Behaviour and Life History of a Large Carpenter Bee (*Xylocopa virginica*) in the Northern Extent of its Range

by

Sean Michael Prager, B.A.

A Thesis

submitted to the Department of Biological Sciences

in partial fulfilment of the requirements

for the degree of

Doctor of Philosophy

Brock University

St. Catharines, Ontario

Abstract

Large carpenter bees (Hymenoptera: Apidae: *Xylocopa*) have traditionally been thought of as exhibiting solitary or occasionally communal colony social organization. However, studies have demonstrated more complex forms of social behaviour in this genus. In this document, I examine elements of behaviour and life history in a North American species at the northern extreme of its range. *Xylocopa virginica* was found to be socially polymorphic with both solitary and meta-social or semi-social nests in the same population. In social nests, there is no apparent benefit from additional females which do not perform significant work or guarding. I found that the timing of life-history events varies between years, yet foraging effort only differed in the coldest and wettest year of 2004 the study. Finally, I that male *X. virginica* exhibit female defence polygyny, with resident and satellite males. Resident males maintain their territories through greater aggression relative to satellites.

Acknowledgements

A project like this does not happen without the assistance of those who choose to contribute some of their intellectual fitness. I only hope they received some indirect fitness. Fiona Hunter was amazing in fighting for me and getting me to defence, I could not have done this without your aid. Jean Richardson provided advice, consul and many hours of data checking. To the other members of my committee: Jeff Stuart, Jon Middleton, Miriam Richards and early on Bob Caroline, your help is much appreciated. I would especially like thank the external reviewer for this thesis; Dr. Jon Barthell not only contributed his time and comments but also donated his bees. Numerous field assistants helped observe and mark bees, without them this would have been difficult. Thank you to the machine shop for lending me tools and to physical plant for your varied assistance. Sandra Rehan and Amy Rutgers-Kelly, you both started as lab mates and have become great friends and comrades. Candice Kerling, there are countless ways in which I appreciate you and your karate kicking self. I would like to that thank all those who have helped me by reading drafts. I would like to thank my friends; Canadian, American and other, you are all great. Finally, I would like to thank all the members of my family, immediate and extended, every one of you has contributed to this in some way and I never could have done this without you.

Table of Contents

List of Tables

List of Figures

General Introduction

Group living exists in almost all major groups in kingdom Animalia, including birds, mammals, insects, crustaceans, and arachnids. Forms of group living may range from simple associations of individuals residing near each other to the complicated and highly specialized forms found in the advanced eusocial insects. Group living may be influenced by a host of ecological and behavioural factors and is often associated with cooperation, a behaviour in which one individual aids another individual. In many instances, cooperation comes at a cost to one or more of the cooperating individuals; when this occurs we refer to it as 'altruism' because one individual (the helped) is increasing its fitness at a presumed cost to the other (the altruist).

There are numerous examples of cooperation and group living. For instance, in birds, cliff swallows signal to others when they locate a food patch (Ward and Zahavi, 1973). This behaviour benefits those individuals that are attracted to the food source and comes at a minimal cost, as there is more resource than an individual can consume (Ward and Zahavi, 1973). The individual that signals will benefit in the long term from living within a larger group that will be more apt to locate food in the future. In other species such as Belding's ground squirrels, calling signals danger, and comes at a potential cost to the signaller; despite evidence of nepotism, this behaviour is thus considered "altruistic" (Sherman, 1977).

Examples of seemingly altruistic behaviour exist in numerous taxa including mammals (naked mole rats, bats), shrimp, and arachnids. However, the extreme forms of "altruism" are mostly in the social insects (thrips, aphids, termites, and especially the Hymenoptera), although, mole rats and some species of shrimp also exhibit extreme sociality. Social Hymenoptera include ants, bees, and wasps. In many social insects, a division of labour exists where members of the worker class forego some or all reproduction in favour of helping another reproduce; in some instances, these workers are sterile. This has the effect of a fitness cost to one individual while she increases the fitness of another (Lin and Michener, 1972). How helping behaviours, that seemingly reduce fitness relative to reproductive or selfish individuals, have evolved has been a dilemma to behavioural ecologists and evolutionary biologists since Darwin. Multiple hypotheses have been proposed to answer this dilemma. Two of these explanations, mutualism (Lin and Michener, 1972) and parental manipulation (Alexander, 1974; Michener, 1974) are primarily behavioural. Kin selection, the third hypothesis, is an explanation based on genetic relationships.

Hypotheses to explain the evolution of altruism

Kin selection

The modern explanation of cooperation and altruism is based on Hamilton's kin selection theory (Hamilton, 1964). Hamilton noted altruism evolves due to an unstated goal of reproduction, i.e. the propagation of alleles. Kin selection is the concept that fitness consists of both the direct evolutionary effects of one's own reproduction and indirectly through non-offspring relatives. Kin selection suggests that natural selection can favour behaviours that are seemingly costly (in terms of fitness) if an individual can offset the loss of its personal reproduction and passing of genes by helping genetically similar relatives. Essentially, if an individual can pass on more genes by helping than by reproducing, the genes for that behaviour would also be passed more frequently and the behaviour would be favoured.

Hamilton codified this concept into the equation known as Hamilton's rule. The equation can be expressed as $r_k b > r_o c$, where r_k is the coefficient of relatedness between the altruist and the kin she is helping to raise, b is the benefit to the recipient of help (in terms of number of kin raised), r_o is the coefficient of relatedness between the altruist and her own offspring, c is the cost of to the altruist of helping (measured as the number of offspring sacrificed). By this inequality, if relatedness is high enough, the number of additional offspring is great, or the cost is very low, it can be beneficial to act altruistically. Changes in the values of c and b may occur due to numerous ecological factors.

In the Hymenoptera, sex determination is through complementary sex determination and results in haplodiploidy, which is also found in thrips (Thysanoptera), some beetles (Coleoptera), mites, ticks and whiteflies (Crozier and Pamilo, 1996; Wilson, 1971). In haplodiploids, males are produced from unfertilized eggs, while fertilized eggs become female offspring. This has the result that males are haploid while females are diploid. Haplodiploidy thus results in unusual patterns of relatedness between relatives. If we assume a female has mated only once, in a haplodiploid system, all of a male's alleles are identical to their mother's, while the mother shares half her alleles with her son (relatedness = 0.5). When a mother produces female offspring, half of each daughter's genome will be from the father and will be shared with all her sisters, because males are haploid. The remainder of the female offspring's genome will be from the mother and thus two daughters will have a 50% chance of inheriting any one gene. When taken to its end, these associations have the consequence that sisters on average share 75% of their genes with an r of 0.75; males are related to sisters on average by 25% with an r of 0.25. In contrast, a female is only related to her offspring by 0.5, and so by kin selection theory, a female may benefit from raising her sisters rather than her own offspring, due to the benefits of inclusive fitness. This unusually high relatedness between sisters has been proposed to promote altruism in the Hymenoptera.

Mutualism

A second explanation for the evolution of altruism is mutualism. Proposed by Lin and Michener (1972), mutualism is a scenario in which both a helper and the individual receiving help benefit from interactions. If both individuals are cooperating evenly, a fair trade-off will occur and each party will benefit equally. Mutualism does not require close relationships among group members and the different group members may perform differing tasks that result in equivalent (or increased) net fitness (Lin and Michener, 1972; Michener, 1974).

Queen control

A third explanation for the evolution of helping behaviour is direct manipulation by queens (Crozier and Pamilo, 1996). Specifically, a queen or dominant may physically force group members to perform a task, or more likely to refrain from reproduction. This manipulation takes the form of aggressive behaviours such as nudging, biting or pushing (Michener and Brothers, 1974) directed towards workers. Such queen manipulation has been well documented in sweat bees (Kukuk and May, 1991; Michener and Brothers, 1974; Richards et al., 2005; Richards and Packer, 1996). In many instances, dominant-subordinate size dimorphism may be associated with a dominant's ability to control workers; thus queens are expected to be larger than subordinates (Richards et al., 2005; Richards and Packer, 1994).

Social organization in insects

In the social insects, there are numerous classifications and definitions for forms of social interaction. At the heart of these definitions are the concepts of division of labour, help in raising offspring, and contact between generations (Michener, 1974). In the simplest form of social organization which is solitary behaviour, there is no overlap between generations and there are is no division of labour. The other extreme is eusociality in which a colony contains reproductive queens and workers that are rarely reproductively active (if at all) but perform large amounts of helping behaviour. Such helpful behaviours may include foraging, nest construction, and nest guarding or defence; examples of advanced (or highly) eusocial insects include honeybees (*Apis* spp.) and many ants. Between these extremes is a series of gradations or types of social organization that are defined by the extent of help, generational overlap and division of labour and some morphological and physiological characteristics. Table I.1 summarizes levels of sociality.

Table I. 1: Explanation of terminology used to describe various forms of social organization. Summarized from Michener (1974) and Wilson (2000).

Term	**Definition**
Solitary and Subsocial	One female lives alone and does all reproduction, foraging and nest work.
Communal	Multiple females share a nest (entrance), do all their own foraging, reproduction and nest work. Females may or may not be related. Females are structurally similar.
Quasisocial	Multiple females (2 to several) share a nest and cooperate in raising brood. All females reproduce. Females are usually of the same generation. Females may or may not be related. Females are structurally similar
Metasocial[1]	Multiple females cooperate to raise brood Females are of multiple generations Not matrifilial No reference made to relatedness Females are structurally similar
Semisocial	One female reproduces, others work. Not matrifilial. Workers are Queen's sisters, or of same generation. Females are structurally similar
Eusocial	Many females of overlapping generations, queens reproduce while workers assist the queen in reproduction and generally do not reproduce. Workers are usually the daughters of queens. There are usually structural differences between queens and workers.

---

[1] This term is derived from Gerling at al. (1983) and has been applied only to *Xylocopa*

Evolution of eusociality in insects

Eusociality has evolved numerous times within the insects, in addition to two times in mole rats (Bathyergidae) (Jarvis and Bennett, 1993) and at least twice in shrimp (*Synalpheus*) (Duffy, 1996; Duffy and Macdonald, 2000; Tóth and Duffy, 2005). Eusociality has also evolved once in the termites (Isoptera) (Wilson, 1971), once in beetles (Coleoptera: Curculionidae) (Kent and Simpson, 1992), twice in the thrips (Thysanoptera) (Chapman et al., 1998; Kranz et al., 2002; Kranz et al., 2000; Kranz et al., 1999), approximately six times in aphids (Homoptera: Aphidae) (Stern and Foster, 1997) and in Hymenoptera. In the Hymenoptera, eusociality is thought to have arisen at least twelve times (Crozier and Pamilo, 1996; Wilson, 1971): once in the ants, at least four times in the wasps (Hines et al., 2007), and five or more times in the bees (Schwarz et al., 2006). Eusociality likely evolved once or twice in the family Apidae, three times within the Halictinae and once in an ancestor of the allodapine bees (Apidae: Xylocopinae) (Schwarz et al., 2006). It is plausible that eusociality has evolved multiple times because differing ecological conditions have acted towards a similar outcome. This is the foundation of comparative studies of social evolution.

Many evolutionary studies of behaviour involve comparisons among species, populations and occasionally broader taxonomic groups (Packer, 1991). The assumption is that closely related groups should be similar by descent and thus should have similar behavioural patterns. When patterns differ, it is then possible to examine the pattern within the ecological context of that specific species, or population. Ecological differences may be responsible for differences in behavioural patterns. Ecological factors should alter the value of a particular behaviour, either via changes in the mortality and survivorship of the individual performing the behaviour, or via that individual's fitness, which in itself is affected by mortality. In studying cooperation and social evolution, ecological factors may be responsible for much of the variation in the costs (c) and benefits (b) that lead to the evolution of group living (altruism) (Alexander, 1974; Lin and Michener, 1972; Schwarz et al., 1998). Thus, applying comparative methodology should lead to an understanding of the variation in factors related to social organization. Naturally, this approach is most effective when similar information is available for multiple species, and making such comparisons requires detailed studies of life history traits in related taxa. Further, choosing the correct taxa is essential, as different taxa allow the examination of different ecological factors.

Reproductive skew models

A feature of almost all group-living organisms is reproductive skew, a common term used to refer to the apportioning of reproduction among group members. In general, a social group with high skew has a single or a few individuals that monopolize direct reproduction. Conversely, in a social group with low skew, reproduction is more egalitarian and there is a more balanced distribution of reproduction throughout the society (Johnstone, 2000; Keller and Reeve, 1994).

Models of reproductive skew attempt to explain the degree of skew in a society by accounting for levels of dominance, and by assuming that some individuals may defer or surrender some reproduction in favour of some delayed return (Dugatkin and Reeve, 1998). Simple skew models include factors that are mostly consistent across taxa and have been applied to insects, birds and mammals (Clutton-Brock et al., 2001; Johnstone, 2000; Reeve and Keller, 1995; Reeve and Jeanne, 2003; Reeve and Keller, 2001). In "optimal skew" models, it is assumed that a single dominant individual has complete control over reproduction (Dugatkin and Reeve, 1998). This contrasts with "incomplete control models" which assume that a dominant individual's control of reproduction is limited (Dugatkin and Reeve, 1998; Reeve et al., 1998). These models take many forms, but are ultimately based on evolutionary game theory models and attempt to predict how various factors may influence the apportioning of reproduction when a single individual is not in control.

There are multiple types of reproductive skew models. Transactional models assume that individuals are conceding reproduction to other individuals within a group for some variety of incentive (Reeve and Keller, 2001). Transactional models are usually divided into "concession models" and "restraint models." In a concession model, a dominant is assumed to control group membership and the proportion of the group's reproduction that subordinates receive. Restraint models assume that the dominant individual controls group membership while subordinates control reproduction. A final group of skew models are the "tug of war" models in which neither the dominant nor a subordinate has complete control and are thus forced to "compromise" as each maximizes their share of reproduction (Johnstone, 2000).

Many newer models extend reproductive skew models to include additional factors such as the possibility of reproducing later (social queuing) or inheriting resources (Johnstone and Kokko, 1999; Ragsdale, 1999). Since factors such as nest inheritance and social queuing have been shown to play a role in the social organization of many taxa, such models are likely to prove useful in understanding how social aggregations may form (Bull et al., 1998; Waser, 1988). It is reported that some species in the genus

Xylocopa are social and often inherit the natal nest (Gerling et al., 1983; Gerling et al., 1989; Maeta and Sakagami, 1995). These species may be useful in empirical tests of these models.

Subfamily Xylocopinae

The subfamily Xylocopinae is one of five subfamilies in the family Apidae. Xylocopinae includes four tribes, the large and small carpenter bees, Xylocopini and Ceratinini, respectively, as well as Manueliini and the Allodapini. The phylogenetic relationships among tribes of Xylocopinae have been investigated; however, there is some uncertainty as to the relationships among groups. Sakagami and Michener (1987) and Roig-Alsina and Michener (1993) placed the Ceratinini and Allodapini as a sister clade basal to the subfamily. There is no agreement on the sister group to the Allodapini-Ceratinini clade, and both of the remaining tribes have been suggested as the basal group (Minckley, 1998). Minckley (1998) and Roig-Alsina and Michener (1993) both identified the family Megachilidae as the next most basal taxon to the Xylocopinae. The allodapine bees have been the subject of many studies of social evolution (Schwarz et al., 1998; Schwarz et al., 2006), while species within the Ceratinini (Sakagami and Maeta, 1995; Sakagami and Michener, 1987) and Xylocopini have been studied to a lesser extent, often with respect to general life-history characteristics (Camillo and Garofalo, 1989; Gerling et al., 1983; Gerling et al., 1989; Hogendoorn, 1996; Hogendoorn and Velthuis, 1993; Michener, 1990; Sakagami and Laroca, 1971; Sakagami and Maeta, 1995; Stark, 1992a; Stark et al., 1990; Velthuis and Gerling, 1980; Velthuis and Gerling, 1983; Vicidomini, 1996).

Species within the Xylocopinae exhibit a variety of social organizations ranging form solitary to eusocial (Michener, 1985; Michener, 1990). While most members of the Xylocopinae nest in some variety of plant material, there is tribal variation in many nesting habits and provisioning strategies. Specifically, there are two provisioning strategies exhibited, one in the Allodapini, which feed their brood progressively and do not separate them into cells (Michener, 1974; Schwarz et al., 1998), and another in the Manueliini, Ceratinini and Xylocopini, all of which use mass provisioning. The mass provisioning species lay eggs on pollen masses and their brood are placed into individual cells. Within the subfamily Xylocopinae there is also variation in the number of generations with univoltine (one brood) and multivoltine (multiple broods) species, and this variation is sometimes associated with climate (Michener, 1990).

Tribe Xylocopini

In their revision of the Xylocopini, Hurd and Moure (1963) recognized three genera within the tribe Xylocopini. The genus *Lestis* Lepeltier & Serville was defined based on its geographic distribution to Australia, *Proxylocopa* Hedicke, was identified as the only genus that was ground nesting, and the genus *Xylocopa* Latreille contains the remaining species. However, Minckley (1998) has since produced a cladistic study that shows these three genera belong to a single monophyletic group comprised exclusively of the genus *Xylocopa* Latreille; this taxonomy was also supported by Leys and by Michener (Leys et al., 2000; Leys et al., 2002; Michener, 2000; Minckley, 1998).

Genus *Xylocopa*

The genus *Xylocopa* has been classified by Minckley (1998) as the sole genus in the tribe Xylocopini and containing 31 subgenera. This position is supported by two molecular studies (Leys et al., 2000; Leys et al., 2002; Minckley, 1998). In his study, Minckley gives a number of approximately 500 species of *Xylocopa* and Michener (2000) presents a similar number. However, the only exhaustive cataloguing of *Xylocopa* lists 750 described species (Hurd and Moure, 1963). Commonly, researchers use the taxonomic scheme of Hurd and Moore, while accepting a single genus (Da Silva et al., 1999; Doolan and Macdonald, 1999; Leys, 2000b; Leys et al., 2002; Michener, 2000; Minckley, 1998; Steen, 2000) .

Xylocopa is widely distributed and can be found on all continents except Antarctica (Hurd and Moure, 1963). Most species of *Xylocopa* are distributed in tropical and subtropical climates but some species may be occasionally found in temperate areas (Hurd and Moure, 1963). Many subgenera are restricted to particular regions or islands with distributions rarely crossing zoogeographical boundaries (Hurd and Moure, 1963; Leys et al., 2002).

Subgenus *Xylocopoides*

The subgenus *Xylocopoides* contains five species, two of which (*virginica* and *californica*) purportedly contain subspecies (Hurd, 1978). This is a chiefly Nearctic subgenus that is distributed in North America mostly in warm and temperate regions. Members of this subgenus can be found in south-eastern Canada, most of the United States and ranging into southern Mexico (Hurd, 1978). Reports on the nesting of this subgenus suggest that all members nest in dry, sound wood, but it appears that the particular substrate choice varies among species (Hurd, 1958; Hurd, 1978; Hurd and Moure, 1963). Reports on foraging suggest that these bees forage on a variety of native and introduced plant species (Hurd, 1978).

Xylocopa virginica virginica

Xylocopa virginica virginica (L.) is one of three subspecies within the species *virginica*, which also includes *Xylocopa virginica texana* and *X. v. krombeini* (Hurd, 1978). *Xylocopa virginica virginica* is the most widely distributed member of the subgenus *Xylocopoides* ranging from southern Ontario to Florida and west to the Mississippi River and into Texas. However, *X. v. virginica's* range overlaps with the other subspecies in Florida and Texas. Further, preliminary molecular data based on COI sequences raise questions about the validity of the subspecies (S. Prager, unpublished data). Since *Xylocopa virginica* are large bees and often nest near humans, they have been the subject of occasional studies and publications, most of which are descriptive in nature. Most notable of these studies are those of Gerling and Hermann (1976) in Georgia and that of Rau (1933) in Missouri. Additional studies have focused on male behaviour (Barrows, 1983; Barthell and Baird, 2004), foraging (Dukas and Real, 1991; Frankie and Vinson, 1977), and specifics of morphology and physiology (Chapman, 1999; Chapman and Abu-Eid, 2001; Chapman, 1986; Williams et al., 1983). None of these studies has managed to combine elements of male and female behaviour in the same population or has examined these bees in the more northern extremes of their distribution.

Aims of this research

It is the overall conceptual goal of this work to further the knowledge of social evolution and animal behaviour. Since much of the work to date on social organization in *Xylocopa* has been on only two species, this will add to the available data for comparative studies. To date, studies of *Xylocopa* social evolution have suggested a complex trade off which results in helping behaviour (Dunn and Richards, 2003; Hogendoorn, 1996; Hogendoorn and Leys, 1993; Hogendoorn and Velthuis, 1993), while another study cites reproductive skew as a mechanism for social evolution in these bees (Steen, 2000). The quantitative data I will present should be helpful in evaluating these explanations and in making comparisons among species. Further, in facultatively social bees, an understanding of factors that influence an individual's decision to help may provide insight into the evolution of obligate social organization (Hogendoorn and Leys, 1993). These factors likely include nest inheritance, queen control and guarding behaviour, all of which are related to hypotheses explaining the evolution of helping behaviour. I will show that *X. virginica* is facultatively social and offer data and suggestions as to why this social polymorphism exists.

In the study presented here, I intend to accomplish a series of goals:

1. I will expand on existing knowledge of *Xylocopa virginica* life history by adding additional data and more quantitative data with respect to social behaviour, foraging and phenology.
2. I will offer the first detailed examination of this bee's social and mating behaviour in the northern extreme of its range, and compare those data with studies conducted in other locations.

3. I will examine variation in life-history traits among years with different weather conditions.
4. Finally, I will examine male behaviour and its relationship to female life history traits. This information should prove useful for future comparative studies with other species of *Xylocopa*, bees and social insects in general.

General background and methods

Many of the data presented in this thesis were generated using the same techniques and in the same study sites. These common data were used to answer different questions in different chapters. Therefore, the same data sets were often analyzed in different manners. For brevity, common techniques and methods are presented here. Individual chapters contain details and variations relevant to the data they present. For purposes of clarity, I also provide definitions for terms commonly used in this document.

Study sites-St. Catharines

I conducted work between 2003 and 2006 at two sites located on the Brock University campus (St. Catharines, Ontario, Canada) (43°07'21", 79°14'37"). The primary site, "Courtyard", was used for observational studies and non-destructive sampling. The Courtyard site was situated between academic buildings and residences, had limited shade and consisted mostly of a concrete walkway through a grass lawn (Figure I.1).

