</td>
<td>0.05</td>
<td>0.27</td>
<td>0.14</td>
<td>0.04</td>
<td>-0.10</td>
</tr>
<tr>
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<td>0.32</td>
<td>0.73</td>
<td>1.00</td>
<td>0.14</td>
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<td>0.07</td>
<td>0.37</td>
<td>0.81</td>
<td>0.53</td>
</tr>
</tbody>
</table>

| Follow a backing bee | 0.49 | 0.05 | 0.22 | 1.00 | 0.48 | 0.35 | 0.18 | 0.36 | 0.55 | 0.21 | 0.07 |
| Follow a walking bee | 0.07 | 0.32 | 0.05 | 1.00 | 0.91 | <.0001 | 0.01 | 0.16 | 0.46 | 0.27 |
| Head-to-head touch | 0.37 | -0.05 | 0.27 | 0.35 | -0.02 | 0.57 | 0.21 | 0.11 | 0.17 |
| Pass | 0.30 | 0.31 | 0.14 | 0.18 | 0.36 | 0.57 | 0.47 | 0.46 | 0.27 |
| Unsuccessful pass attempt | 0.10 | 0.58 | 0.04 | 0.11 | 0.55 | 0.21 | 0.47 | 0.42 | -0.22 |
| Push | 0.12 | 0.53 | -0.10 | 0.23 | 0.21 | 0.11 | 0.16 | 0.42 | -0.02 |
| Sting | 0.34 | 0.44 | 0.00 | 0.66 | 0.73 | 0.27 | 0.26 | 0.16 | 0.88 | 1.00 |