A second site, "Farmhouse", was used for destructive sampling of nests. The Farmhouse site was located on the southern end of the campus, approximately 400 metres from the Courtyard site, near a farmhouse, an old-field meadow, and parking lots. This site was smaller than the Courtyard site and was well shaded by numerous large trees. In addition, this site consisted entirely of lawn, dirt, and wood shavings rather than the concrete of the Courtyard site. These factors made the Farmhouse site two to three

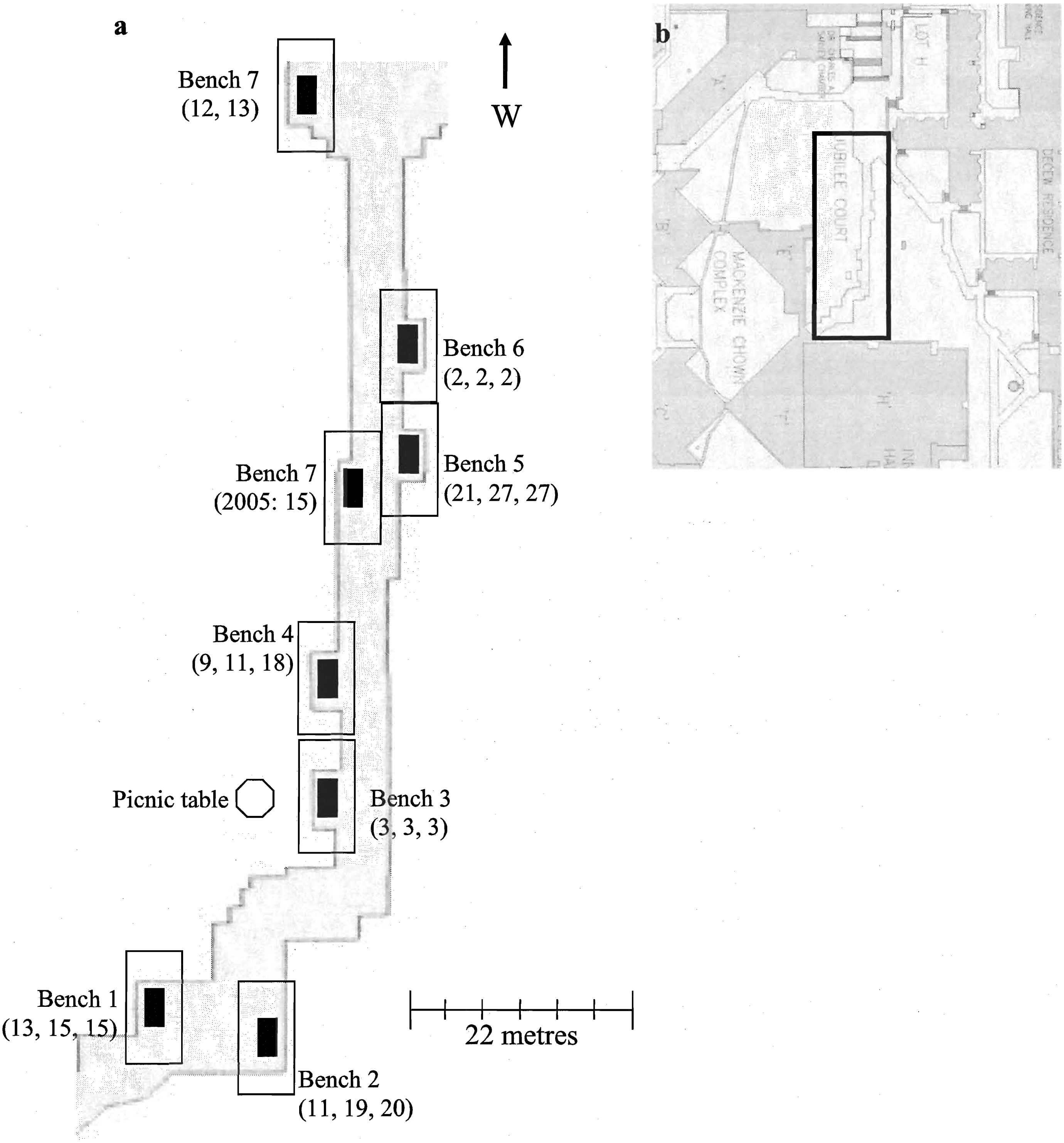


Figure I.1: a. Scale diagram of Courtyard site showing location of each bench and its 1m surrounding perimeter. The number of nests in a bench in each year (2003, 2004, and 2005) is given in brackets. b. Expanded view of courtyard and surrounding buildings (hatched areas). Box indicates path along which benches are located. Note that bench 7 was moved prior to the 2005 season.

degrees cooler than the Courtyard site for most of the day. The Farmhouse site was used exclusively as a source of nests for destructive sampling.

In both study sites, nests were contained within wooden benches (Figure I.2a and b). Study sites were defined as the area encompassing a perimeter of approximately 10 metres from the edge of each bench, and all the area contained within. This area constituted approximately 900 m^2 in the Courtyard site and 400 m^2 at the Farmhouse site. In 2003, the Courtyard site contained seven cedar benches and a total of 81 nest entrances. In 2003, the Farmhouse site contained 6 cedar benches and 35 nest entrances. All the nests at the Farmhouse site were destructively sampled in 2003 and no new nests were constructed in subsequent years, although benches remained. The Courtyard site was used for all non-destructive sampling work. Prior to the start of each season, each nest entrance was marked with an alphabetical identifier (used once per bench); the same identifier was assigned to a nest in every year. In 2006, I obtained five nests from the eves trough of a pavilion in Burgoyne Woods, a city park in St. Catharines, Ontario, located 2 km north of the Brock University Campus. These nests were not subjected to preliminary study prior to collection, and were primarily used for architectural analyses (Chapter 2). Females collected in Burgoyne Woods nests are included in some morphological analyses (Chapter 1).

Phenology and life cycle

Here I summarize the life-cycle of both female and male *Xylocopa virginica* in chronological order. The season for *X. virginica* females begins when they become active after a period of overwintering that typically lasts from late September until late

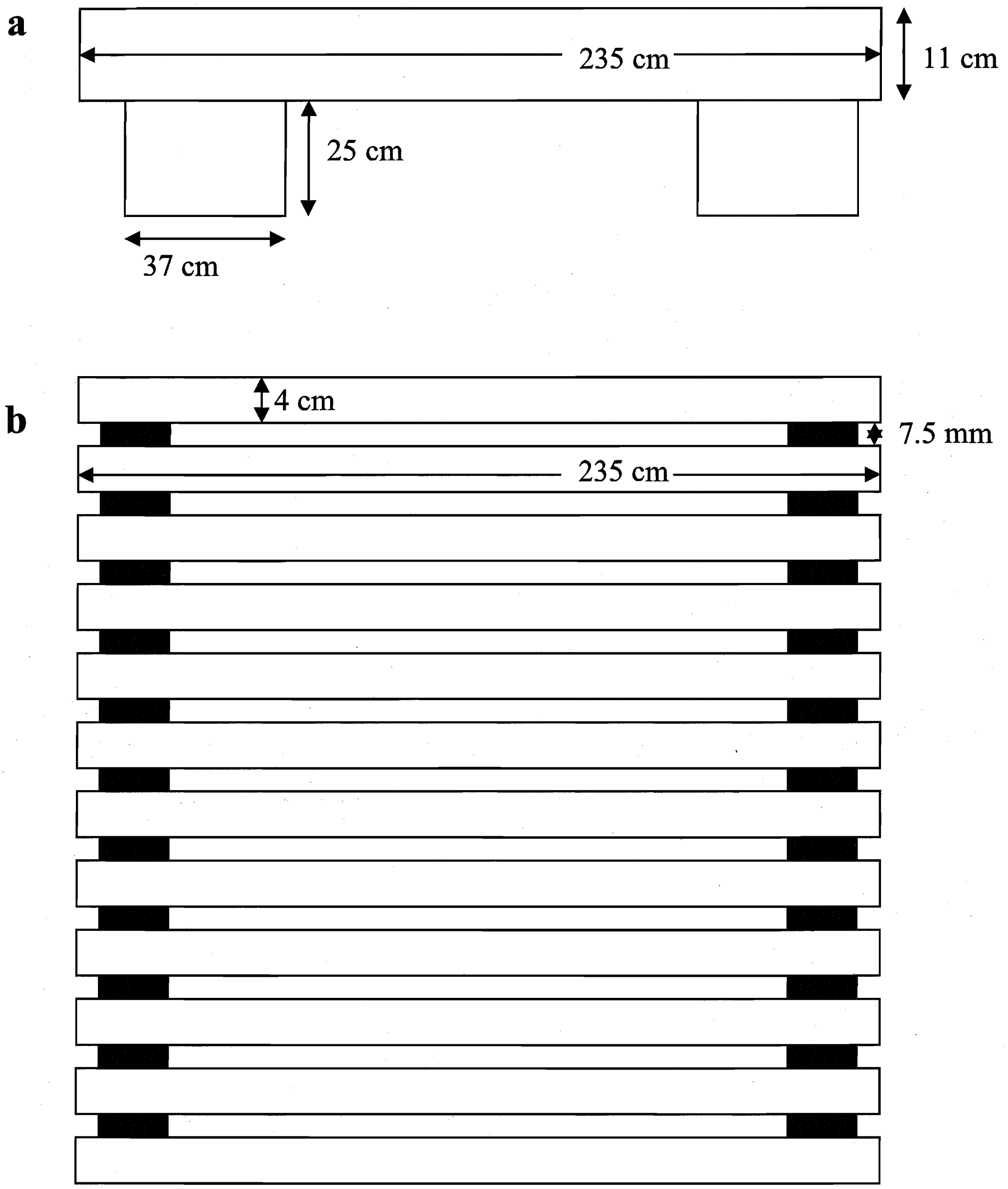


Figure I.2: **a:** Diagram (side view) of typical bench containing *X. virginica* nests as contained in both the Farmhouse and Courtyard sites. **b:** Top view of a typical bench containing *X. virginica*. Not to scale.

April. In mid- to late April, the beginnings of bee activity are indicated when debris and dead individuals appear below nest entrances, indicating nest renovation has begun. Shortly after nest renovation has begun, females can be seen making flights that do not involve pollen collection.

At about this same time (mid-April) males begin to emerge from nests; four to six weeks later, males can be observed "hovering" over the benches and occasionally at trees or flowers in the study site. Males are thus active after nest construction or renovation begins. Following nest renovation, pollen foraging by females begins and continues for about 6 weeks (usually from late May until early July). Males are therefore present near nests before females begin foraging. Brood begin to eclose in late July or August, at which point a second period of foraging begins; this period lasts into September. Young females (and males) overwinter together in their natal nests along with surviving foundresses. Detailed life-cycle information is provided in chapter 3.

Bees obtained from other sources

Some analyses required bees that could be behaviourally classified as having performed specific tasks outside of a nest or as having been active during a particular time. To generate sufficient sample sizes for these analyses, *X. virginica* were collected using other methods. First, bees were collected "on the wing" at various locations on the Brock University campus and around St. Catharines using a hand net. Second, bees collected for a separate project (Rutgers-Kelly, 2005) were dissected and included in analyses. These bees were collected in sweep nets or directly collected on flowers and thus were known to have been foraging. Finally, bees captured incidentally or that died during marking were included in some analyses. Bees collected using these methods constitute the remainder of the "outside" females dissected and analyzed in chapter 1.

Destructive sampling of nests and collection of contents

All destructively sampled nests from Farmhouse were collected and opened using the same protocol. The Farmhouse nests were collected in summer, after females had stopped provisioning broods. Nests collected in other St. Catharines sites and in Maryland were collected with procedures modified as indicated. Appendix 1 gives collection dates for all nests collected in St. Catharines. The evening prior to collecting a nest, I plugged nest entrances with non-sterile cotton wool secured with duct tape, to ensure that all adults were inside the nest; *X. virginica* are inactive at night, returning to nests in early evening. To expose the nest, I planed the boards a layer at a time using a 6" block plane (Stanley model 12-220) until nests were visible. When the nest was exposed, the remaining wood was carefully removed using forceps, razor blades, and a penknife.

Once nests were fully visible, all adult inhabitants were collected, their wings were removed (to facilitate measurements), and the specimens were stored at -80°C in 50 ml conical centrifuge tubes. At the time of nest opening, I recorded the contents of each brood cell (Figure I.3) and the developmental stage of all brood (very small, small, medium, large, or fully-grown larvae; white, pink, red, brown, or black-eyed pupae; 1/4, 1/2, 3/4, or fully pigmented pupae; adult); the use of developmental stage gives more gradation than instars. Larvae and pupae were left *in situ* and raised to adulthood. When brood reached the adult stage or died, their brood cell position and final development stage was recorded and they were stored at -80°C or in 95% ethanol (redistilled in the

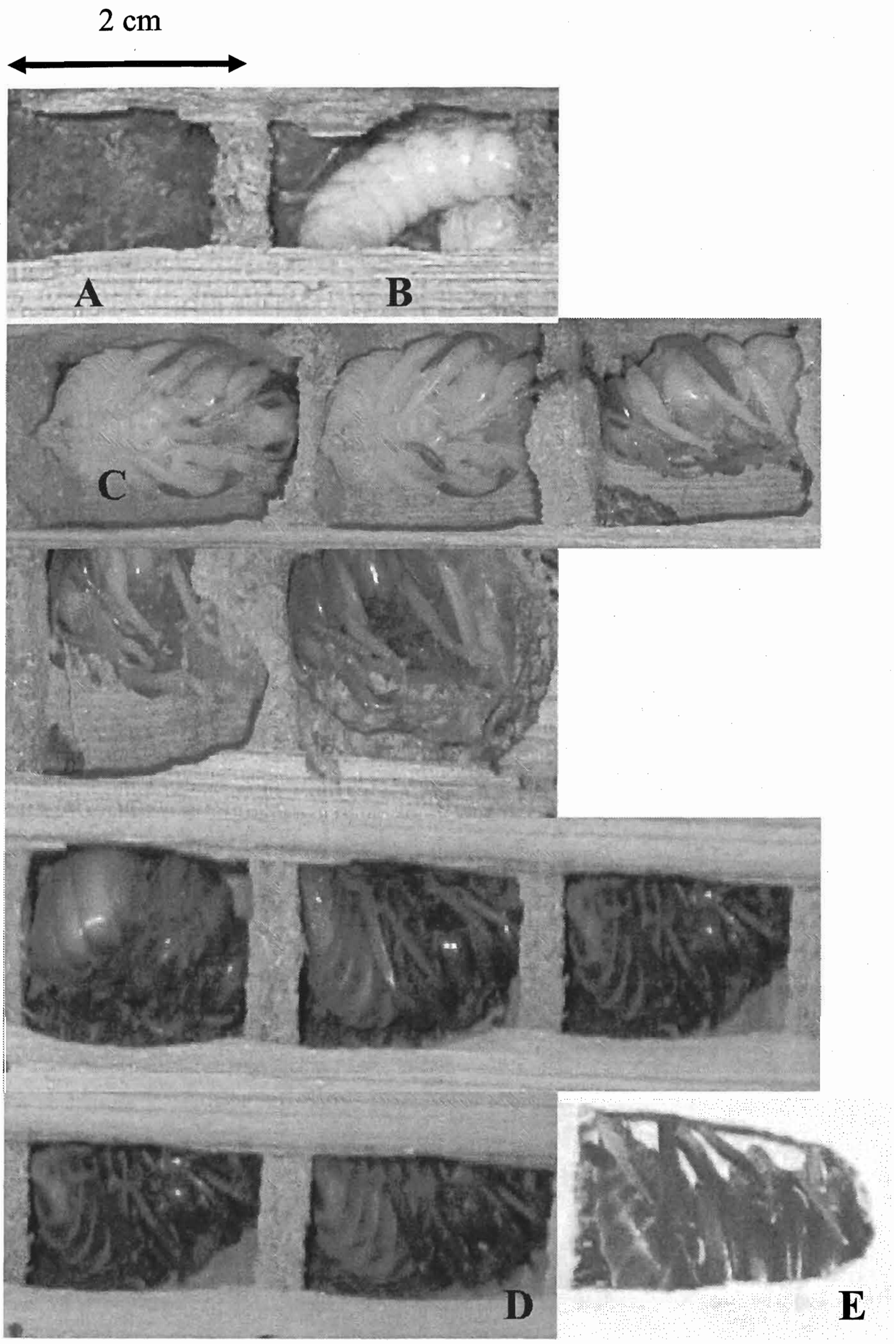


Figure I.3: Individuals of various brood stages. Stage progresses from left to right, **A** is a pollen mass, **B** is a medium larva. **C** through **D** are sequential pupal stages identified by the proportion of pigmentation; **C** is a ½-pigmented pupa and **D** is a fully pigmented pupa. **E** is an imago.

lab). The majority of nests collected at the Farmhouse site contained broods in advanced stages, suggesting that females had finished laying and broods were complete at the time of collection.

Some data could not be obtained from all destructively sampled nests. Specifically, in three nests, females escaped after the nests were returned to the lab and the females could not be assigned to a specific nest. Five nests did not contain any adult females when collected. Two nests were collected without brood or adult females (i.e. were empty). For all analyses, I use data from as many nests as possible and thus sample sizes vary. Appendix 1 gives the exact contents of each nest. Appendix 2 gives the number of destructively sampled nests used in each analysis.

Paint marking for identification

In order to track the activities of individual bees, bees were individually marked using Testors lead-free, all-purpose enamel paint applied with a small brush or transfer pipette to the abdomen and thorax. Since *X. virginica* is sexually dimorphic, colour combinations were used once on each sex; colours were chosen for visibility at a distance. In every year (2003-2005), I used the same procedure. Bees were captured using a hand net and transferred into a 50 mL centrifuge tube that was then chilled on ice to reduce movement and prevent bees from grooming themselves before the paint dried. After marking bees and taking head capsule width measurements bees were released at the bench closest to where they were caught.

Intensive marking was done at the beginning of each year (as soon as bees were seen flying) until mid-June when behavioural observations began. One person would walk the length of the study site and capture any bee that was outside of a nest.

Previously marked individuals were released immediately, while unmarked individuals were retained for marking. During the initial intense periods of marking in each season, approximately five hours per day was spent capturing and marking individuals, depending on weather (*X. virginica* does not fly in the rain, when it is too cold (below 14°C)), and occasionally when it is too hot (above approximately 30°C). Following intense periods, marking continued every two to three days or whenever an unmarked individual could be caught without interrupting other activities. I continued marking throughout the entire study period, so almost all individuals that flew regularly in the study site were marked.

Videoscope observations of nests

In 2004 and 2005, I was able to inspect nests in the Courtyard site without destroying them using an Everest VIT Inc. VP300™ video boroscope (henceforth 'videoscope'), which has an adjustable light source and a camera lens on the end of a flexible probe that can be inserted into a nest. Nests were inspected to ascertain the minimum number of individuals present, the sex of visible individuals, the identity of marked inhabitants, and the presence of pollen stores and brood. Nests were also examined to determine their basic shape, the number of branches, and in some instances, the nest's position within the board. As some segments of nests were not accessible to the probe (either because the distance exceeded the length of the probe or the nest tunnel changed direction on too sharp an angle), the number of females and branches always represented a minimum number.

Benches were inspected approximately every third day in random order, with all accessible nests observed from 10 July to 22 September 2004 and from 17 May to 1

September 2005. The duration of observations varied as was necessary, from approximately 10 minutes to 3 hours based on the ability to determine occupancy, female activity, and technical considerations.

Observations of females at nest entrances

I observed individually identifiable females as they entered or exited their nests to determine the number of foundresses occupying each nest and their daily activity patterns. In 2003, preliminary observations were taken to determine when females first began foraging for a day and when they finished activity for a day. In three days of observations in June, no females were seen entering or exiting a nest prior to 0845 h or after 1800 h. In general, activity began around 0930 h and ended at approximately 1630 h. A period of behavioural observation began with the first indication of daily activity and lasted 6 hours. However, if during the initial 30 minutes of the day's observation, no activity occurred at any bench, then conditions were presumed inappropriate for bee activity and observations were cancelled for that day. Thus, observations should closely approximate daily activity. In 2003 and 2004, the order of observation was randomized with respect to bench. In 2005, observations were associated with a removal experiment (Chapter 1) and could not be randomized with respect to order of observation. During the observations, the identities of all individuals arriving, entering and departing from a nest were recorded, as well as the time of day and whether pollen was visible on the legs. Behavioural observations began in mid-June and continued until late August. Each nest was observed at least once per week. Observations were conducted daily until foraging activity became less frequent, at which point observations were conducted as activity patterns made practical (i.e. some indication that activity would occur). As the number of nests in bench 5 (Figure I.1) increased, it sometimes became necessary to split observations at this bench so that three hours were spent facing each side of the bench.

Terminology

Here I present definitions for terminology used throughout this thesis. In all chapters, foundress refers to an adult female that is present at the start of a season (before brood are laid). Foundress is used in opposition to the term female that indicates any adult female, regardless of timing. Since some females may leave a nest for purposes other than collecting pollen to provision brood, forager specifically refers to a female observed carrying pollen at any time during the season; not all females that are observed flying outside a nest are foragers. "Inside females" describes females collected along with a destructively sampled nest for which there is no behavioural evidence of their having left the nest. Conversely, "outside females" refers to a female (regardless of collection method) for which there is behavioural evidence that she left the nest (i.e. she is marked or was caught on the wing). Male always refers to any adult male. The term haplometrotic is used interchangeably with solitary to describe a nest containing a single foundress. Pleometrotic refers to nests containing multiple foundresses. A colony refers to the adult and immature bees within a nest; nest refers to the physical structure (Michener, 1974).

Chapter One: Foundress associations in *Xylocopa (Xylocopoides) virginica*

Introduction

Facultatively social bees can offer special opportunities to examine the evolution of helping behaviour and multiple-foundress associations through comparative studies (Dunn and Richards, 2003; Richards, 2000; Richards, 2003; Richards and Packer, 1994). Comparative studies have commonly been made within the bee family Halictidae and to a lesser degree in members of the tribe Allodapini (Michener, 1990; Packer, 1993; Sabrosky, 1962; Schwarz et al., 1998; Schwarz et al., 2006; Schwarz et al., 1997). However, sporadic studies of the genus *Xylocopa* (Apidae: Xylocopinae: Xylocopini) suggest that these bees, often considered solitary or communal, may also be useful in such studies of social evolution.

Some species of *Xylocopa* have previously been studied with respect to social organization and evolution. The most extensive studies of *Xylocopa* to date are of the species *X. pubescens* and *X. sulcatipes*. Studies of *X. pubescens* suggest that sociality (semisocial or metasocial) can arise when a female loses her nest and social position to a usurper. When this occurs, the usurped female may have previously laid eggs, but only the usurper remains reproductive; to date, there is no genetic evidence to confirm maternity in these nests (Hogendoorn and Velthuis, 1999; Michener, 1990; Stark et al., 1990). Similarly, both the usurped and usurper may have foraged, but only the usurper continues to forage (Dunn and Richards, 2003; Hogendoorn and Velthuis, 1999). In *X. sulcatipes* more than one female of the same age may occupy a nest, but only one female collects food, the others remaining in the nest (Gerling et al., 1983; Stark et al., 1990).

Overall, sociality has been demonstrated in eight species of *Xylocopa* (Camillo and Garofalo, 1989; Gerling et al., 1989). Some other species of *Xylocopa* have been considered, and studies suggest that they may be solitary or facultatively social (Balduf, 1962; Maeta et al., 1985; Michener, 1990; Stark, 1992a; Steen, 2000; Steen and Schwarz, 2000; Van Der Blom and Velthuis, 1988; Velthuis and Gerling, 1983; Velthuis et al., 1984; Vicidomini and Priore, 1999; Watmough, 1983).

In eastern North America, there are two species of *Xylocopa*. *X. micans* resides in southern Georgia and Florida and is mostly unstudied (Hurd, 1978; Hurd and Moure, 1963). The more common species of *Xylocopa* in eastern North America is *X. virginica* (Hurd, 1978; Hurd and Moure, 1963). *X. virginica* ranges from Florida to southern Ontario and from the Atlantic Ocean to the Mississippi River. Due to its large size, this species is conspicuous and has been the subject of occasional study (Balduf, 1962; Barrows, 1983; Chapman and Abu-Eid, 2001; Frankie, 1977; Sabrosky, 1962), mainly concentrating on male behaviour. There have been two extensive publications on the behaviour of female *X. virginica*, a book chapter by Rau (1933) that is mostly qualitative, and a study by Gerling and Hermann (1976) conducted in Georgia. Gerling and Hermann (1976) suggested that females can nest in solitary or in multi-foundress nests with older females performing the nesting activities while younger bees remain in the nest and perform little or no work (Gerling and Hermann, 1976). They proposed that social aggregations might result from limited nesting sites. In addition, in their study Gerling and Hermann (1976) noted that females are often in their second spring (twice overwintered) when they reproduce.

In this study, I examine a population of *Xylocopa virginica* found at the northern extent of the species' range and look for evidence of sociality. To this end, I examine the roles of individuals in multi-foundress nests with respect to division of labour and reproductive skew and I examine factors related to fitness in multi-foundress nests and solitary nests. In particular, I test the hypothesis that *X. virginica* nests socially, resulting in nests with larger or more successful broods than solitary nests. The quantitative data I present here both expands on the more qualitative studies of Rau (1933) and of Gerling and Hermann (1976), and provides novel information on populations at the northern extreme of the species' range.

Methods

Nest contents and productivity

The first requirement for social behaviour is the presence of multiple foundresses within a nest. Further, I hypothesised that multi-foundress nests would be more productive than solitary nests. I used three methods to determine the number of foundresses within nests. The first method was to count the number of foundresses contained within destructively sampled nests. While this method was the most reliable and gave an absolute number, it required the destruction of nests and thus nests examined with this method could not be used in subsequent studies. The second method was videoscope observation, which allowed counts of individuals within nests and was non-destructive. The third method was observation of females as they entered or exited nest entrances and was also non-destructive. Observational methods provided a minimum estimate of number of foundresses residing within a nest because females were potentially out of the range of the videoscope or did not leave the nest. Since neither video observation nor observation at nest entrances provided exact numbers, the use of multiple methods provides greater confidence in the estimates of foundress number.

I calculated both the number of individual brood per nest and the production per foundress as indications of brood size. These were both based on a direct count of the number of completed brood cells in destructively sampled nests from Farmhouse in 2003. Following the maturation of broods, maximum brood size was adjusted by subtracting cells in which individuals died of natural causes. The result was two brood sizes, one that represented the initial female investment (number of cells) and one that represented the number of surviving brood. Foundress productivity was calculated for each nest as either the number of brood cells, or number of surviving individuals, per foundress. Nests without brood or foundresses were excluded from productivity calculations. These analyses were only conducted for the 2003 Farmhouse nests because other years and sites had insufficient numbers to control for differences between locations or years. Nests that did not contain females but did contain brood were included as zero foundress nests for some analyses on brood, but are excluded from analyses of productivity.

Brood parasites

Some brood were parasitized by the bombyliid fly *Xenox* (formerly *Anthrax*) *tigrinus* (De Geer genus revised by Evenhuis). These flies are undetectable until larvae reach advanced stages (at least the medium larva stage). However, once this stage is achieved, the flies are large and obvious. Since all individuals from a brood were raised to maturity, individuals that were parasitized could be easily identified, as could the cells in which parasites had been residing. I compared both the degree of nest parasitism (proportion of nest parasitized) and the number of parasites per brood cell for solitary and multi-foundress nests. When possible, flies and pupal exuvia were gathered and stored in 95% ethanol just after eclosion for potential future use.

Foundress survival and colony longevity

Since *X. virginica* nests in wood, nests can be reused in multiple years. The ability to reuse nests means that they are occupied for multiple seasons, likely by the same family of bees. Nest reuse is therefore an indirect measure of colony longevity when reuse is by relatives. I used data from videoscope observations to determine whether nests occupied in 2004 (the first year of video observations) were re-occupied in 2005 and likewise if nests used in 2005 were re-used in 2006. Active nests were defined based on the presence of brood cells, cleanliness (no fecal pellets or pollen bits present) and seemingly light coloured walls (an indication of nest tunnel maintenance). Conversely, nests that were not provisioned and contained debris such as broken cell partitions and rotten pollen were classified as inactive nests. In 2006, additional re-use information was available via females trapped directly at nest entrances as part of an unrelated study (Peso, M., 2008, MSc thesis). Since daughters are expected to inherit a nest, when a nest was occupied and is then vacant the following year, it indicates the death of the colony. Starting in early April, and continuing until September, nests (and the pavement below the nest entrances) in the Courtyard site were inspected daily for the presence of indicators of occupancy, nest renovation (preparation or elongation of an existing nest for reuse), and construction (creation of a new nest). These indicators included the presence of sawdust, debris (old pollen, cell partitions) and dead pupae under nest entrances. These data were used to supplement data from video observation or for nests that were not accessible to the videoscope.

Behavioural roles of females

To explore potential differences in behavioural roles among females, I compared various morphometric characteristics among females that were known to have left a nest (outside females) and females that were collected inside nests (inside females). The outside set included all females collected in St. Catharines that were not collected along with a nest, as well as marked females collected with a nest, and these data were pooled across years except for analyses of size.

Morphological measures of work

When females were captured for marking, I measured head capsule width (HW) using an Edmund Scientific 6x pocket comparator equipped with a reticle. In females that were collected away from the study site, I also measured forewing length (CVL) as the length of the costal vein from its intersection with the subcostal vein to the notch near the prestigma, using a Zeiss Stemi SVII binocular dissecting microscope equipped with an eyepiece reticle (Figure 1.1). Based on the assumption that in social nests queens would be larger (to facilitate physical manipulation of workers) and that workers are more likely to leave the nest to forage, I hypothesised that bees collected outside the nest would be smaller than those collected within nests. I used these size data to test this hypothesis.

I measured wing wear (WW) for all collected bees. Wing wear is a proxy for flight activity; greater wear indicates that a female flew more (Cartar, 1992). Wing wear was scored on a scale of 0-5 (Figure 1.1) using a Zeiss Stemi SVII binocular microscope. A score of zero indicates an intact forewing distal edge whereas a score of five indicates

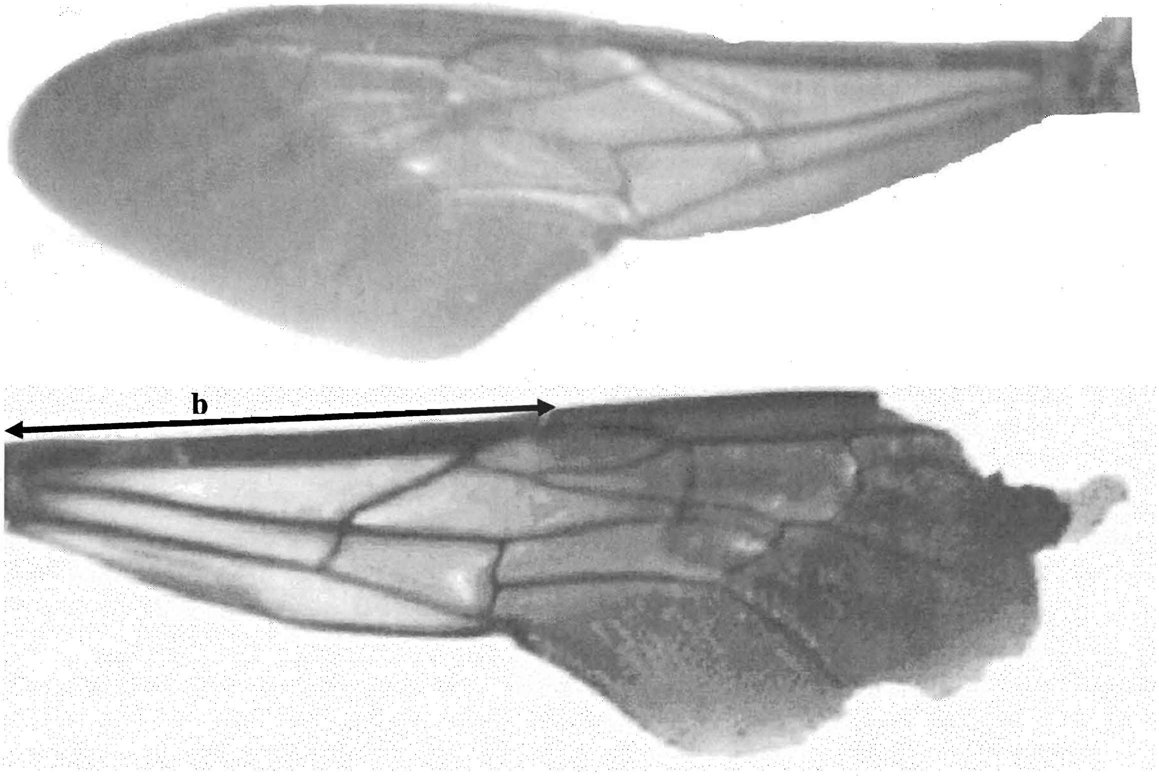


Figure 1.1: Unworn (top) and worn (bottom) *X. virginica* wings. The worn wing would receive a score of five while the unworn would receive a score of zero. Forewing length as measured from subcostal vein to “nick” near prestigma (b).

that the distal edge of the forewing was completely torn away by nicks or tears (Richards, 2000; Richards et al., 2003).

To determine how adult females might differ in terms of nest construction and maintenance activities, I scored mandibular wear (MW) in all collected females on a scale of 0-5. As with wing wear, greater activity is expected to result in greater wear (Richards, 2000; Richards et al., 2003). A score of zero represented no wear, as indicated by sharp, shiny, unblemished mandibles. A score of five represents completely worn mandibles, which are dull and possess rounded ends. To confirm the repeatability of the scoring methods, sub-samples were scored by naïve observers; scores matched at a 95% confidence interval. I also calculated a total wear (TW) score by adding wing wear and mandible wear, for a score between 0 and 10 that indicates the total amount of work that a female was performing.

Morphological measures of reproduction

All adult females collected were dissected to assess ovarian development (OD) and largest oocyte size. While both these variables measure reproductive readiness, OD better indicates the investment a female has made to reproduction overall, while largest oocyte size indicates how near a female is to laying an egg. I scored ovarian development by assessing the proportional development of each developing oocyte relative to the size of a fully developed egg, on a scale from 1/4 to 1 (Michener, 1974) and summing oocyte scores into an overall ovarian development score (OD).

Females with thickened ovaries but no developing oocytes were scored as 0.1. Females with no developed oocytes and thin ovaries received a score of zero. Scores of zero were pooled with scores of 0.1 into a single category (0), as both indicate no developed oocytes. All oocytes were measured, allowing analyses based on oocyte sizes (relative to a fully developed oocyte) in addition to ovarian development score. In all analyses of reproduction, females were only considered if they were collected during the brood laying period: between when a female was first observed with pollen and when the provisioning of brood ended for the population. The completion of provisioning was determined via nest collections in 2003 and via videoscope and observation at nest entrances in other years (no females returning with pollen or observed working on brood cells).

In addition to ovarian development, mating status was determined whenever possible. Only mated females can produce daughters, and thus mated females are more likely to produce full broods. Matedness was determined based on the presence of visible sperm in spermathecae (sperm storage organs) after dissection; mated females have opaque spermathacae while those of unmated females are clear (Wyman and Richards, 2003). Determining matedness in *Xylocopa* is notoriously difficult and error prone (R. Minckley, pers. comm.). Females that had significant fat deposits, were decomposing, highly damaged, or had recently consumed large amounts of pollen were difficult to assess accurately and were excluded.

Cross-tabulation of reproduction and work

Indicators of wear (total wear) and indicators of reproduction (largest oocyte and OD) were compared in cross-tabulation. Two sets of analyses were performed. In the first analysis, cross-tabulations were tested using chi-square goodness of fit tests to determine if values differed in distribution from even (the same values for each condition). In the second analysis, the frequencies of various combinations of work and reproduction were compared to hypothetical distributions. Hypothetical distributions were based on the observed sample size of 75 and constrained to have a minimum number of five individuals in all cells, in order to meet this assumption of the chi-square analysis. Thus, for solitary behaviour the hypothesized distribution had 60 individuals in the high work and high reproduction category, and five females in each of the remaining three categories (high work/low reproduction, low work low/low reproduction, low work/high reproduction). I also created an expectation that represented eusociality (and similarly semi-sociality). Since this social organization has workers that are mostly non-reproductive, this distribution placed half of all individuals in the high work and low reproduction category; this assumes that half of all foragers are workers. The minimum value of five individuals was placed in the low work/low reproduction, since all individuals would be expected to either work or reproduce. The remaining individuals were divided evenly among the remaining categories because both conditions can be expected for eusocial (or semi-social) queens.

Comparisons of females from multiple foundress nests

Morphological comparisons

Measures of work and reproduction were recorded for all females in the population, regardless of whether they came from a solitary or multi-foundress nest (this is unknown for many females). Sociality, however, predicts division of labour among females within a nest. Therefore, I next considered morphological differences among the subset of females that came from multi-foundress nests. Only nests collected at the Farmhouse site with known foundresses were included in these analyses of wear and reproductive measures.

Behavioural comparisons

In addition to morphological data, I also used methods based on behavioural observation to examine potential roles of females within multi-foundress nests. These methods included a forager removal experiment, and comparisons of foraging rates for foragers from multi-foundress versus solitary nests.

Forager removal experiment

In the summer of 2005, I performed a foundress removal experiment on multi-female nests in the Courtyard site to determine whether the absence of a forager leads to an assumption of foraging duties by other females. Initial observations for this experiment were conducted on 80 nests in four benches, and began on 2 June 2005, prior to any brood emergence, and immediately following the first observation of a female entering a nest with pollen.

I used videoscope counts and observations at nest entrances to ascertain which nests were pleometrotic; single foundress nests were excluded. I estimated foraging rates based on the numbers of departures and arrivals per observation period and assessed these along with nesting density to determine which benches had the most active nests. The most active benches, those containing the most nests with foraging females, were chosen as they allowed me to conduct more experiments (observations) simultaneously. From these observations, I selected 20 (of the initial 80) nests based on the criteria that the nests contained multiple females, but only a single identifiable female was observed leaving and entering with pollen. These determinations were based on at least three days (9 hours) of observation. I then removed the actively foraging females from 12 of these nests on either 21 or 22 June. Eight of the females previously identified as foraging were not observed during the removal period on 21 or 22 June and were never removed; these nests were not considered further. After removing the designated foraging female, observations were conducted on each of the two days following the removal, and then at least once a week until all female activity in the site ceased. Final observations were conducted on 5 September 2005. Interspersed with observations at nests, videoscope observation was used to confirm the presence of females within nests from which a female had been removed. Removed females were later dissected and treated as "outside bees."

Two sets of control nests were used for this experiment. The first set consisted of unmanipulated multi-foundress nests contained in the same bench as the nests from which foundresses were removed. The second set of control nests consisted of unmanipulated nests from additional benches within the Courtyard site. Both sets of control nests were observed in the same manner as experimental nests. Nests subject to removal and those in the first control set were observed simultaneously. Nests in the second set of control nests were observed on alternate days.

Comparisons of foraging rates

If the additional foundresses in multi-foundress nests act as guards, foragers from multi-foundress nests are expected to spend more time away from the nest than those from solitary nests. I addressed this by comparing foraging rates between females from solitary and multi-foundress nests, based on observations of females observed at nest entrances. Data were collected in 2004 and from the control nests in the removal experiments of 2005. I labelled as solitary those nests that had a single observed female entering or exiting nests and only a single female in videoscope observations. If more than one female was observed entering or exiting nests, or in videoscope observations, the nest was deemed multi-foundress. As there are no videoscope data for 2003, this year was not included in analyses. There were limited data for 2004, and thus most calculations are only for the 2005 season.

Statistical analyses

Distributions of wing and mandible wear in inside versus outside females were compared using Kolmogorov-Smirnov (K-S) tests performed using a custom worksheet in Microsoft Excel®. K-S examines differences between two data sets without assumptions of distribution and is sensitive to differences in both location and shape of distribution (Zar, 1999). K-S critical and p-values were taken from tables in Zar (1999). K-S was used in these analyses because these data are essentially ordinal. Analyses of the size relationship between CVL and HW was performed as an ANCOVA with a model including CVL and HW as ranks in addition to type of female (inside or outside) and year. Year was included as a categorical variable to control for size differences between years. Chi-square analyses on frequency of multiple foundress nests, reproductive measures, and for cross tabulations were performed in Microsoft Excel®. Chi-square was used on reproductive measures because OD scores are not truly continuous. Regression analyses on brood productivity, correlation of wing and mandible wear, and Wilcoxon 2-sample analyses on rates of parasitism were performed in SAS 9.1.3. In all analyses, significance was set at alpha equals 0.05.

Results

Nest contents and productivity

The simplest indication of social organization is the number of foundresses contained within a nest: sociality requires multiple foundresses. Destructively sampled nests contained zero to six live adult females (foundresses) with a mean of 2.1 ± 1.5 live foundresses per nest (n=29 nests) (Figure 1.2a). In the courtyard site, the mean number of foundresses per nest observed with the videoscope in 2004 and 2005 combined was 2.3 ± 1.1 (n=56 nests) (Figure 1.2b). Based on females observed entering and exiting nests during observations in the 2003, 2004, and 2005 field seasons, the average number of females per nest was 2.0 ± 1.4 females (n=127 nests). All three methods indicated that some nests are solitary (1 female) while others contained multiple foundresses.

I next considered the frequency of haplometrotic (single foundress) versus pleometrotic (more than two foundresses) nests. Among destructively sampled nests from 2003, 8 of 26 nests (31%) were solitary, with the remainder (69%) containing multiple females. Observations at nest entrances revealed that, averaged across all years, 52% (66/127) of nests were solitary. However, the proportion of solitary nests varied among years. In 2003, more than half (69%) of all nests observed had multiple females at the nest entrance, while in 2004 and 2005 only 18% and 19% of nests, respectively, had multiple females entering or exiting (Figure 1.3). Videoscope observations done in 2004 and 2005, however, revealed differences in the number of solitary foundress nests. In 2004, 34% (13/38) of nests examined had a single foundress, while in 2005, 24% (9/38) of nests contained a single foundress; the frequency of solitary and multi-foundress nests differed significantly between 2004 and 2005 (Chi-square: χ^2_1=0.03, p<0.001).

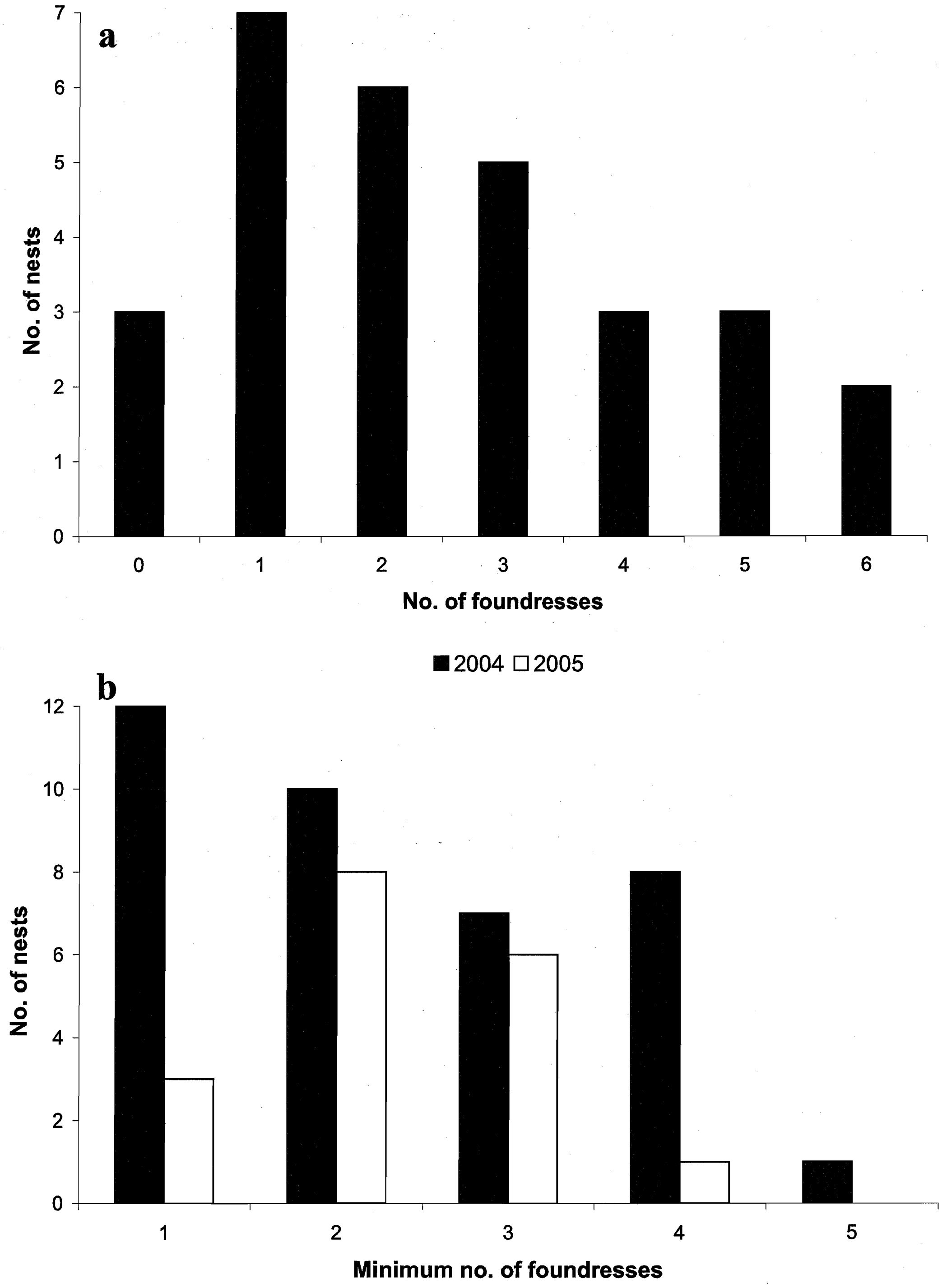


Figure 1.2: a. Frequency of adult females from 29 destructively sampled nests collected nests at the Farmhouse site in summer 2003. Note that zero foundresses indicate that the nest had indications of use, but no foundresses. b. The minimum number of females observed in videoscope observations of nests at Courtyard in 2004, during the active foraging season (black bars, n=38 nests) and 2005 (white bars, n=18).

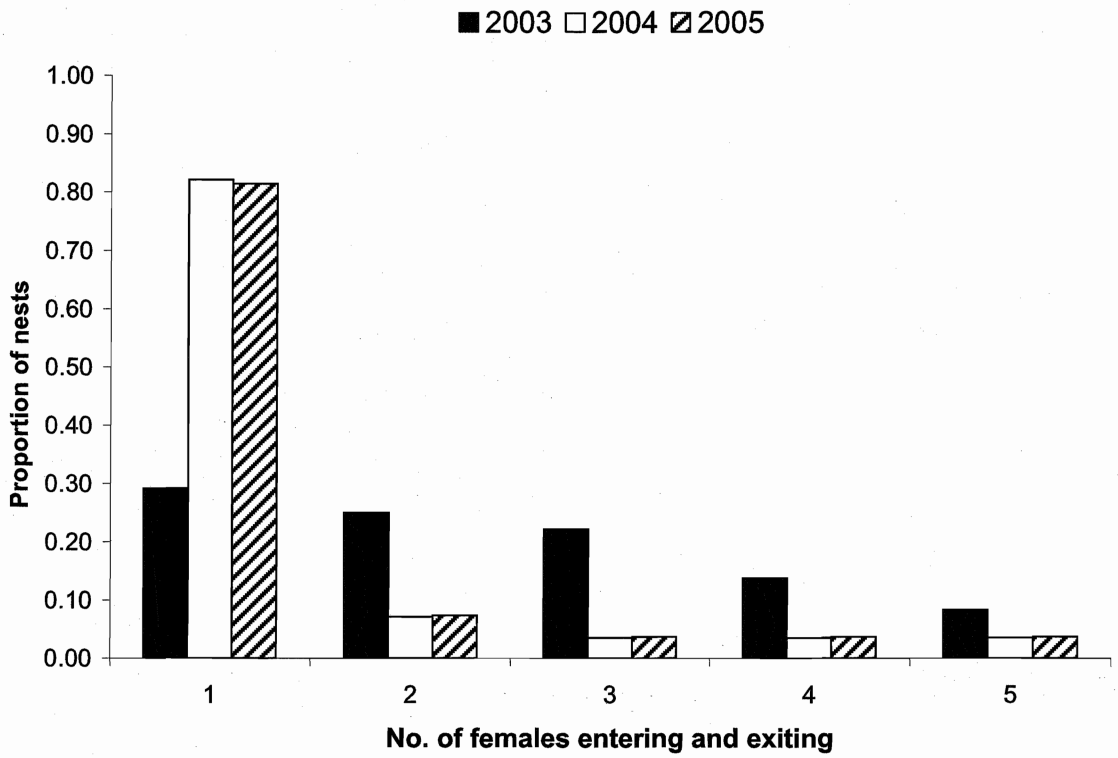


Figure 1.3: The proportion of nests with one to five females observed entering or exiting nest entrances during the 2003 (n=72 nests), 2004 (n=28 nests) and 2005 (n=27 nests) bee season. All females are included regardless of whether they were ever observed carrying pollen.

Both brood size and foundress productivity (number of offspring per foundress) reflect social structure. In communal nests, brood size per foundress will be approximately even, as all foundresses should be reproducing. In most other multiple foundress associations, we expect either larger absolute brood size or greater productivity if social nesting is to be beneficial. In the Farmhouse population, additional foundresses did not result in larger brood or in more surviving individuals in a brood. There was no significant relationship between the number of foundresses and the number of brood cells (Regression: $F_{1,24}=4.41$, $p=0.59$), or the number of surviving brood (Regression: $F_{1,24}=0.07$, $p=0.79$). As a result, the number of brood cells per foundress decreased significantly with increasing number of foundresses (Regression: $F_{1,23}=20.72$, $p=0.001$) (Figure 1.4), as did the number of surviving offspring per foundress also decreased with the number of foundresses (Regression: $F_{1,22}=10.66$, $p<0.004$; $n = 23$ because survivorship data are missing for one nest). This implies that there was no fitness benefit from multi-foundress nesting in terms of brood size.

Likewise, multi-foundress nests did not benefit through increased guarding against predation or parasitism. The most significant source of mortality I observed in *X. virginica* brood was parasitism by the bombyliid fly *Xenox* (formerly *Anthrax*) *tigrinus*. Forty-one percent of occupied nests collected in 2003 were parasitized and the number of nests parasitized did not differ between solitary and multi-foundress nests (Wilcoxon 2-sample test: $W_{27}=151.5$, $p=0.6$) (Figure 1.5a). There was also no difference in the proportion of parasitized cells (flies per brood cell) between solitary and multi-foundress nests (Wilcoxon 2-sample test: $W_{22}=57.5$, $p=1.0$) (Figure 1.5b).

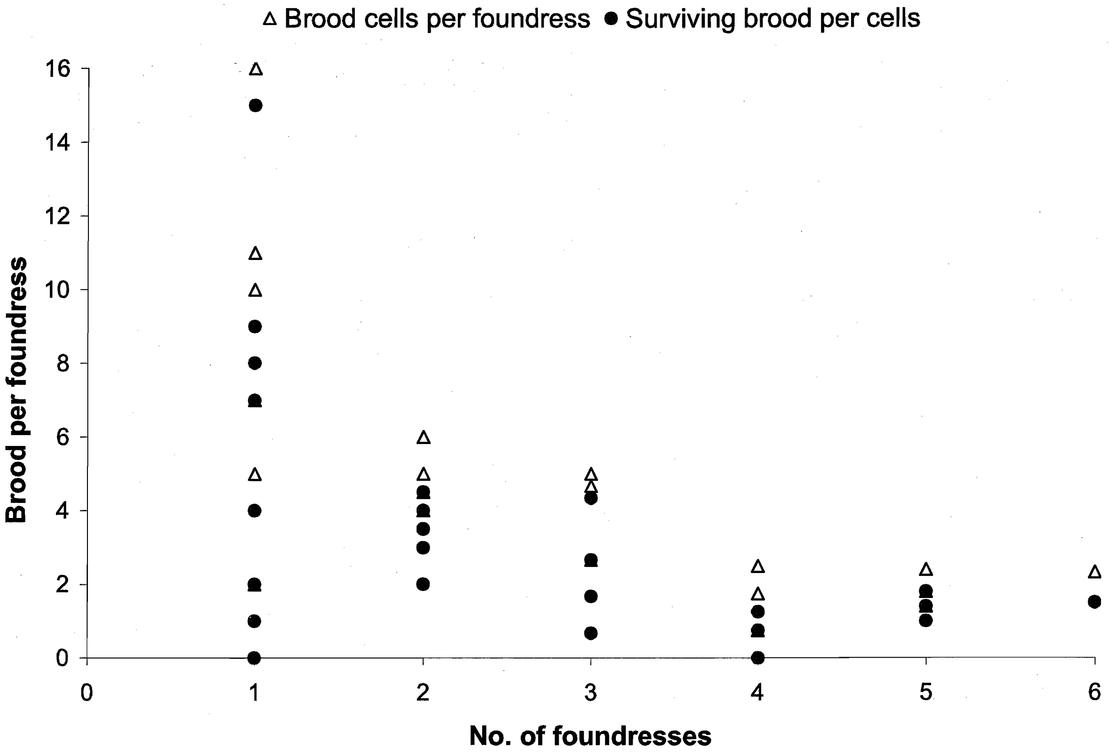


Figure 1.4: Productivity at Farmhouse (number of offspring per foundress) decreased significantly with increasing number of foundresses. This was true for both brood cells (open triangles) (Regression: $F_{1,23}=20.72$, $r^2=0.48$, $p=0.0001$), and surviving offspring (filled circles) (Regression: $F_{1,22}=10.66$, $r^2=0.32$, $p=0.004$).

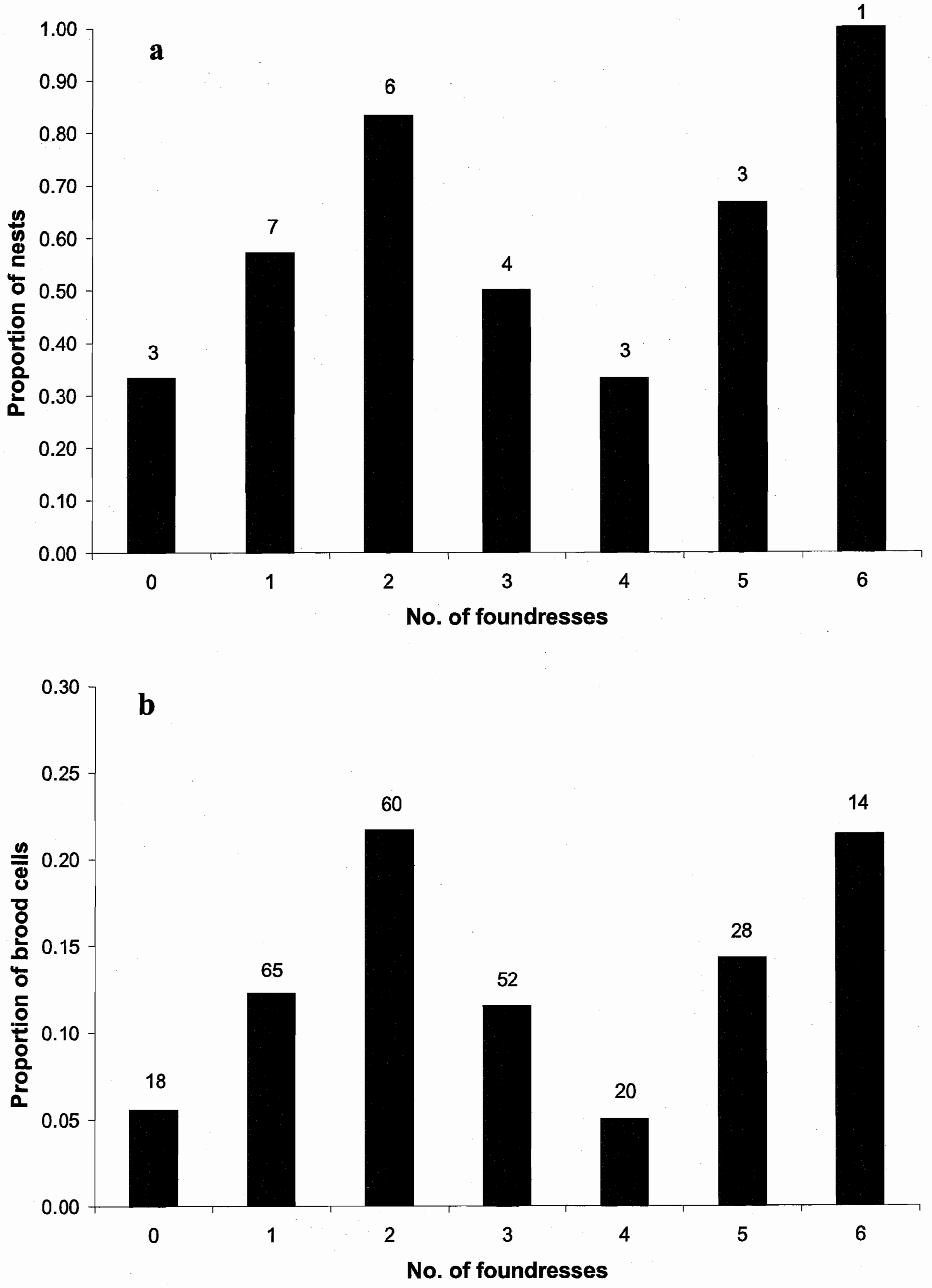


Figure 1.5: a. Rate of parasitism for nests with differing numbers of foundresses. Numbers above bars are sample sizes, given as total number of nests. b. Proportion of parasitized cells for all nests combined with 1 to 6 foundresses. Numbers above bars are total number of cells.

Foundress survival and colony longevity

Additional foundresses may result in less work performed per individual (or for a subset of individuals) resulting in longer life spans or greater survival for some individuals. Because a brood must be laid by at least one female, all nests containing a brood must also have contained a foundress, at least initially. Among all destructively sampled nests containing brood and collected in summer, two of 27 (7%) contained no adult females but contained brood, indicating that the original foundress which laid the brood died or abandoned the nest. Eight of the remaining nests contained a single female and a brood. Three of these eight nests (37.5%) contained a female that was unworn (did no substantial flying or nest building); these were probably two-foundress nests where the active foundress died or abandoned the nest. Among the 17 nests with multiple foundresses, one (6%) contained no worn females but contained brood. In this nest, the female that foraged (flew) and constructed nests appears to have died leaving only the less active foundresses. Putting this together, six foragers appear to have died, with two coming from solitary nests and four from multi-foundress nests. Therefore, foragers from solitary nests do not appear to have decreased longevity compared to those from multi-foundress nests. Thus, there was likely limited opportunity for additional foundresses to takeover egg laying or foraging and limited benefit from social nesting in terms of increased longevity for the forager.

Social structure may influence colony survival, because multi-female nests may have more females to inherit the nest. I examined this by comparing nest re-use between years for solitary and multi-foundress nests. Between 2004 and 2006, two solitary nests examined were not reused (n=19), and six multi-foundress nests were not re-used (n=50).

There was no significant difference in the proportion of re-used solitary nests and re-used multi-foundress nests (Chi-square: $\chi^2_1=0.34$, $p>0.05$).

Behavioural roles of females

Morphological measures of work and reproduction

Foraging, expected to be the most common task performed by workers (reproducing is the most common task of queens), requires leaving the nest. Therefore, I compared females that were known to have left a nest (outside females) with those collected within nests that had apparently never left the nest (inside females). Specifically, I compared morphological measures associated with common tasks performed by workers and queens in most social bees, to look for evidence that inside and outside females have different roles.

Females cannot reproduce without a nest and reproduction must therefore involve some nest construction or renovation. I used mandibular wear (MW) as a proxy for estimating the amount of effort devoted to nest construction and renovation. Outside females had higher mandible wear scores than inside females (K-S test: $D=51$, $p<0.001$, $n_1=139$, $n_2=102$), with more scores of four and five, and fewer scores of zero (Figure 1.6).

I used wing wear (WW) scores to examine differences in flying effort for inside and outside females (Figure 1.7). Wing wear differed significantly between inside females and outside females (K-S test: $D=35.4$, $p<0.001$, $n_1=151$, $n_2=115$). Outside females exhibited fewer scores of zero and more scores of two, three, four and five; inside females exhibited more scores of zero and one (Figure 1.7). To determine whether some individuals do both nest construction and tasks involving flying, I examined the

Figure 1.6: Distribution of mandible wear scores (0-5) for females by location of collection. White bars indicate "outside females" (n=102); black bars indicate "inside females" (n=139), (see text for explanation of female groups). Females are pooled across years and collection sites.

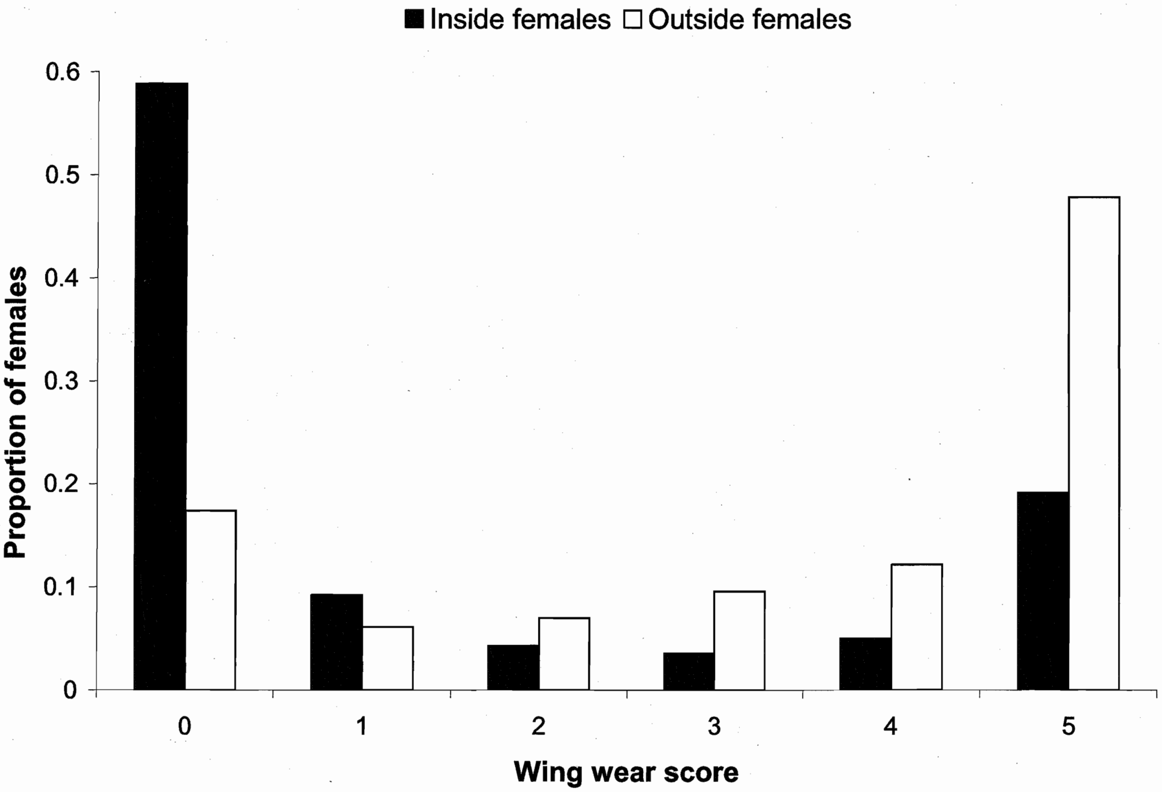


Figure 1.7: Distribution of wing wear scores (scale is 0-5) for adult females by location of collection. White bars indicate "outside females" (n=115); black bars indicate "inside females" (n=141) (see text for explanation of female groups). Females are pooled across years and collection sites.

relationship between wing wear and mandible wear. In these populations, mandibular wear was highly correlated with wing wear (Spearman rank correlation: $r_{256}=0.52$, $p<0.001$). Based on this correlation, I used total wear (TW), the sum of WW and MW, for each individual in future analyses.

I used both ovarian development (OD) and maximum oocyte size to examine differences in reproductive status between females. OD score best reflects the overall investment a female has made in producing eggs, while maximum oocyte size indicates how close a female is to laying an egg; a female may have a high OD score but no large oocytes that are ready to be laid. More than half (49 of 82 = 60%) of all females exhibited an OD score of zero (Figure 1.6). The number of undeveloped (OD=0) and developed (OD > 0) females differed significantly between inside and outside females (Chi-square: $\chi^2_1=4.6$, $p<0.05$), with more outside females having an OD > 0 than expected by chance (Figure 1.8). Inside females were also more likely to have only undeveloped oocytes, while outside females more often had one-half, three-quarter and fully developed oocytes (Chi-square: $\chi^2_4=27.9$, $p<0.01$) (Figure 1.9).

Since only mated females can produce female brood, most reproductive females should be mated. I examined the number of mated females within destructively sampled nests. Overall, most nests contained a single mated female, although some nests contained no mated females, or multiple (up to four) mated females (Figure 1.10). Amongst all females collected during the active foraging period that could be assessed for mated status (n=153), 56 females were mated and 64% of mated females were "outside females". As mated females should be reproductive, I inspected mated females for developed oocytes (Figure 1.11). Mated females possessed larger oocytes than unmated females (Chi-square: $\chi^2_4=27.9$, $p<0.001$), with almost 70% of unmated females

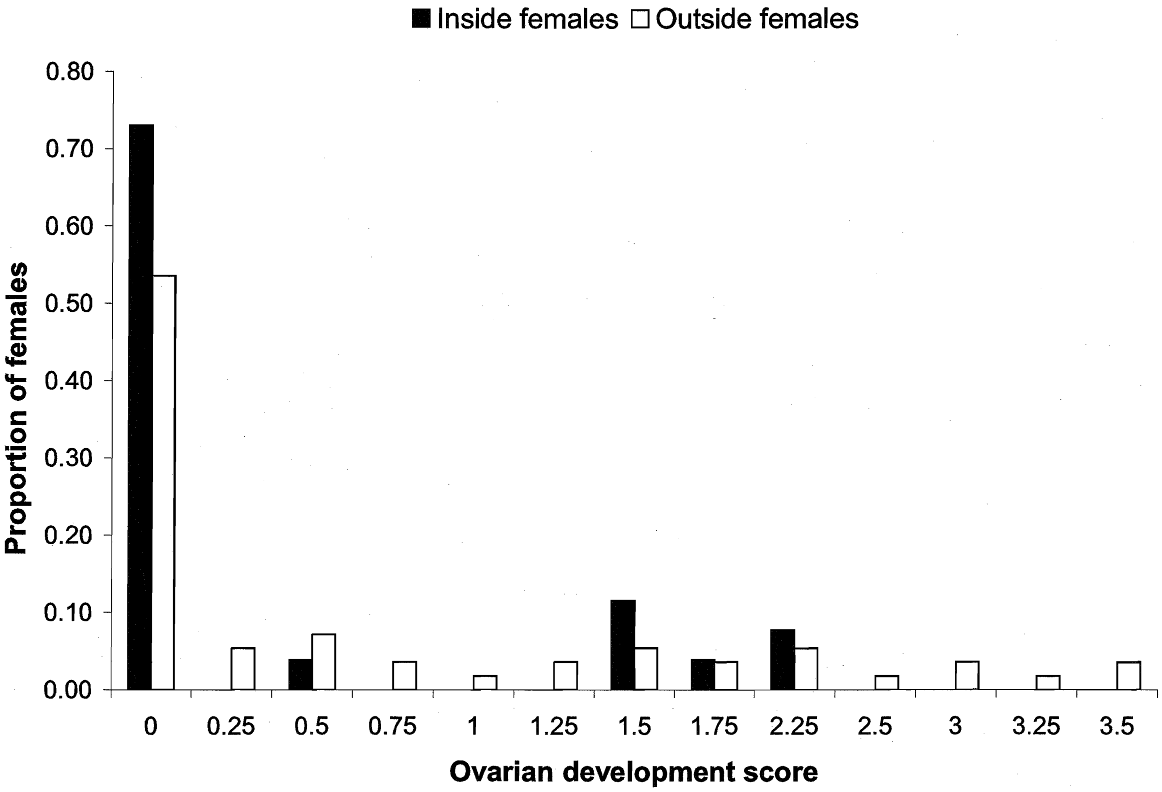


Figure 1.8: Distributions of total ovarian development score for inside (n=30) and outside females (n=78). Total ovarian development score is the fractional size of all ovarioles summed within the individual. Proportions are based on the total number of females in that class. Only females collected during the active foraging period are included. Data are pooled across years and across seasons.

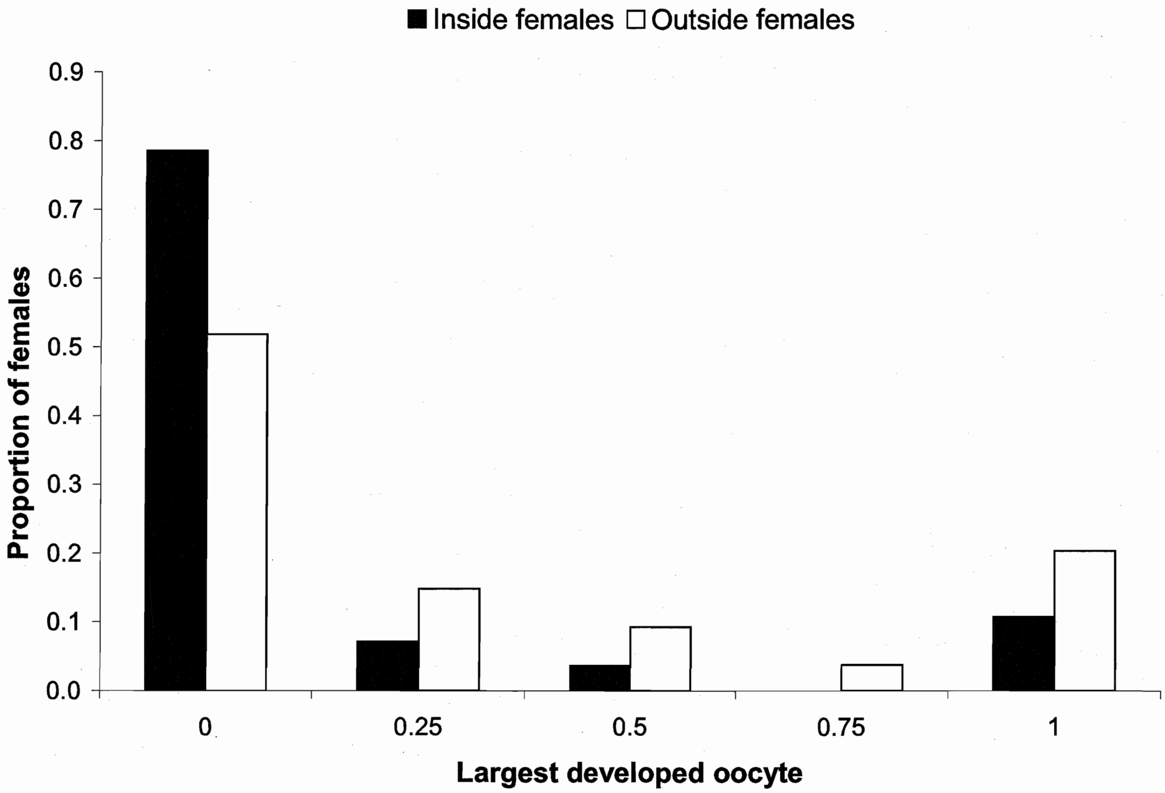


Figure 1.9: Proportion of inside (n=28) and outside females (n=54) with no developed oocytes, or a largest developed oocyte that is one-quarter developed, one-half developed, three-quarters developed or fully developed. Data are pooled across years and study sites. Only females caught during the active foraging period are included.

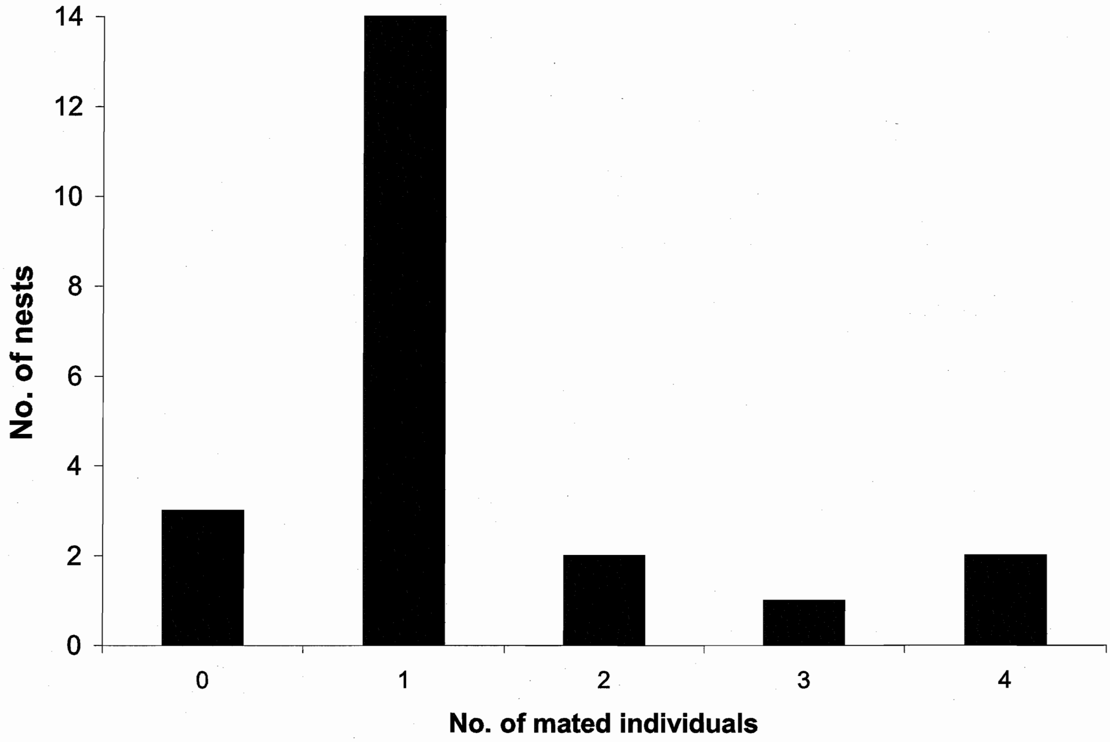


Figure 1.10: The number of nests with varying number of mated individuals (n=22). Nests with no worn and no mated females are excluded (n=3), as are nests where matedness could not be determined for all individuals (n=3). Nests with no foundresses were also excluded (n=2).

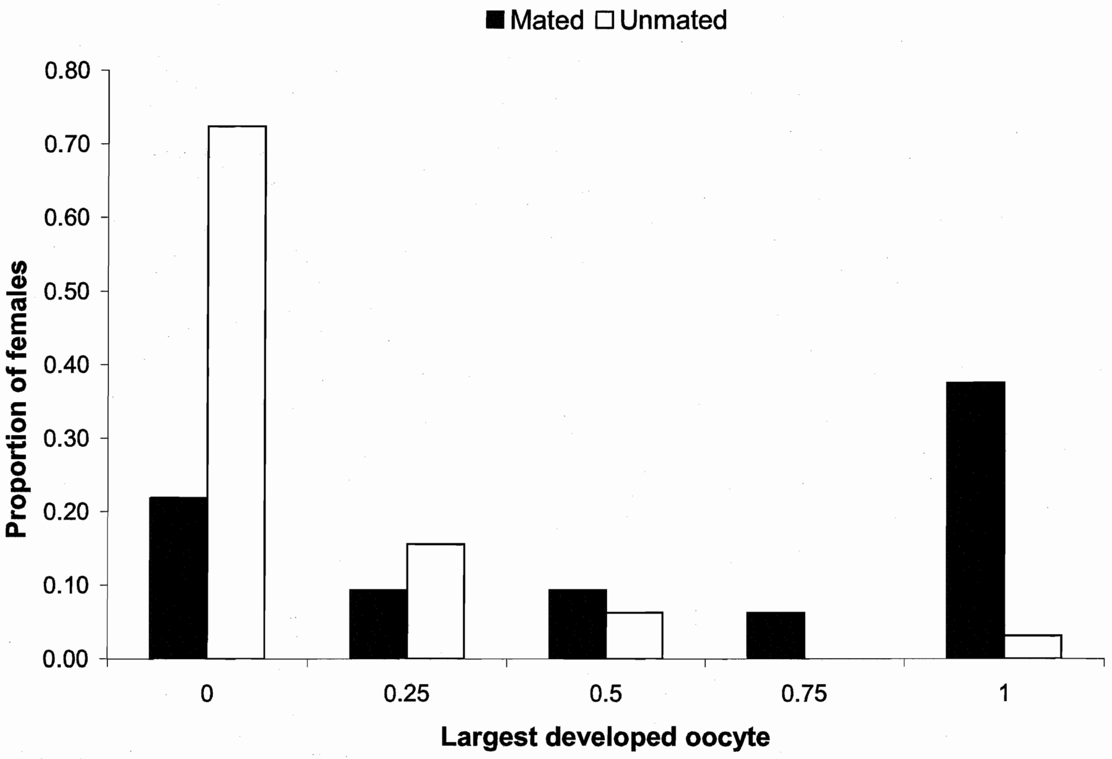


Figure 1.11: Proportions of mated females and unmated females with no developed oocytes, a largest developed oocyte that was one-quarter developed, one-half developed, three-quarters developed or fully developed. Sample sizes were 56 mated females, 97 unmated females for a total of 153 individuals. All years and sites were pooled, and data were included only for females collected during the active foraging season.

having no developed oocytes; mated females exhibited more one-half, three-quarter and fully developed oocytes than unmated females (Figure 1.11).

In the social Hymenoptera females of different roles typically vary morphologically, particularly in size because larger females may be capable of manipulating smaller ones. I compared measures of size between inside and outside females to examine whether size differences occurred. First, I compared head capsule width (HW) and costal vein length (CVL) to test whether these were suitable measures of size. Both HW and CVL were treated as ranks, as the underlying distributions were not normally distributed. An analysis that controlled for the female type (inside or outside) and year (as category), revealed a significant positive correlation between HW and CVL (Regression on ranks: $F_{1,202}=9.65$, $p<0.001$), and a significant effect of year (Regression on ranks: $F_{3,199}=3.94$, $p=0.009$) (Figure 1.12). Size did not vary between types of females (Regression on ranks: $F_{1,202}=0.08$, $p=0.78$). I confirmed the inside versus outside difference by specifically testing HW between inside females and outside females and found no significant difference (Wilcoxon 2-sample test: W=14988.0, p=0.6, n=266); because CVL and year were not included in this analyses, sample sizes were larger. This suggests that any division of inside nest tasks and outside nest tasks is not based on size, and that morphological castes do not exist.

To examine the relationship between work and reproduction in adult females, I created cross tabulations of wear and ovarian development (Table 1.1). First, I examined the relationship between work, measured as total wear (TW) and ovarian development (OD). I divided females into two wear groups: unworn (TW=0) and worn (TW>0); I also divided females into two OD groups: "high OD", defined as an OD greater than or equal to 0.25, and "undeveloped", a score less than 0.25 (Table 1.1). Frequencies in each

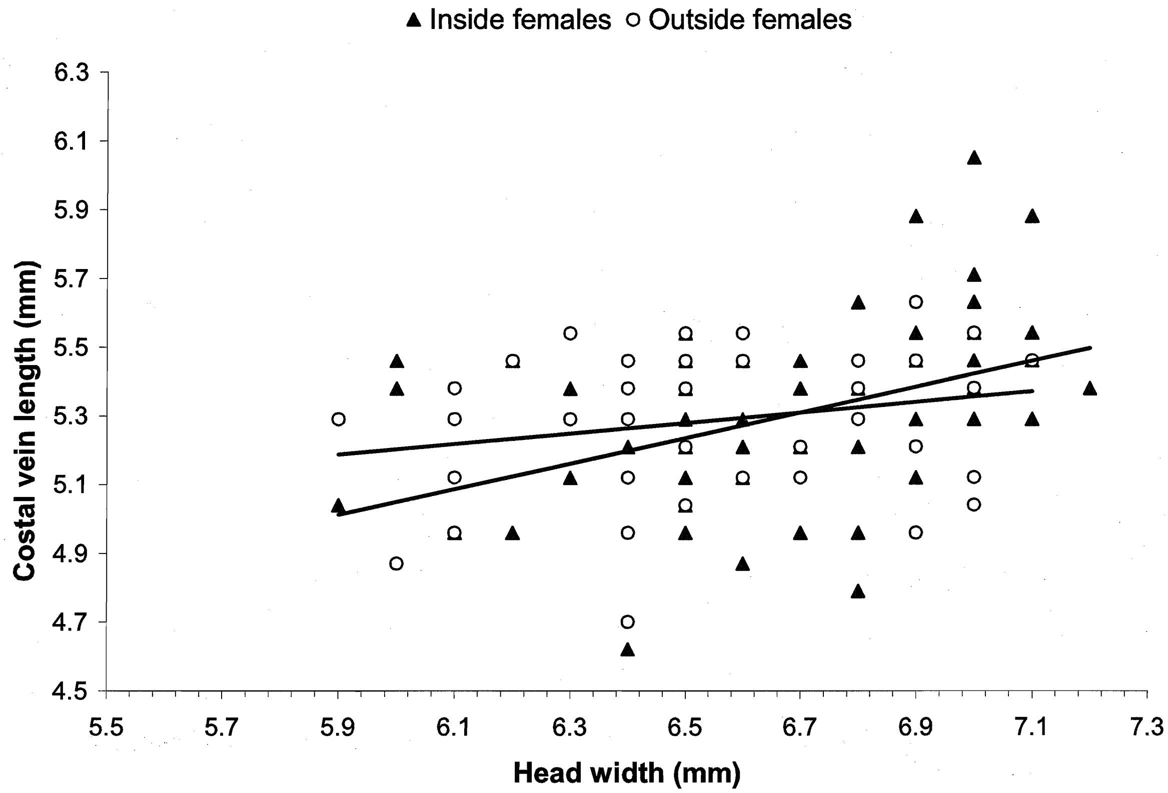


Figure 1.12: The relationship between head width (HW) and costal vein length (CVL) with female type and year as covariates. HW was correlated with CVL ($F_{1,202}$=9.65, p<0.001). There was no significant effect of female type (inside or outside females) ($F_{1,201}$=0.08, p =0.78). There was a significant effect of year ($F_{3,199}$=3.94, p=0.009), but not a significant interaction between HW and year ($F_{3,200}$=1.50, p=0.22).

Table 1.1: Cross tabulation of total wear (TW) (wing wear + mandible wear) and reproduction measured as ovarian development (OD). High OD is defined as a total score above 0.25. Unworn is defined as a wear score of zero, worn is any wear score greater than zero. All females are from the period following the first observation of a female with pollen, and before complete nests were collected. Total sample size is 75 females. There was a significant difference detected from random (all values equally distributed among cells) (χ^2_3=13.05, $p<0.005$). This was also significant when compared to an expected distribution for solitary (communal) bees (χ^2_3=158.22, $p<0.001$), and eusocial bees (χ^2_3=39.6, $p<0.001$).

	No OD (≤0.25) n=45	High OD (>0.25) n=30
Unworn (TW=0) n=24	17 (23%) Solitary=5 Eusocial=5	7 (9%) Solitary=5 Eusocial=16
Worn (TW >1) n=51	28 (37%) Solitary=5 Eusocial=38	23 (31 %) Solitary=60 Eusocial=16

class differed significantly from even when OD and wear were examined in cross-tabulation (Chi-Square Goodness of Fit: χ^2_3=13.05, $p<0.05$). I further made comparisons with two hypothetical (expected) distributions based on 75 individuals (the sample size) (Table 1.1). For these distributions, I imposed a minimum frequency of five individuals in a category. Because all solitary foundresses should both perform all nest tasks and reproduce, the expected distribution under this scenario had most individuals in the worn and high OD category; the observed distribution differed significantly from this (Chi-square Goodness of Fit: χ^2_3=158.22, $p<0.001$).

I also created a hypothetical distribution based on eusociality, assuming half of all females that foraged were workers and that the minimum number of females were both unworn and had no OD. I also assumed an even probability of highly worn, highly reproductive females and of unworn, highly reproductive females; both conditions are expected for eusocial queens. This hypothetical distribution also differed significantly from the observed (Chi-square Goodness of Fit: χ^2_3=39.6, $p<0.001$).

I next examined the relationship between work and reproduction when reproduction was defined as the possession of an oocyte at least one-half developed and worn was again defined as total wear greater than zero. When reproduction was defined in this manner, the observed distribution (Table 1.2) varied significantly from the assumption that all conditions are equally probable (Chi-Square Goodness of fit: χ^2_3=87.6, $p<0.001$). In particular, a seemingly high percentage of unworn, undeveloped females (31%) was present (Table 1.2). When the observed distribution was compared to expectations for solitary and eusocial colonies, a significant difference existed from both the solitary (Chi-square: χ^2_3=80.5, $p<0.001$) and eusocial (Chi-square: χ^2_3=241.8,

Table 1.2: Cross tabulation of total wear (TW) (wing wear + mandible wear) and reproduction measured as largest oocyte. Oocytes smaller than one half developed are considered undeveloped. Unworn is defined as a wear score of zero, worn is any wear score greater than zero. All females are from the period following the first observation of a female with pollen, and before complete nests were collected. Total sample size is 75 females. There is a significant difference in the distribution relative to equal distribution (χ^2_3=87.6, $p<0.001$). This was also compared to an expected distribution for solitary (communal) bees and was significantly different (χ^2_3=80.5, $p<0.001$), and an expectation for eusocial bees (χ^2_3=241.8, $p<0.001$).

	No OD (≤0.5) n=55	High OD (>0.5) n=20
Unworn (TW=0) n=24	23 (31%) Solitary=5 Eusocial=5	1(1%) Solitary=5 Eusocial=16
Worn (TW>1) n=51	32 (43%) Solitary=5 Eusocial=38	19 (25 %) Solitary=60 Eusocial=16

$p<0.001$) expectations. Both sets of cross-tabulations suggested wear and reproduction in the study population differed from both those expected for solitary (or communal) and eusocial bees. This suggests that these bees differ in allocation from both typical communal, solitary, eusocial, and semi-social colony social organizations.

Comparisons of females from multiple-foundress nests

Morphological comparisons

Since the population patterns suggested differences between females that left a nest and those that remained within a nest, I examined foundresses from multi-foundress nests collected at the Farmhouse site to determine the relative roles that inside and outside females might play. I counted the number of worn females within a nest to determine if multiple females in the same nest were performing work. Five of 17 multi-foundress nests contained more than one foundress that exhibited some wear (wing, mandible, both) (Figure 1.13). I also examined multi-foundress nests to determine if more than one foundress was likely to be reproductive. Only two of 17 nests contained more than one highly developed foundress ($OD \geq 0.5$), while six of 17 nests contained a single highly developed foundress (Figure 1.14). Both nests that contained more than one highly developed female also contained multiple worn females. Reproductive foundresses are expected to both be mated and to possess developed oocytes. I was able to assess this in 10 of the 17 multi-foundress nests and found that among all foundresses within these nests, four nests contained a single mated foundress; whereas six nests contained multiple mated foundresses (Figure 1.15). Finally, since size often determines roles within nests (larger females can better control small females), I compared head capsule widths between worn and unworn foundresses within the same nest (Figure 1.16).

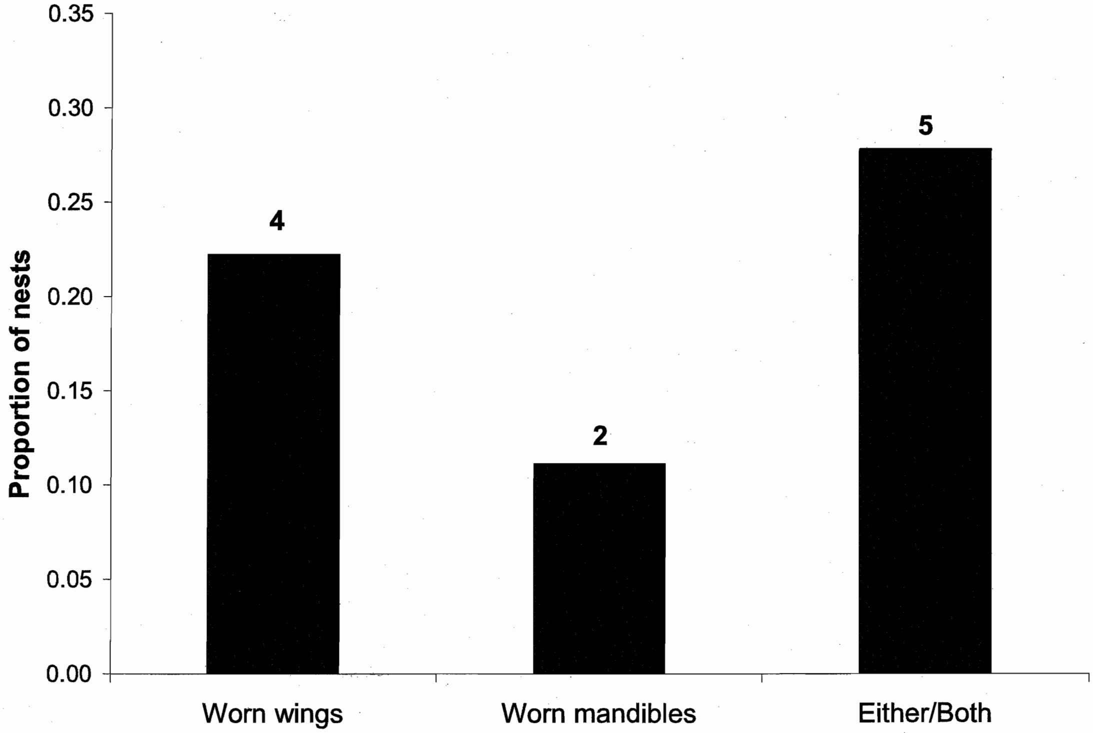


Figure 1.13: The proportion of 17 multiple-foundress nests with two or more worn individuals. The "either/both" category includes females with worn mandibles, worn wings or both and is not the sum of the wings and mandibles categories. Worn is defined as any wear score greater than zero. Numbers above bars are number of nests within the category.

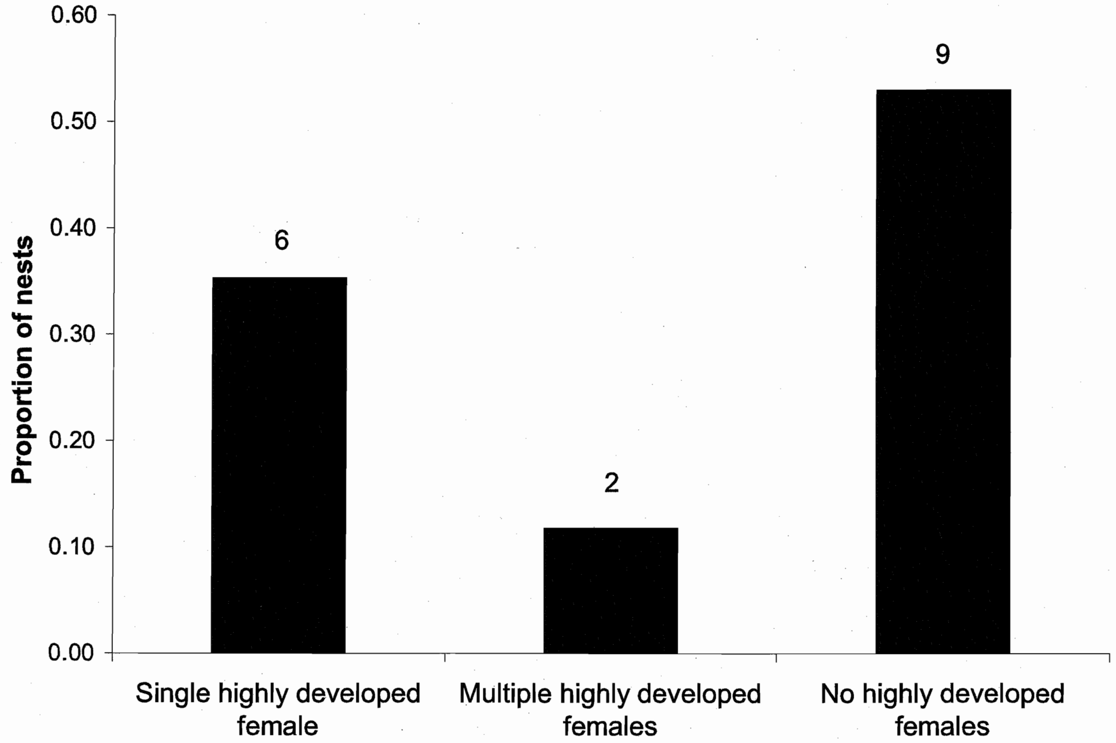


Figure 1.14: The proportion of multiple-female nests with a single, multiple or no highly reproductively-developed foundresses (OD≥0.5). Sample size is 17 nests; numbers above bars are number of nests.

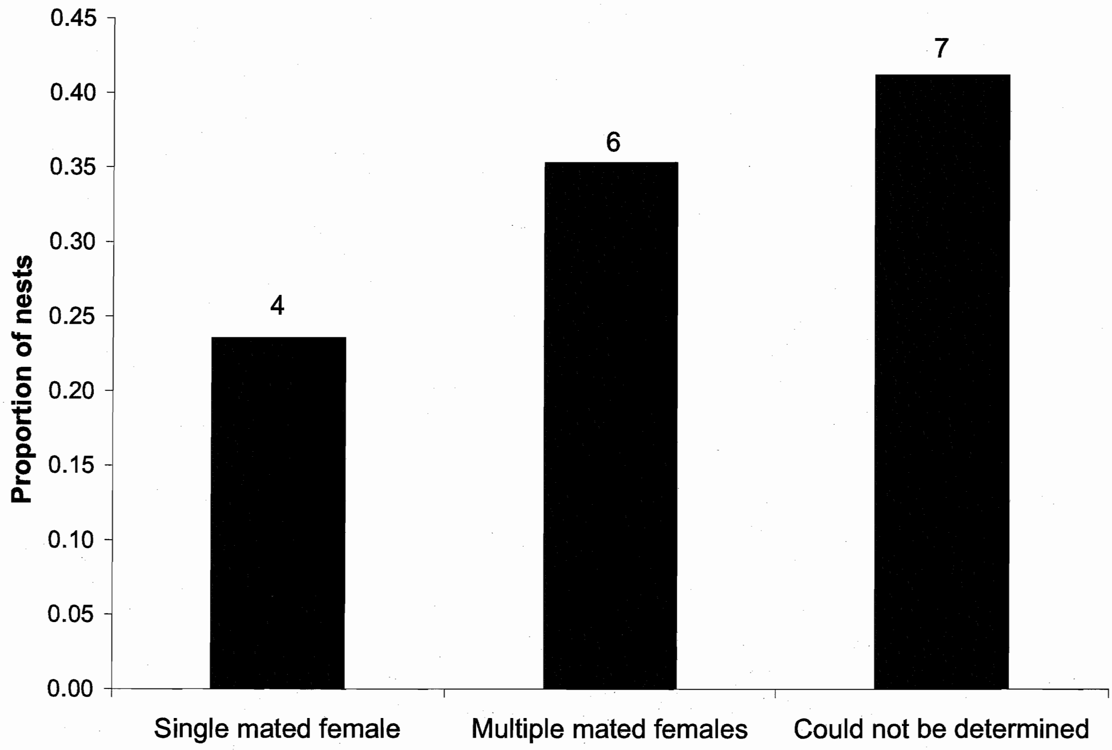


Figure 1.15: The proportion of multi-foundress nests that have no, a single or multiple mated foundresses. Sample sizes are given as number of nests. "Could not be determined" nests contained foundresses for which mated status could not be determined.

Figure 1.16: Head capsule width of each worn and each unworn foundress in a given multi-foundress nest. All individuals from a nest are represented by the same symbol (n=17 nests, n=65 foundresses).

Worn and unworn foundresses did not differ in size (Wilcoxon 2-sample test: W=987.50, n=65, p=0.64).

Behavioural comparisons

To investigate whether some foundresses may have been "hopeful reproductives" (females waiting to inherit the nest) or replacement queens, I removed active females from some nests. If females are hopeful reproductives, they are expected to begin foraging and laying eggs in response to the imposed absence of other reproductive or foraging females. After removing the actively foraging female from 12 nests, I observed no replacement females exiting these nests during the foraging period (Figure 1.17). In contrast, I observed females entering and exiting the entrances of control nests from the same bench and in additional control nests observed on alternate days.

In the removal experiments, there was a spike of flight activity that occurred afterwards in nests that were known to contain pupae (Figure 1.17). This spike could be seen in both the experimental and control nests. In control nests, this spike may represent foundresses collecting pollen to feed offspring: in four different nests on three days in August 2004, I observed both females and males licking pollen off the legs of a marked female. The feeding of new newly eclosed juveniles by foundresses prior to overwintering, has been reported for this species (Gerling and Hermann, 1976). Activity was also observed in nests from which the forager was removed (Figure 1.17); these females did not typically return with pollen and were unmarked, suggesting that they represented newly eclosed females foraging on their own.

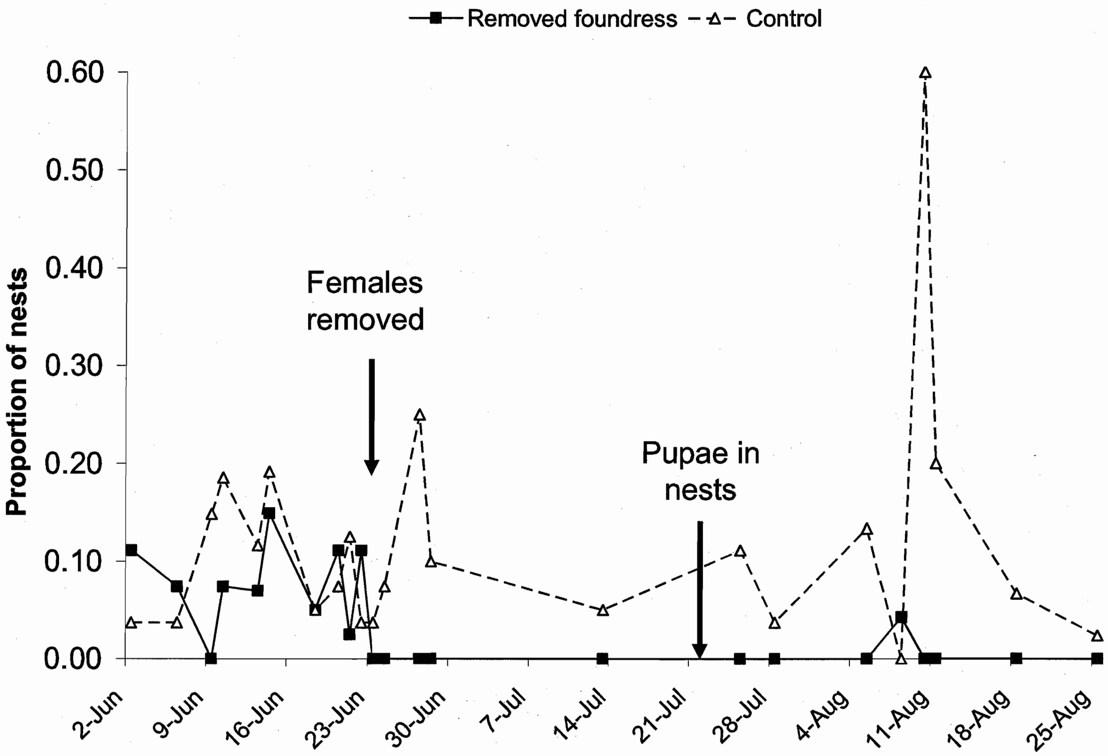


Figure 1.17: The daily proportion of nests at which a female was observed arriving with pollen in six-hour observations. Solid line indicates experimental nests from which the identified foraging foundress was removed. Control nests are a combination of nests from the same benches as experimental nests observed on the same day, and nests from alternate benches observed on alternate days. The first arrow indicates the date alpha females were removed. August activity occurred after brood provisioning had finished and broods started to eclose.

Removal experiments were also intended to test whether unworn and undeveloped females were replacement foragers. If a foraging foundress dies prior to eclosion of the brood, it might be necessary for another foundress to take on feeding tasks. I would have observed this as unmarked females flying and returning with pollen after brood emerge. Three such events occurred on August 8; one instance involved a single female making two trips with pollen and in two instances, a single female made a single trip for a total or three occurrences in 12 nests. However, it is also possible that these were newly emerged females and not old foundresses.

Finally, females that are not flying or constructing nests may be acting as guards. Having guards would allow a foraging foundress to leave the nest with less risk from parasites, predation, or nest take-over. This may allow increased time away from the nest, either through more trips, taking more time per trip, or both. I examined the number of foraging trips taken by foragers (females known to have carried pollen) from solitary and multi-foundress nests based on observations at nest entrances (Figure 1.18) and found no statistical difference in 2004 (Wilcoxon 2-sample test-W=35.5, n=18, p=0.5) or in 2005 (Wilcoxon 2-sample test: W=76.8, n=55, p=0.15). There was also no difference in the average time spent away from the nest during foraging trips between solitary nests and multi-foundress nests (Figure 1.19) (unequal variance t-test: $t_{7=}$-1.3, p>0.08); although, appropriate data for these analyses were limited.

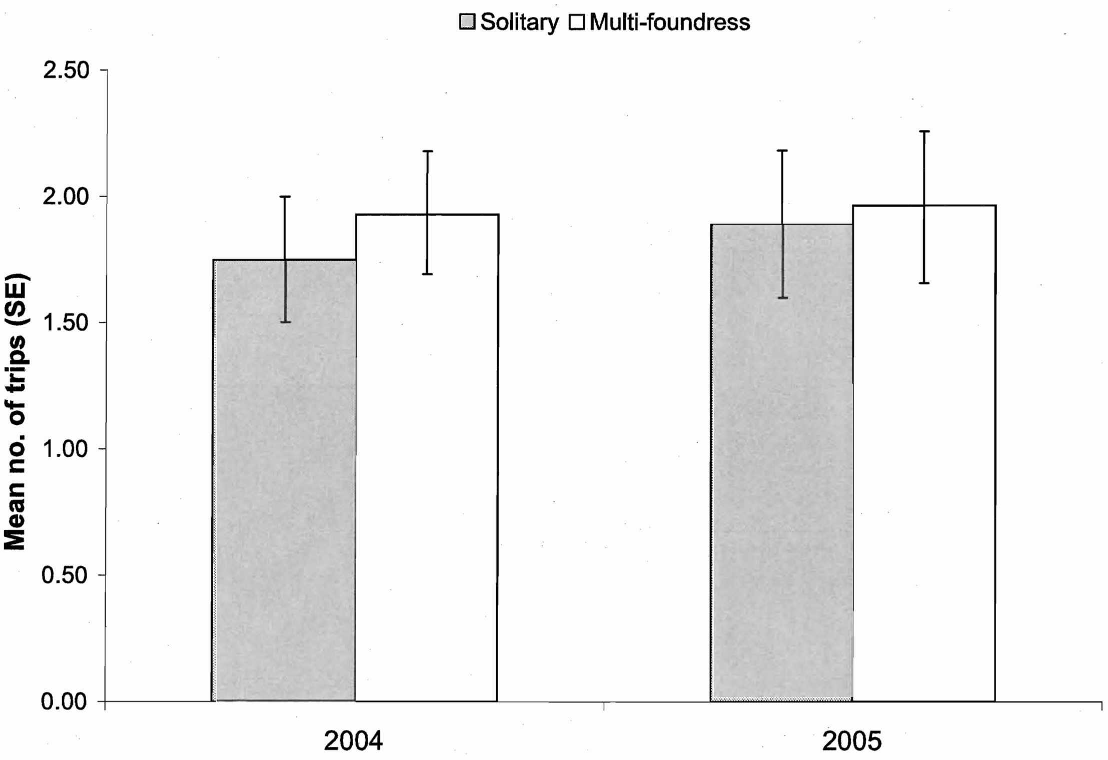


Figure 1.18: The mean number of foraging trips per female per nest for females from solitary (n=4 females in 2004, n=24 in 2005) nests (grey) and from multi-foundress nests (white) (n=14 females in 2004, n=31 in 2005). Solitary versus multi-foundress nest designation was determined based on videoscope observation. Trips only include females known to be foragers (females that were observed carrying pollen at least once during the season).

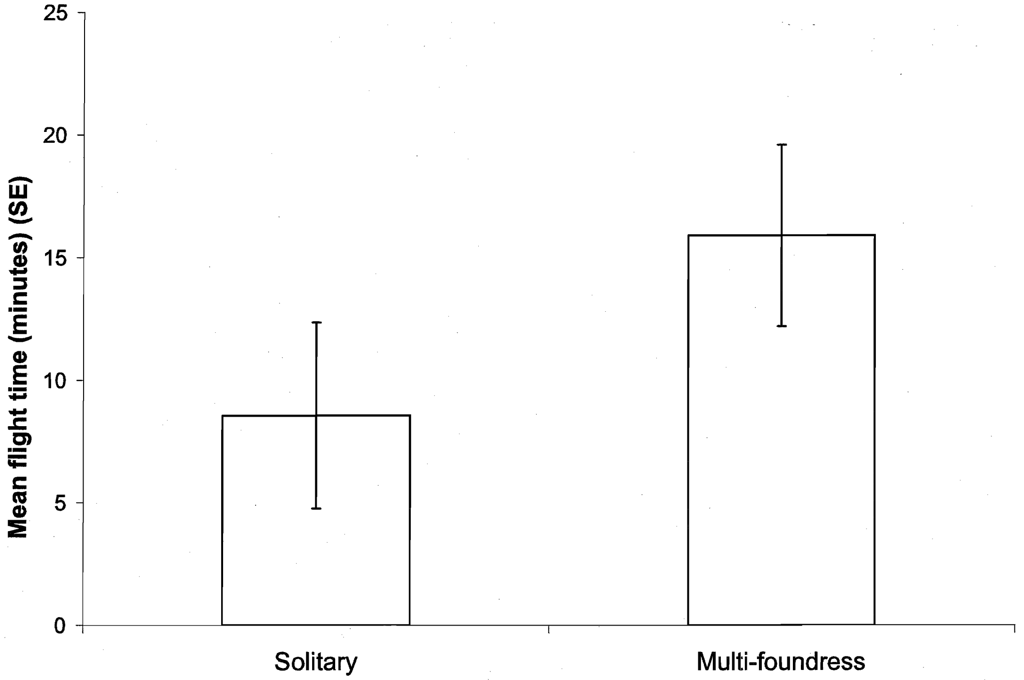


Figure 1.19: The mean flight time (time from departure to arrival) for foragers in single foundress (n=3) and multi-foundress nests (n=4).

Discussion

The social organization of *Xylocopa virginica*

In the population studied, *Xylocopa virginica* shows a social polymorphism. Some nests are solitary with a single foundress while other nests are social and contain up to six foundresses. Multi-foundress nests do not have a common social organization. These might more appropriately be described as multi-female nesting aggregations, where one female apparently establishes dominance early in the season and then utilizes the nest as would a solitary foundress. The remaining females reside in the nest and perform few, if any, tasks. These seemingly inactive foundresses may also function as guards, similar to what has been observed in *X. combusta, X. sulcatipes,* and *X. pubescens* (Bonelli, 1976; Stark, 1992a), may be "hopeful reproductives", or may be replacement foragers. Each of these possibilities is discussed below.

Examining the relationship between wear and reproduction in cross-tabulation (Table 1.3), it is possible to interpret what behavioural role a female with a particular combination of wing/mandible wear and reproductive status fills. When I examined females within the population, I found that some females show signs of performing work and reproduction, the typical condition for solitary and communal females. This pattern can also occur if one individual foundress performs foraging, nest construction and reproduction even in multiple foundress nests. The population also contained females that showed signs of only working or only reproducing; these resemble workers and queens in semi-social and some eusocial colonies.

Table 1.3: Cross tabulation of the expected relationships between work (wear) and reproduction (OD or largest oocyte) in females of different castes and forms of social organization.

	No OD (≤0.5) or no large oocytes	High OD (>0.5) or oocyte larger than ½ developed
Unworn	Other (failed foundress, hopeful reproductive, replacement forager)	Queen-like (advanced eusocial)
Worn	Worker-like	Solitary, Communal, Queen-like (primitively eusocial)

Very few of the females I examined were in the highly reproductive category (highly reproductive and unworn); this suggests an absence of advanced eusocial-like queens. It is more likely that these females were preparing either to "sneak" an egg into a brood cell prepared by another female, or to obtain a nest via takeover later in the season. Steen (2000) has provided genetic evidence that some *Xylocopa bombylans* nests contain brood from more than one mother and I found multiple reproductively developed and multiple mated females within a single nest, both of which suggest that some *Xylocopa* females may sneak eggs.

Females that are worn and not reproductively developed generally fall into the class of workers. This is possible with *X. virginica*, and it has been reported that in the *Xylocopa* subgenus *Lestis* two females may forage while only a single female reproduces (Steen and Schwarz, 2000). At Farmhouse, I occasionally observed nests with multiple females entering or exiting nests, and with multiple worn individuals; some of these nests had multiple females carrying pollen as they entered or exited (see Chapter 3 for further discussion). I propose that some of the lesser-worn females in these nests are worn because they began to establish a nest and failed. The result is that their ovaries regressed (although I observed no evidence of reabsorbed eggs in dissection). This possibility is supported by the observation of multiple females entering and exiting a nest before brood cells are actively prepared (Chapter 3).

The final combination of wear and reproduction is the situation in which a female is unworn and undeveloped, again a condition indicative of high reproductive skew. This condition is anticipated if the unworn and undeveloped females are guards, hopeful reproductives, or insurance against the death of the foraging female. However, in my video observations, marked females conducted the most aggressive guarding in nests, so it is unlikely that unworn and undeveloped females are guards. In addition, unguarded (solitary) nests experienced equal parasitism. Removals demonstrated that additional foundresses do not leave the nest when the primary foraging female is absent, suggesting that these females are not assuming foraging roles. Similar experiments with *Xylocopa pubescens* found that only 8% of subordinate females became egg layers when the dominant was removed (Hogendoorn and Leys, 1993; Hogendoorn and Velthuis, 1993; Steen and Schwarz, 2000). While I cannot eliminate the possibility of egg replacement in already provisioned brood cells, the evidence for this is limited and it is seemingly infrequent (Chapter 2), which corresponds to observations in other species. I suggest that non-reproductive, low work females are likely those that lost the competition for the nest, and failed to establish their own nests. In most instances, losing females would be dead and we would not observe them; however, in this system they instead apparently remain mostly passive within the nest.

Adaptive significance of solitary versus multi-foundress nesting in *Xylocopa virginica*

There was no apparent difference between multi-foundress and single foundress nests in terms of brood size or colony longevity. Further, as the number of foundresses within a nest increases, the number of brood produced per foundress decreases. These findings are consistent with those for other species of *Xylocopa* (Hogendoorn and Leys, 1993; Watmough, 1983) and suggest that any fitness advantages of solitary or multi-foundress nesting are not from greater brood survivorship, larger overall brood sizes, or increased productivity.

Recent work on *Xylocopa* in the subgenus *Lestis* (Steen and Schwarz, 2000) and on reproductive skew theory (Ragsdale, 1999) has suggested that when a resource is of enough value, the division of reproduction among females (skew) can be extreme with a single female dominating reproduction, and that multi-female nesting might still occur, even if no tasks are being performed by subordinates. This is a likely scenario in *X. virginica*. Gerling and Hermann (1976) suggested that, in Georgia, suitable boards for nesting are limited, and it is costly to construct a new nest even when nesting substrates are available, and therefore the benefit of inheriting a nest is high. In my study population, many nests are permanently abandoned and likely no longer suitable (Chapter 3). In addition, while the site contained many panels without nests, there is an apparent preference for some benches and panels over others (Chapter 2). Further, when additional artificial nests were added or artificial entrances drilled, these went unused. Therefore, it is possible that there is less suitable room for nesting than it may appear. Nesting materials are considered to be limiting for other species of *Xylocopa* (Gerling and Hermann, 1976; Hogendoorn, 1991; Hogendoorn, 1996; Hogendoorn and Leys, 1993; Watmough, 1983). This means that a female who loses a fight for dominance may be better off waiting in a nest that she will inherit rather than attempting to establish her own nest or attempting a nest takeover. This alternative will be particularly attractive given the time involved with constructing a new nest, which might limit time available for provisioning (Chapter 2). In *X. virginica*, because nests are frequently re-used (Chapter 3) and re-use is likely to be by relatives of the reproductive (dominant) foundress, a dominant may choose to allow some females to live and attempt to establish a colony the next season, resulting in indirect fitness for the dominant. Dominant females may also benefit if additional females act indirectly as guards. Gerling and Hermann

(1976) have suggested that a female may be effective in guarding simply by residing in a nest, and may not need to perform any particular action. However, my data suggest that there is no benefit of guards in terms of foraging time. In such a situation, there might still be additional benefit to the dominant in some form, but little cost to the queuing female(s).

In other species of *Xylocopa,* there are high rates of mortality due to predation by invertebrates, and guarding is common (Gerling et al., 1989; Hogendoorn and Velthuis, 1995; Steen, 2000; Watmough, 1983) (Appendix 4). High predation would make any guarding beneficial and, in part, would explain the evolution of multi-foundress nests. It has been suggested that in halictine bees parasitic and predatory pressure is a major factor in the evolution of multi-foundress nesting (Lin, 1964). In my study populations, brood mortality is almost exclusively due to parasitism by bombyliid flies that do not enter the nest (Gerling and Hurd, 1976); similar parasitism was observed in Georgia where the flies lay eggs outside the nest (Gerling and Hurd, 1976). This means that guards cannot effectively reduce mortality, and that solitary nests are at no more risk than multi-foundress nests. In addition, I observed nests containing various invertebrates (wasps, earwigs, and other bees) while inhabited by *X. virginica*, which also suggests that guarding is not essential in some populations. In Chapter 2, I further discuss parasitism as it relates to nesting behaviour, and I suggest that nest structure is also adapted to reducing parasitism.

Some authors (Hogendoorn and Leys, 1993; Hogendoorn and Velthuis, 1993) have suggested that guards mostly prevent pollen robbery or nest usurpation (which I rarely observed), which could also be prevented by a female returning to the nest more frequently, resulting in shorter foraging trips. However, I found that solitary females make the same number of foraging trips as those from multi-foundress nests, and that they spend the same amount of time away from the nest. This suggests that additional females do not allow for more effective foraging *X. virginica* via guarding. Finally while the finding that few nests contained more than one highly developed female is suggestive, genetic analysis of females from nests will be required to determine whether a single foundress laid all members of a brood, or conversely to prove that multiple females oviposit.

I suggest that guarding may be an ancestral behaviour to many species of *Xylocopa* and perhaps the genus. This trait is exhibited in various forms throughout the genus, but is not adaptive in northern populations of *X. virginica* where few invertebrate predators exist and nest usurpation is likely rare. Anecdotal evidence suggests that *X. virginica* may be more recent to southern Ontario and so nesting materials may be less limited (discussed in Chapter 2) than in habitats that are more southern. This could alter the value of inheriting a nest and thus the decision to cooperate.

Broader implications of *Xylocopa virginica* social structure

There are three broad explanations for the evolution of altruism in social insects. The first of these is kin selection as proposed by Hamilton (Hamilton, 1964; Hamilton, 1972), an explanation based upon genetic relatedness. The other two explanations for the evolution of altruism in social insects are more behavioural and ecological in nature. Lin and Michener (1972) have proposed mutualism, the situation where a helper and a reproductive both receive direct fitness from their interactions, as a mechanism for the evolution of altruism and eusociality. Other authors have suggested that manipulation of workers by a queen may lead to the evolution of helping (Crozier and Pamilo, 1996).

This thesis, and other studies of *Xylocopa*, provides some of the necessary information to test the competing hypotheses of mutualism and queen control as they relate to the evolution of social nesting and altruism.

If social nesting in *Xylocopa virginica* evolved via mutualism, some behaviour would have to benefit both the foundress that is reproducing and foraging and the females that are seemingly not performing significant tasks. Guarding is a behaviour that might accomplish this. If a guarding is a trade-off, as has been proposed for *X. pubescens* (Dunn and Richards, 2003; Hogendoorn, 1996; Hogendoorn and Leys, 1993), then it is possible that both individuals achieve a benefit. In these populations, such a benefit would arise if the seemingly underproductive female sneaks eggs, or if she gets to inherit a nest in exchange for guarding; guarding would potentially help her own offspring or would serve as a guarantee to the quality of the nest. However, since guarding is seemingly not beneficial in the study populations, it is questionable how much benefit the dominant foundress actually receives. Future studies of *Xylocopa* behaviour in both this species and others may help to clarify these possibilities. It would be particularly useful to examine parasitism in other ecological settings where selection pressures may be different.

X. virginica is a good species for studying queen control as a mechanism for social evolution because it can be contrasted with other species known to have some queen control, and because division of labour is non-traditional. In many species of bee, divisions of labour and reproduction are maintained via physical manipulation on the part of the dominant (queen) (Kukuk and May, 1991; Richards et al., 2005). In *Xylocopa Lestis*, aggressive interactions occur among foundresses, as do instances of two foraging females within a nest; these instances may be related to dominance and queen control, although, these bees also show size variation between "castes" (Steen, 2000). I did not detect size differences in the study populations. In the study populations, less active foundresses may be under queen control where the dominant female prevents them from reproducing in most instances. Under the usual expectations of queen control we would expect a dominant to monopolize reproduction while forcing subordinates (workers) to perform the risky tasks such as foraging. Neither of these expectations is met fully in these populations. First, most foundresses in nests do less work than the primary reproductive females; dominants are thus not able to gain the presumed advantage of having workers. Second, some nests contain multiple females which are mated or reproductively developed which suggests that dominants may not be able to prevent other foundresses from reproducing in all instances. It is thus intriguing that this species appears to have evolved to the point of tolerating conspecific females and to have some control, yet has not evolved a proper worker caste. This deserves further study as it may be an "exception that proves the rule" scenario and raises many questions, such as: Does the lack of size difference make it impossible for females to be fully dominant? Are dominants capable of distinguishing between various females within the nest? Is the benefit of workers too small to result in the evolution of queen control or a true worker caste?

Conclusions

This study suggests that as in many species of *Xylocopa,* multi-foundress nests of *X. virginica* are associations of a single dominant female that is acting like a solitary female by performing work and reproduction and subordinate females that are seemingly underproductive and perform limited work. In southern Ontario, it is likely that guarding is of limited benefit due to the lack of invertebrate predators and nest usurpation. If nestmates are related, there might be enough indirect fitness for the dominant foundress to offset any costs from allowing subordinates to remain in a nest. Since fighting may be costly, it is probably not beneficial for the dominant to incur the risk or use the energy required to kill other females rather than allowing them to remain and possibly gaining future fitness, particularly if subordinates do not impose a significant cost in efficiency or personal reproduction for the dominant female. Some of the differences between my study population and those studied in Georgia or Missouri may be due to the seemingly recent expansion of *X. virginica's* range into southern Ontario where nesting sites may be less limited and seasons may be shorter. Future studies of *X. virginica* behaviour may add to our knowledge of queen control mechanisms, reproductive skew, and the role of parasitism in the evolution of sociality.

Chapter Two: Relationships between nest architecture and behaviour in *Xylocopa virginica*

Introduction

Nests constitute a large portion of the environment in which social insects spend their life-cycle. Most bees, ants, and wasps lay their eggs within nests and these nests presumably influence reproductive behaviour and life-history. Consequently, the influence of nesting biology on aspects of behaviour is a common topic in social insect biology. Studies of nesting behaviour consider a diverse array of topics including basic nest architecture, the relationships between nesting and parasitism, and the influence of nests on efficiency with respect to tasks such as nest construction, brood laying and foraging.

A common topic in the study of wasps is the role of nest architecture in the evolution of sociality and a colony's efficiency due to division of labour. Jeanne (1975), for example, has demonstrated that elements of nest architecture are related to brood sizes: nests hang from a pedicil and if the nest is too large and heavy it will fall and the colony will fail. Karasi and Wenzel (1998) discussed the relationships between nest structure and a colony's efficiency and noted that these factors are also related; inefficiency will lead to smaller nests and consequently smaller broods. Studies of bees have examined the functional significance of architectural elements as they relate to aspects such as mortality and parasitism (Packer, 1991; Packer et al., 1989). These studies note that different nest structures and positions may serve to protect the colonies from parasitism. Finally, nest structure has been investigated with respect to its influence on social evolution in various taxa (Hansell, 1993).

The subfamily Xylocopinae consists of four tribes, all of which nest in stems, twigs, or wood (Daly et al., 1987; Maeta et al., 1996; Michener, 1974; Michener, 1990; Minckley, 1998). The exception is the ground nesting behaviour of the subgenus *Proxylocopa* (Hurd, 1978). Some of the tribes in this subfamily contain social species and the evolution of sociality is linked to nest structure and availability in at least one of these tribes, Allodapini (Bull and Schwarz, 1996). In the Allodapini the absence of cell partitions leads to increased contact between females and social nesting may occur as a result of limited nesting substrate (Schwarz, 1986; Schwarz et al., 1998; Schwarz et al., 1997).

The genus *Xylocopa* gets its common name (the large carpenter bees) from its tendency to burrow nests into wood, stems or reeds. Since these nesting behaviours are characteristic of the genus, descriptive studies of nests have often been a topic of research conducted on *Xylocopa* (Dunn and Richards, 2003; Maeta et al., 1996; Steen and Schwarz, 2000; Thoenes and Buchmann, 1994; Vicidomini, 1996). *Xylocopa* construct nests of two basic shapes, linear and branched that vary in the number of "tunnels" (Gerling et al., 1989). The chronology and order of nesting events such as constructing cells and laying brood has been linked to nest architectural factors such as nest shape and architectural complexity (Steen and Schwarz, 2000; Velthuis et al., 1984). Further, the architecture of a nest may influence other elements of life-history such as brood size, provisioning efficiency, and susceptibility to parasitism. For instance, the available space within a nest can influence brood size and number of foundresses. Likewise, the developmental stage of an individual offspring reflects the time since an egg was laid, which occurs immediately after the cell is provisioned (Gerling and Hermann, 1976). Therefore, the developmental stages of brood can be used to infer the order in which brood cells were provisioned. Since completed brood cells impede access to points of a tunnel beyond them, the number of termini within a nest may influence brood provisioning strategies. In linear nests, foundresses might provision one terminus at a time, completely filling one end of the tunnel with brood before commencing in the other, or they may alternate between the two sides (Steen, 2000; Steen and Schwarz, 1998). In branched nests, the possibilities increase; for example, one branch might be provisioned entirely and then the remaining termini might be provisioned in an alternating pattern (Steen, 2000; Steen and Schwarz, 1998).

In *Xylocopa*, as in other Hymenoptera, females can choose whether an oocyte is fertilized, giving them control over the sex of oviposited eggs. This control leads to a great deal of speculation about the order and patterns of sex allocation in these bees. The linear and fixed nature of brood cells in *X. virginica* nests makes this species especially suitable for examining patterns of sex allocation, particularly with respect to nest architecture.

Nest architecture of *Xylocopa virginica* has been studied twice previously, by Rau (1933) in Missouri and later by Gerling and Hermann (1976) in Georgia. These studies both concentrated on description of the nest architecture. Similar descriptive work is available in Appendix 10. Here I extend studies of *X. virginica* nesting behaviour. Specifically, I examine the role that nest architecture has on the order of brood laying, brood sizes, parasitism and foundress number of a southern Ontario population of *X. virginica*.

Methods

Study sites

Nests used in this study were collected at two sites in St. Catharines, Ontario. Between 13 July and 26 August, 2003 nests were collected from the Brock University Farmhouse site. In 2006, an additional five nests were obtained from a pavilion in Burgoyne Woods, a pubic park 2 km from the Brock University campus. All nests were excavated according to the methods in Chapter 1 and the general methods.

Nest architecture as it relates to brood laying order and size

The number of brood cells in a nest may be constrained by nest architecture. To determine if constraints exist, I counted the number of brood cells in each terminus and compared this to the length of termini and the total length of nests using linear regression. In order test the hypotheses that eggs are laid starting from the end of a terminus, and one branch at a time, a rank that reflected developmental stage (pollen ball, larva, unpigmented pupa, pigmented pupa, adult, as described in general methods, Figure I.3) was assigned to each individual; earlier developmental stages were assigned lower values. I also assigned a number value to each cell that reflected its position within a terminus (Appendix 10). These numbers were assigned sequentially from the terminus to the entrance gallery along each branch so that lower numbers indicate a greater distance from the gallery.

I examined the relationship between developmental stage of offspring and their position within a terminus, and a nest, to test the hypothesis that brood are laid starting from the end of a terminus and ending near the entrance gallery. Since developmental stage reflects brood laying order, younger brood stages were expected near the entrance gallery (i.e., in cells of higher number). Analyses were performed via ANCOVA with the model, brood development = cells from terminal end + number of cells (within nest); this tests an effect of position on age.

To test the hypothesis that brood laying was sequential within termini and among termini, I used ANOVA with a model where the dependent variable was brood stage (converted to a rank), and the predictor was terminus nested within nest. This tests for a pattern between when an individual was laid and terminus nested within nest. Consequently a significant effect means each terminus is provisioned completely prior to the start of another, while no significance would indicate a random positioning.

The relationship between nest architecture and sex allocation

I examined whether there were patterns of sex allocation with respect to the sex of an individual offspring and its position within the nest. I counted the number of instances in which cells were provisioned in a particular sequence (male followed by female, female followed by female, female followed by male, male followed by male). These frequencies were compared to an expectation that all sequences were equally likely using a chi-square-goodness of fit test performed in Microsoft Excel. To determine if there was a nest wide pattern of sex allocation, I examined the relationship between sex and position within the nest using logistic regression performed with the SAS 9.1.3 (SAS Institute Inc, Cary, NC). PROC Logistic. Two different models were used. The first model treated sex as a binary categorical response variable with unknowns excluded (male, female) and the second model treated unknowns as an additional category (male, female, unknown). Both models used the numerical position of individuals within a terminus as a predictor. The model used binary logits for categorization of sex as male or female, and used cumulative logits when the sex was categorized as male, female or unknown; all models used Fisher's scoring technique (Stokes et al., 2000).

Relationships between nest architecture and parasitism

While Chapter 1 demonstrates no role of parasitism in the social behaviour of *X. virginica*, relationships between parasitism and nest architecture are commonly studied. Further, it is possible that the architecture of a nest protects against parasitism making guards unnecessary. Similarly, it is also possible that nest architecture influences the degree of parasitism in a nest indirectly by its influence on brood size. I tested four hypotheses relating nest architecture to parasitism:

1) I tested the hypothesis that branched nests would contain more *Xenox tigrinus* (the most common parasite of *X. virginica*) compared to linear nests using Wilcoxon 2-sample tests.

2) I tested the hypothesis that longer nests would contain fewer parasites, because it is more difficult to reach brood cells. I tested this using linear regression with the number of parasites as a dependent variable and the total length of a nest (see Appendix 10) as a predictor.

3) I tested the hypothesis that nests containing larger broods (more individuals) would also contain more parasites. This analysis was conducted using linear regression of brood size (number of cells) versus number of parasites.

4) Finally, I examined the positional patterns of parasitism, to test the hypothesis that any cell within a nest is equally likely to be parasitized. This was conducted using a logistic regression model that treated parasitism as a binary response variable (parasitized or unparasitized) and position as the number of cells from the terminus (Appendix 10). PROC logistic (SAS) was used with binary logits and Fisher's scoring method.

Relationships between nest architecture and number of females

To test the hypothesis that branched nests will contain more foundresses than linear nests, I compared the number of foundresses in each nest type using Wilcoxon 2-sample tests. I used ANCOVA to test the hypothesis that the spatial and architectural elements of nest (total length, empty space, space occupied by brood cells and whether nests were branched) will influence the number of foundresses within a nest. These variables may influence the space in which foundresses reside, and therefore the number of foundresses within a nest. I used an ANCOVA model that included the total length of the nest, the amount of space occupied by brood cells, the space not occupied by brood cells that could accommodate them and whether nests were branched or linear as predictors, and the number of foundresses as a dependent variable. Initially, interaction terms were included for all variables, but no interactions were significant and the final model excluded these terms with no noteworthy difference. The measure of space occupied by brood cells violated assumptions of normality, and was thus log transformed to meet the assumptions of GLM. To determine if there were associations between the number of working females and nest architecture, ANOVA was also performed replacing the number of foundresses with the number of worn females (an indication that the female either foraged or constructed nests - see Chapter 1 for explanation) and again with the number of females with worn mandibles (an indicator of nest construction or renovation).

Sample availability considerations

Not all variables were available for all analyses (Appendix 2). In particular, some nests were collected without foundresses (n=3) or brood (n=3); one nest contained neither. For three other nests, it was not possible to assign foundresses to a particular nest. Details for nests collected in Ontario are available in Appendix 1. Appendix 2 gives details of nests included in each analysis. Sex could not be determined for parasitized individuals, nor for larvae that failed to pupate.

Results

Relationships between nest architecture and brood

Branched nests from Farmhouse (n=13) contained significantly more brood cells (11.1 ± 4.9) than linear nests (n=14) (7.2 ± 4.1) (Wilcoxon 2-sample test: W=236.5, n=26, p<0.01). In nests collected from Farmhouse in 2003; 73% (16/22) of nests showed an ordered pattern of brood laying in which a single terminus was provisioned before moving to the next branch (Table 2.1). In 86% (19/22) of nests, age was sequential within a branch (Table 2.1). I tested whether brood laying was sequential within termini and among termini using ANOVA with a model where the dependent variable was brood stage (converted to a rank), and the predictor was termini nested within nest. The overall model was significant (ANCOVA: $F_{56,187}$=5.68, p<0.01), as was the effect of nest (ANCOVA: $F_{29,187}$=9.41, p<0.01), which suggests that the age of broods varies among nests. The terminus (nest) factor was also significant (ANCOVA: $F_{27, 187}=1.68$, $p<0.05$), indicating that the variability between termini was greater than the variability within a terminus.

The relationship between nest architecture and sex allocation

I found no significant pattern of sex allocation. The number of instances in which cells were provisioned with females consecutively (n=16), a female followed by a male cell consecutively (n=18), male cells consecutively (n=22), and males followed by females (n=16) were not different from the expectations of an equal probability (Table 2.1) (Chi-square goodness of fit: $\chi^2_3=1.33$, $p>0.7$). I also found that the pattern of sex allocation is not statistically associated with an offspring's position in the nest, and therefore with the order in which it was laid. These patterns are consistent when the model treated sex as a binary category of either male or female (Logistic regression: $Q_{w1}=0.81$, $p=0.37$) and when the model treated individuals of unknown sex as an additional category (Logistic regression: $Q_{w1}=0.59$, $p=0.44$).

Relationships between nest architecture and parasitism

I found no significant relationships between nest architecture and parasitism. There was no difference in the number of parasites between branched and linear nests (Figure 2.1, Table 2.1) (Wilcoxon 2 sample test: W=176.5, n=27, p=0.79); on average linear nests contained 1.4 ± 1.6 parasites while branched nests contain 1.2 ± 1.5 parasites (Table 2.1). In addition, the number of parasites at Farmhouse was not related to brood size, and large broods did not contain more parasites than smaller broods (Regression: $F_{1, 22}=0.76$, $p=0.39$). Nest length did not influence the rate of parasitism and no statistical

Table 2.1: The developmental stage and sex of brood relative to its position within the nest. Cells represent the number of cells between a given cell and the end of the branch in which it is located. Lower numbers are farther from the nest entrance gallery, while larger numbers are closer to the gallery. Developmental stages are given as: pollen ball (PB), larva, unpigmented pupa (UP), pigmented pupa (PP), and adult. Individuals that developed into females are indicated by F, those that developed into males are indicated as M, U indicates an individual of unknown sex. Parasitized cells could not be sexed and are not assigned a developmental stage. Empty cells contained no pollen or offspring.

Nest	Branch	Cell 0	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8	Cell 9
A	A	Larva-M	Larva-U	Larva-M	Larva-U	Larva-U	Larva-U	Larva-U			
	B	Larva-U	Larva-U								
B	A	PB-U	PB-U	PB-U							
BB	A	Parasitized	UP-U								
	B	Adult-F	Adult-F	PP-F	Parasitized	UP-M	UP-U	UP-U			
C	A	Larva-U									
CC	A	PP-M	PP-M	PP-U	PP-U						
	B	UP-M	UP-M	UP-M	UP-F	UP-M					
	C	PP-M	PP-F								
D	A	PB-U	Larva-U	Larva-U	Larva-U						
	B	Empty	Larva-U	Larva-U							
DD	A	PP-M	Dead-U	PP-M	Parasitized	UP-F	Empty	Parasitized			
	B	PP-F	UP-M	UP-U	UP-M	UP-M					
	C	Parasitized	UP-F	Empty							
E	A	Larva-U	Larva-U	Larva-U							
	B	Dead	Dead	Larva	Larva-M	Larva-U					
EE	A	PP-M	PP-M	PP-M	PP-M	PP-U	PP-M	PP-U			
	B	UP-M	UP-M	UP-M	UP-U	UP-U	UP-F	UP-U			

Nest	Branch	Cell 0	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8	Cell 9
F	A	Larva-U	Empty								
	B	Parasitized	Parasitized	Parasitized	Empty	Larva-U					
FF	A	PB-F	PB-F	PP-M	PP-U	PP-U	PP-M	PP-M	PP-M	UP-M	UP-F
GG	A	UP-F	UP-F	UP-F	UP-U	UP-F					
	B	Empty	UP-U	Empty	PP-f	Empty	PP-U				
H	A	UP-F	UP-F	UP-F	UP-M	Larva-U	Larva-M				
	B	Dead									
I	A	UP-F	UP-M	PP-M	Dead						
	B	UP-F	UP-M	UP-M	UP-F	Dead					
	C	Larva-U	Parasitized								
J	A	Parasitized	Larva-F								
K	A	Parasitized	Larva-F								
	B	PB	UP-F	UP-M							
	C	Parasitized	PP-U	Parasitized	UP-M						
L	A	UP-M	UP-F	PB	PB	Parasitized					
	B	UP-M	UP-F	UP-F	UP-F						
M	A	Parasitized	UP-F	UP-F	UP-F	UP-M	UP-F				
	B	UP-M	UP-F	UP-M	UP-M	Parasitized	Parasitized				
N	A	UP-M	UP-F	UP-F	UP-M						
	B	Larva-M	Dead	Larva-F	Larva-M						
O	A	Larva-U	Larva-F	Larva-U							
P	A	Empty	Empty	Empty	Empty						

Nest	Branch	Cell 0	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8	Cell 9
Q	A	UP-M	Parasitized	UP-M	UP-U	UP-F	UP-M	UP-M	UP-U	UP-U	
R[a]	A	UP-M	UP-F	UP-M	UP-F						
	B	UP-U	UP-M	UP-F	UP-M						
S	A	UP-M									
	B	UP-F	UP-M	UP-M	UP-M	UP-F					
	C	UP-M									
T	A	UP-F	UP-F	UP-U	UP-U	UP-U	UP-U	UP-M	UP-U	UP-U	
	B	UP-U	Parasitized								
	C	PP-M	PP-M	PP-M	dead						
U	A	PP-F	UP-F	Parasitized	UP-F	Dead					
	B	Dead									
V	A	UP-M	UP-F	UP-M							
	B	Dead	PP-U	PP-U	PP-F	PP-F	UP-M	UP-F	UP-F		
W	A	UP-M	Parasitized	UP-M	UP-M	UP-M	UP-U	UP-F			
	B	PP-U	PP-M								
	C	Parasitized									
X	A	Parasitized	UP-F	UP-U	UP-M	UP-F					
Y	A	Parasitized	Parasitized	UP-M	Parasitized	Parasitized	Larva	UP-M			
	B	UP-F	Larva-U	UP-F							
Z	A	PB	PB	Parasitized	Parasitized						
	B	PP-F	PP-M	Parasitized	Larva	PB					
	C	PP-U	PP-U	UP-U	dead	UP-F	Parasitized				

---

[a] Nest R contained a third branch but brood could not be assigned positions

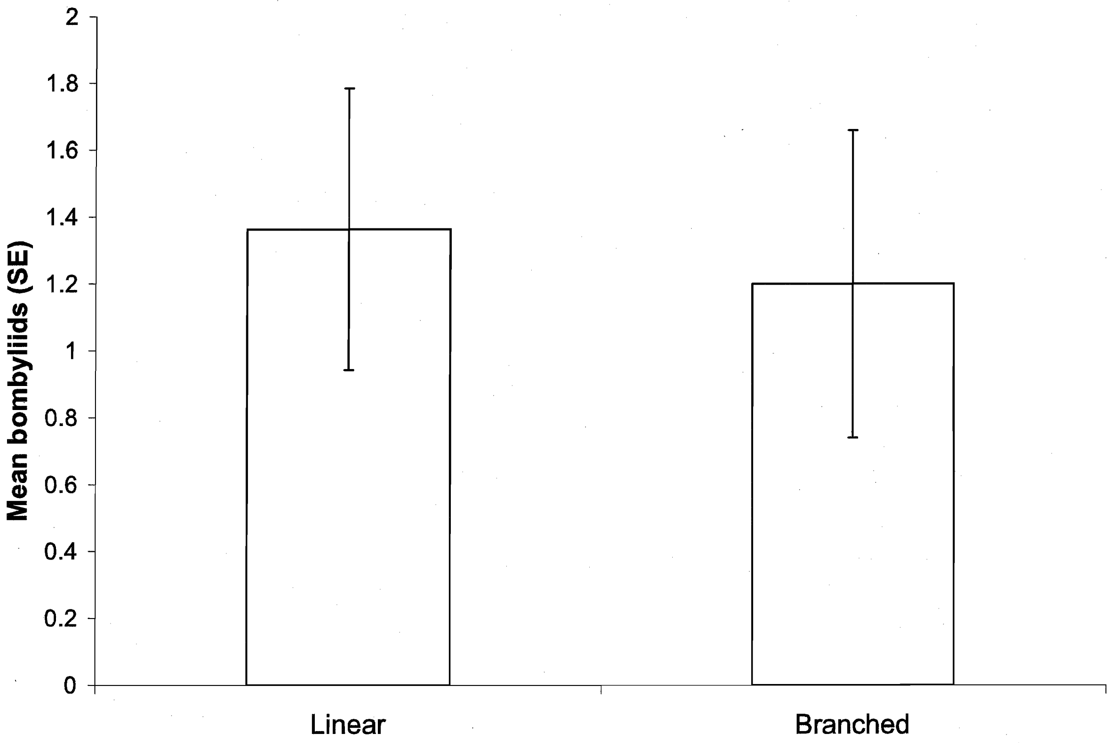


Figure 2.1: The mean number of parasites and standard error in linear nests (n=14) and branched nests (n=13). In both linear and branched nests, the minimum number of Bombyliids was 0 and the maximum was 5.

relationship existed between the number of flies and the total length of the nest (Regression: $F_{1, 25}=0.62$, $p=0.44$). Finally, there were no significant relationships between the position of a brood cell within a terminus and whether it was parasitized (Logistic Regression: $Q_{w1}=2.4$, $p=0.12$) (Figure 2.2, Table 2.1).

Relationship between nest architecture and number of females

The number of females occupying a nest was significantly greater in branched nests than in linear nests (Wilcoxon 2-sample test, $W=94$, $n=27$, $p<0.05$). On average, branched nests contained 3.3 ± 2.1 foundresses while linear nets contained 1.9 ± 1.0 females. Similarly, when Courtyard nests were examined by videoscope (see general methods), branched nests contained more females than linear nests (2.7 ± 1.4 in branched versus 1.3 ± 0.6) in linear nests (Wilcoxon 2-sample test, $W=472.0$, $n=27$, $p<0.001$).

I tested the hypothesis that nest architecture influences the number of foundresses using an ANCOVA model with the total length of the nest, the amount of space occupied by brood cells, the space not occupied by brood cells that could accommodate them and whether nests were branched or linear as predictors, and the number of foundresses as a dependent variable; interaction terms were not included. While the overall model was significant (ANCOVA: $F_{4, 18}=3.34$, $p=0.03$), none of the effects terms were significant (Table 2.2). When a similar analysis was performed replacing foundresses with the number of females that exhibited total wear greater than zero (see Chapter 1 for details), the model was not significant (ANCOVA: $F_{5, 15}=0.98$, $p=0.45$). Finally, to determine if females worked cooperatively to construct longer nests, which would have more room for brood cells, I tested the relationship between the number of foundresses with worn

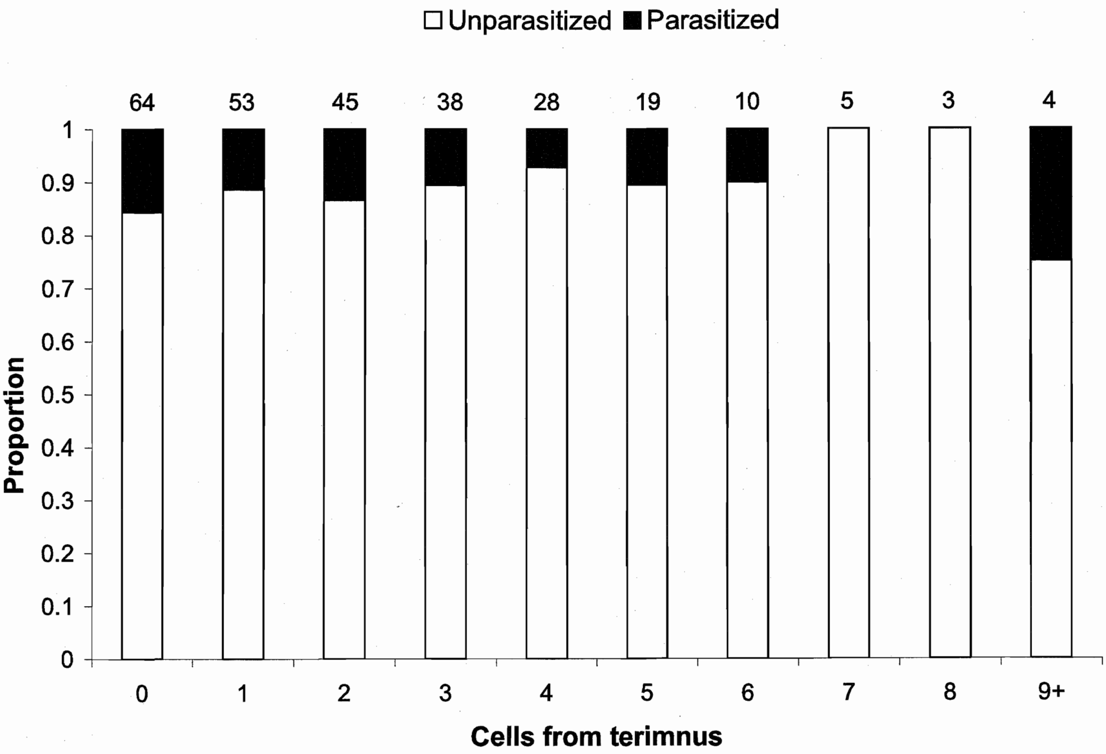


Figure 2.2: The relationship between parasitism and position within a nest. Cells from terminus (x-axis) was measured as the number of cells from the end of a branch farthest from the entrance gallery. The y-axis gives the number of parasitized cells in that position across all nests. Numbers above bars indicate the total number of cells in that position across all nests.

Table 2.2: The effects of nest architectural characteristics on the number of foundresses within a nest. Results are based on type III sums of squares.

Characteristic	F	p-value	df
Total length	0.16	0.7	1
Unused (empty) space	0.00	0.99	1
Length occupied by brood cells (log transformed)	3.59	0.07	1
Branched or linear	2.31	0.15	1

mandibles (those that constructed or renovated nests) and total length of a nest and again found no significant relationship (ANOVA: $F_{2,17}=0.23$, $p=0.8$).

Discussion

Brood development and nest architecture

Many authors have speculated on the developmental rates of *Xylocopa*, concentrating on the order and mechanisms of emergence (Gerling and Hermann, 1976; Rau, 1933; Skaife, 1952). Specifically, Gerling et al. (1978) claimed that *X. virginica* nests in Georgia have a mechanism that synchronizes the development time of individuals, but only presented a speculation as to the mechanism. In Niagara, individuals varied significantly in developmental stage, within and between branches; the oldest individuals were at the end of a branch. However, the order of brood stages within a branch was not perfect suggesting no synchronizing mechanism exists in Niagara populations. This finding also indicates that some variation may exist in developmental rate; although, it seems unlikely that individuals in the same nest would develop at substantially different rates. It is not clear if lack of synchronization results from lab rearing brood or if this is a population difference, but since it was apparent in nests collected late in the season it seems to be a natural phenomenon.

In *Xylocopa* that provision cells sequentially, it is not possible to access a previously provisioned cell without destroying the cell partitions built after it; although these cells could be rebuilt later as is seen in some species of *Ceratina* (Michener, 1974; Michener, 1990; Sakagami et al., 1977) and Halictini (Michener, 1974). In solitary cavity nesting bees, such as some species of *Osmia* (Bosch and Vicens, 2006) and *Ceratina* (Sakagami and Maeta, 1977) a pattern exists where cells are ordered by age within the nest; the oldest progeny are located at the branch's terminus. I observed the same pattern in *X. virginica*, which provisions cells sequentially, a single branch at a time, and did not appear to revisit cells once completed. However, I did detect some variation within branches, which is open to multiple explanations. Variation may be expected if pollen masses differ in nutritional value, and thus individuals develop at different rates. The variation observed in the age of cells within branches, may also indicate that eggs are occasionally replaced, since a replacement egg would be younger than those on either side of it. This scenario implies that cells are revisited, for which there is little evidence. If foundresses worked together to provision some brood cells, it might be seen as variation in the age of individuals within a branch; since cooperatively constructed cells would be built and provisioned more quickly and the individual would be older relative to individuals in surrounding cells. While unlikely, this is not incompatible with some findings in Chapter 1. In strictly communal species, I would expect to find multiple nest branches as is reported for *Xylocopa sauteri* (Iwata, 1964; Michener, 1969). I would also expect each branch to contain brood of approximately the same age in equivalent cell positions within a branch. This is because each female should be constructing and provisioning cells on her own, in a branch specific to her. Neither of these patterns was visible in *X. virginica*, and therefore brood age patterns do not support communal social organization in this species.

Relationships between nests and sex allocation

I did not detect any pattern in the allocation of sexes within brood cells with respect to position or laying order. There was neither a pattern with respect to nest, or within consecutive brood. My findings contradict those of Stark for *X. sulcatipe*s (Stark, 1992b) and Watmough's for the average of 15 *Xylocopa* species; both studies found female biased broods (Watmough, 1983). While Gerling and Hermman (1976) did not report sex ratios directly, they noted that nests of overwintering *X. virginica* from Georgia contained on average 4.5 ± 2.21 females and 3.4 ± 2.15 males. Since broods overwinter in their natal nest, this would suggest a female biased sex ratio. However, this number likely includes old foundresses (those attempting to overwinter a 2nd time) in addition to newly emerged brood. Hence, the number of females may be inflated. Similarly, while she did not give analyses, Steen reported data for two species of *Xylocopa* in the subgenus *Lestis* in which she showed nests with both more male brood and nests with more female brood (Steen, 2000). It is possible that sex ratio varies with species of *Xylocopa*; this may be related to social organization, differences in the relative "value" of males versus females in a given environment, or it may reflect seasonal variation.

Relationships between parasitism and nesting

The primary source of mortality in southern Ontario populations of *X. virginica* brood is bombyliid parasitism (Chapter 1). Nest architecture may influence parasitism rates. Packer (1988) compared mortality from parasitism between ground nesting sweat bees and trap nesting megachilid bees and concluded that mortality from parasites is higher in trap nesting bees. This contradicts Michener (1985) who suggested that ground-nesting bees should suffer greater mortality from parasites (Michener, 1985). In nests collected from the Farmhouse site, 41% of nests were parasitized which corresponds to the range observed in other twig nesting bees (40%-44%).

In multi-foundress nests of *X. virginica*, females do not appear to guard by plugging or blocking the nest entrance. It is possible that guarding instead is performed on a per branch basis with one female per branch; this would require extra space in termini to accommodate the guarding female. Dhaliwal and Kapil (1968) have suggested that extra space in nests of *X. fenestrata*, which nests in bamboo, is for purposes of nest defence (Dhaliwal and Kapil, 1968) and this has also been suggested for species of *Ceratina* (Daly, 1966). I have observed space that is not dedicated to brood cells or entrance gallery in nests of *X. virginica* from the Farmhouse population (Appendix 10). On the other hand, in Chapter 1, I demonstrated that multi-female nests do not benefit from reduced parasitism. Similarly, in a study of *Halictus ligatus* in southern Ontario, Packer found no difference in levels of parasitism for pleometrotic nests, which were guarded versus solitary nests (Packer, 1988). These similar results may suggest that, in general, guarding by a female is not an effective defence against bombyliid parasitism in *X. virginica*.

Relationships between nests and social structure

After the completion of brood provisioning and until broods eclose, much of the space within tunnels is occupied by brood cells. However, many nests have space that is not filled with brood cells. Some unused space is a function of transitional areas, and cannot be used especially in branched nests, while other space is within termini and presumably could be filled with cells (Appendix 10). It is possible that this seemingly empty space is necessary to accommodate additional foundresses. If a single female founds a nest, a branched nest may not be necessary until there are additional adult females to benefit from branches. However, there does not seem to be a relationship in which larger nests or those with more unused space are associated with more foundresses. Similarly, longer nests are apparently not the result of more females performing construction (more females with worn mandibles). This is an important observation in relation to division of labour as it implies that the additional females discussed in Chapter 1 do not benefit a colony by creating nests that can accommodate larger broods.

Numerous authors have discussed potential interactions between social behaviour and nest architecture (Gerling and Hermann, 1976; Michener, 1974; Michener and Kerfoot, 1967; Steen and Schwarz, 1998; Steen and Schwarz, 2000). Some have suggested that branched nests may allow for variable brood laying strategies, as females may choose to either lay in alternating termini or sequentially in a single branch at a time. Sequential brood laying may explain the larger broods seen in branched nests; using a single branch at a time may allow a foundress to work with less obstruction from other females than in linear nests.

Steen has suggested that both polymorphisms in provisioning strategies (laying sequentially vs. alternating between branches), and branched nests are related to social behaviour (Steen, 2000; Steen and Schwarz, 2000). In branched nests, workers can provision cells simultaneously, as in *X. sulcatipes* (Stark, 1992a). This is not possible in linear nests where at maximum two cells can be accessed at a time. In multi-foundress nests of *X. virginica*, a single female (or occasionally more) perform(s) most brood related tasks including nest construction (Chapter 1) (Gerling and Hermann, 1976; Rau, 1933). This form of task allocation would negate the benefits of alternating between branches, as only a single cell is provisioned at a time and thus order is less pertinent. It is possible that branched nests may be useful in reducing the number of interactions between foundresses, because additional foundresses can reside in those branches of a nest not being provisioned. This would reduce the number of passing events required and in turn the time required to deposit pollen. However, in observations of foraging, I found no difference in the total handling time per female for haplometrotic and pleometrotic nests (Chapter 3). Although mean handling time may be a better indicator, it still appears that branching does not influence how efficiently females handle pollen and construct cells. This indicates that unlike many wasp species, the efficiency with which *X. virginica* provisions cells is not influenced by nest architecture.

Conclusions

In this study, I have shown associations between branched nesting and the number of foundresses within a nest, but little evidence for an influence of nest architecture on task differentiation. I also found that broods are provisioned consecutively one branch at a time with no overall bias or sequence of sex allocation. Nest architecture data suggests that *X. virginica* does not have a communal social organization. Similarly, additional females do not appear to aid in nest construction. I conclude that branched nesting in *X. virginica* is advantageous, resulting in more space to accommodate brood and foundresses. However, nest architecture does not substantially influence the efficiency of provisioning brood or sociality.

Chapter Three: Annual variation in demography and activity of *Xylocopa virginica* in southern Ontario

Introduction

Ecological factors are thought to be a major force in the evolution of sociality within bees and wasps (Cronin and Schwarz, 1999a; Strassmann and Queller, 1989; Yanega, 1993). In addition to predation and parasitic pressures, many elements related to climate may influence the social structure of a colony or related behaviours. In the literature on primitively social bees, a vast amount of effort has been dedicated to studies of how social variation is related to environmental factors such as weather and length of season. These factors often vary with latitude (Packer, 1990; Sakagami and Munakata, 1972) or altitude (Eardley, 1983; Eickwort et al., 1996) and result in differing levels of sociality. Solitary behaviour is apparently more common in high altitudes and northern latitudes relative to increasing social organization at lower altitudes and more southern locations. It has been demonstrated that in warmer drier years primitively social sweat bees are less strongly eusocial than in colder, wetter years (Richards et al., 1995). Similar patterns have been proposed for species in the genus *Xylocopa* (Maeta et al., 1996). Specifically, local climatic and weather factors may influence sociality by limiting or increasing the time available for brood production and thus resource availability.

The members of the subfamily Xylocopinae are becoming increasingly popular in studies of primitive sociality. Studies on members of this subfamily demonstrate that at least three tribes (Ceratinini, Xylocopini and Allodapini) exhibit some form of sociality (quasi-social, semi-social or weakly eusocial) in addition to solitary and possibly communal behaviour. In some members of this group, weather influences brood development resulting in variation in social organization. In particular, studies of the allodapine bee, *Exoneura robusta*, demonstrated that both the timing and the duration of brood development varied among sites with differing climates (Cronin, 2001; Cronin and Schwarz, 1999b; Cronin and Schwarz, 1999c). Conversely, no such variation was seen in the related species *Exoneura angophorae* (Cronin and Schwarz, 1999b).

In many bees, foraging is a primary link between the environment and elements of life history such as brood size. Females must collect pollen to provision brood and in many species more pollen (Bosch and Kemp, 2004; Bosch and Vicens, 2006; Tepedino and Torchio, 1982) or pollen with more protein (Roulston and Cane, 2002) can lead to larger individuals. In other bees, pollen is allocated differently among sexes (Paini and Bailey, 2002; Tepedino and Torchio, 1982) or among females of different castes (Richards, 2004). Given that weather may affect a female's ability to forage, there is a potential for interactions between weather and the size, sex or morphology of broods. Packer has shown that in *Augochlorella striata* individuals from northern habitats forage less and contain fewer workers than the same species in more southern populations (Packer, 1990). Richards (2004) has shown significant influences of weather on foraging and consequently on brood sizes and social organization of *Halictus ligatus*. Finally, a study of the megachilid bee *Osmia pumila* illustrated associations between foraging and parasitism which indirectly link foraging to reproductive success (Goodell, 2003).

The genus *Xylocopa* (the large carpenter bees) has become common in studies of social evolution. *Xylocopa* is the sole genus in the tribe Xylocopini (Leys et al., 2002; Minckley, 1998), and is the largest, most speciose and most widely distributed of the Xylocopinae (Steen and Schwarz, 1998). Some species of *Xylocopa* exhibit facultative forms of sociality and sociality may be influenced by how long a female spends away from her nest or from limits in the availability of nesting substrates (Dunn and Richards, 2003; Hogendoorn, 1996; Hogendoorn and Leys, 1993; Hogendoorn and Velthuis, 1993; Maeta et al., 1996; Stark, 1992a). *Xylocopa* is widely distributed and varies in social organizations, which makes it a good group for studies of how intra and interspecific variation in demography, phenology (life-cycle) and activity such as foraging influence sociality.

X. virginica is one of the two species of *Xylocopa* in eastern North America, and is the more widely distributed; its range spans from Florida to southern Ontario, west to Texas and the Mississippi River. Like most *Xylocopa* species, *X. virginica* creates nests by burrowing into wood. Nests may be linear or branched and typically are in softwood structural timbers (Chapter 2). Female behaviour has been extensively studied three times. Gerling and Hermann (1976) reported on the behaviour and life history of this bee in Georgia in a single season, Rau reported anecdotally on the behaviour and life history of this species in Missouri (Rau, 1933), and this thesis provides a detailed study of a southern Ontario population.

In this chapter I: (1) describe the phenology of this bee in the northern most extent of its range, (2) determine whether and how phenology varies among seasons, (3) examine relationships between climate and phenology, (4) assess the influence of climate on population size, (5) examine seasonal and annual variation in foraging effort, and (6) examine annual variation in morphological size. I predict that southern Ontario presents a shorter overall "bee season" (the period in which *X. virginica* is active), with a breeding season that has more cold and wet days (resulting in fewer suitable days) than most *X. virginica* habitats, and that this results in the season starting later and ending earlier. I also predict that annual variation in weather will influence foraging effort and that females may alter foraging effort over the course of the season. Both of these are expected if females respond to loss of "good bee days" by increasing effort on other days. Finally, I expect that broods provisioned in cold and wet years will be smaller in size and in number of individuals.

Methods

Study sites

Most observational data are from the Courtyard site (see General Methods). Destructively sampled nests were collected at the Brock Farmhouse site in 2003 and at an additional location in Port Dalhousie, St. Catharines, Ontario in 2006. The Port Dalhousie nests were all contained in a single pine board collected from a picnic pavilion which was otherwise surrounded by a large lawn and a forested area. These nests were not examined prior to their collection in 2006.

Phenology and life cycle of *Xylocopa virginica*

In any single year, I used all pieces of available data to establish dates for events in the *X. virginica* life cycle. This was necessary because not all data were available in all years. Specifically, nests were only collected in 2003 and 2006, videoscope observations were not available in 2003, and most data from 2002 were derived from a pilot study. In 2006, the courtyard population was the subject of another study (Peso, M., 2008, MSc thesis) which involved trapping bees at nest entrances, making some data unavailable. Finally, data were available from biodiversity sampling carried out between

2003 and 2006 on the Brock University Campus and at the contiguous Glenridge Quarry Restoration Park (Rutgers-Kelly A., 2005, MSc thesis), but the availability of these data varied among years.

In every year of this study, data were available from individuals that I marked and from observations at nest entrances (although these were limited in 2006); full details of protocols are provided in the general methods. Briefly, starting in early April, the area around and below nests at the Brock Courtyard site were inspected daily for indicators of activity. These indicators included the presence of fallen sawdust, debris (old pollen, cell partitions) and ejected dead pupae. At the same time as these "spot checks", field notes were made indicating temperature, precipitation, general bee activity (or lack of activity), and other evidence of activity.

Demographic indicators of phenology

Many demographic measures are associated with phenology. These include when individuals pupated, and when they were marked. Since an individual female must provision a cell before oviposition, the developmental stage(s) of brood represent the time since the brood was provisioned. If foundresses began foraging at the same time in different years, then assuming similar developmental times, brood in their nests would be at approximately the same developmental stage at the same point in a given year. I compared developmental stages of brood from nests collected 19-July 2003 at Farmhouse with nests collected 19-July 2006 at Burgoyne Woods. The contents of cells were classified as pollen ball, larva or pupa and the frequencies in 2003 were compared with those from 2006 using a chi-square test of independence.

The timing of some events can also be inferred from data from marked bees. Since individuals were marked immediately after they were first observed, the number of individuals marked on a particular day reflects the number of individuals active that day. Further, the cumulative number of individuals marked on a given day reflects the number of active bees in the population on that day, and thus overall. In every year from 2003-2005 all individuals were marked according to the protocol in the general methods. In 2006, individuals were marked in association with another student (Peso, M., 2008, MSc thesis) and according to a different protocol. I examined the cumulative number of marked individuals at different points in the season in each year as an indicator of seasonal and annual patterns of emergence and population size. This is discussed further in Chapter 4.

Analyses and quantification of weather and climate trends

Sources of data

Climate data were obtained from Environment Canada meteorological stations in the proximity of Brock University. Data for 2002, 2003, 2004 and part of 2005 were obtained from the weather station at Port Weller, Ontario (WMO ID-71432, 43°15' N, 79°13'W); this station is located 14.3 km from Brock University. Data from this station were not available from October 2005 through September 2006, so data from the Niagara Falls station (43°1.800' N, 79°4.800'W) were used for this period. Port Weller is 79 m above sea level, whereas the Niagara Falls station is 182.90 m above sea level.

Historical climate comparisons were made using the 30-year normal (mean) because it is the internationally recognized and used measure (Guttman, and Plantico, 1987). Thirty-year climate data were retrieved in summarized form from the

Environment Canada online database of Canadian climate normals. The nearest weather station for which historical data were available was the St. Catharines A weather station (43°12.00' N, 79°10' W), 12.1 km from Brock University and 98.1 m above sea level. Since raw data were not available at the time of retrieval, some variables were not available for analyses. The summarized weather data are available from http://www.climate.weatheroffice.ec.gc.ca/climate_normals/index_e.html.

Weather and climate trends

Since many bees can (or do) not fly when the temperature is too cold (Willmer and Stone, 1997) or is too warm (Cooper et al., 1985; Roberts and Harrison, 1998), (but see Abrol, 1992) temperature is likely to influence daily activity patterns. Temperature can be represented by the mean daily, mean daily minimum and mean daily maximum temperatures and heating or cooling degree-day accumulation for a given year. Degree days in a time period are calculated as the difference between a reference value and the average outside temperature and are indicative of the overall temperature patterns throughout the season. In all analyses, degree-days were calculated using 18 °C as the reference temperature. Bees in southern Ontario were rarely active below 14 °C, so degree-days were also calculated based on this value. There was no noticeable difference in patterns or results between 14 °C and 18 °C, so only 18 °C degree-day calculations were used in analyses.

Since many bees including *X. virginica* are not active when it rains, measures of precipitation reflect days on which a bee was inactive. *X. virginica* females do not forage in rain, and on many occasions, observations ended early due to rain during the observation period. On three documented occasions, females did not forage for a second day following significant precipitation. Anecdotally, this suggests that precipitation is a particularly important weather factor. I consider precipitation both as the total precipitation in a season and as the number of days with precipitation.

Bee season weather varied among the five years covered by this study and relative to the 30-year mean (Table 3.1), with respect to both precipitation (Figure 3.1) and temperature (Figure 3.2). In order to better classify and group years with respect to weather variation, I conducted a principal component analyses (PCA) on several measures related to temperature and precipitation. Since the maximum number of variables that can be included in PCA cannot exceed the number of data points (in this case years), PCA was repeated using different combinations of weather variables to determine the combination that explained the most variation. These combinations were based on seven climate traits: (1) mean daily temperature, (2) mean maximum daily temperature, (3) mean minimum daily temperature, (4) total cooling degree-days, (5) total heating degree-days, (6) total precipitation, (7) total number of days with measurable precipitation.

After all combinations were tested in the PCA, the variables that explained the most variability, and that were retained included: mean daily maximum temperature, mean daily minimum temperature, total cooling degree-days, total precipitation, and total days with measurable precipitation (Table 3.2). PCA was performed on data from two periods; the first analysis was based on the spring and early summer (April-July). These months were chosen under the assumption that spring weather is more likely to influence phenology. The second analysis included all months in which bees were active. This period was termed the "bee season" and was defined as 1 April to 30 September. Both analyses gave similar results and so only bee season analyses are reported.

Table 3.1: Summary of weather conditions for the *X. virginica* active season (April-September) in St. Catharines, Ontario for the years 2002-2006 and the 30-year mean (1971-2000). For 30-year normal temperatures, the average extreme is presented; the absolute extreme for the period (1971-2000) is presented in brackets. Bolded weather conditions are those included in the final principal components analysis (Table 3.2).

Weather condition	**2002**	**2003**	**2004**	**2005**	**2006**	**30 year normal**
Mean daily temperature						
Maximum	21.5	19.4	19.9	21.5	22.5	21.7
Minimum	13.6	12.0	12.6	13.9	12.2	11.2
Warmest temperature achieved	33.2	31.5	30.4	33.6	36.5	29.7 [37.4[a]]
Coolest temperature achieved	-1.6	-5.2	-3.8	0	-3	4.2 [-9.1[b]]
Total cooling degree-days (18°C)	489.6	314.1	256.5	529.5	282.5	328.4
No. of days with ppt>0 mm	52	80	67	51	73	65.8
Total precipitation	74.1	80.0	97.6	54.3	67.6	77.8
Number of "good bee days" [c]	89	81	105	79	110	NA[d]
Comparison to 30-year mean	Average, Warm	Wet, Cold	Wet, Cold	Dry, Hot	Dry, Warm	

---

[a] Warmest day on record, July 1998

[b] April 1982

[c] Days with no precipitation and temperature greater than 14°C

[d] The necessary daily information for calculating this was unavailable

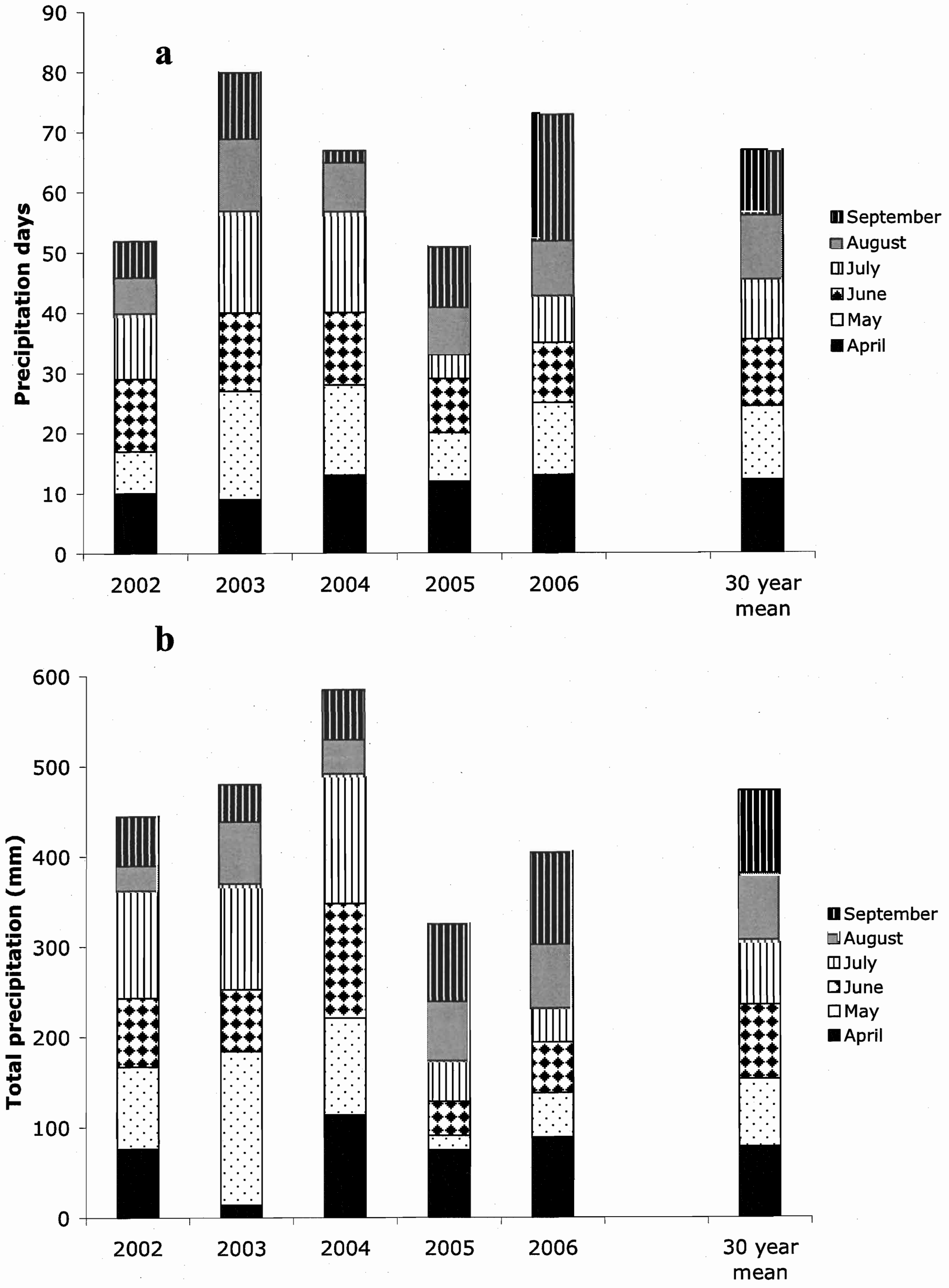


Figure 3.1: The total number of days with precipitation **(a)** (ppt>0 mm) and the total precipitation **(b)** for the *X. virginica* active season (April-September 30), in 2002-2006. Data for thirty-year means (1971-2000) were obtained in summarized form from Environment Canada and included no more than two consecutive missing years.

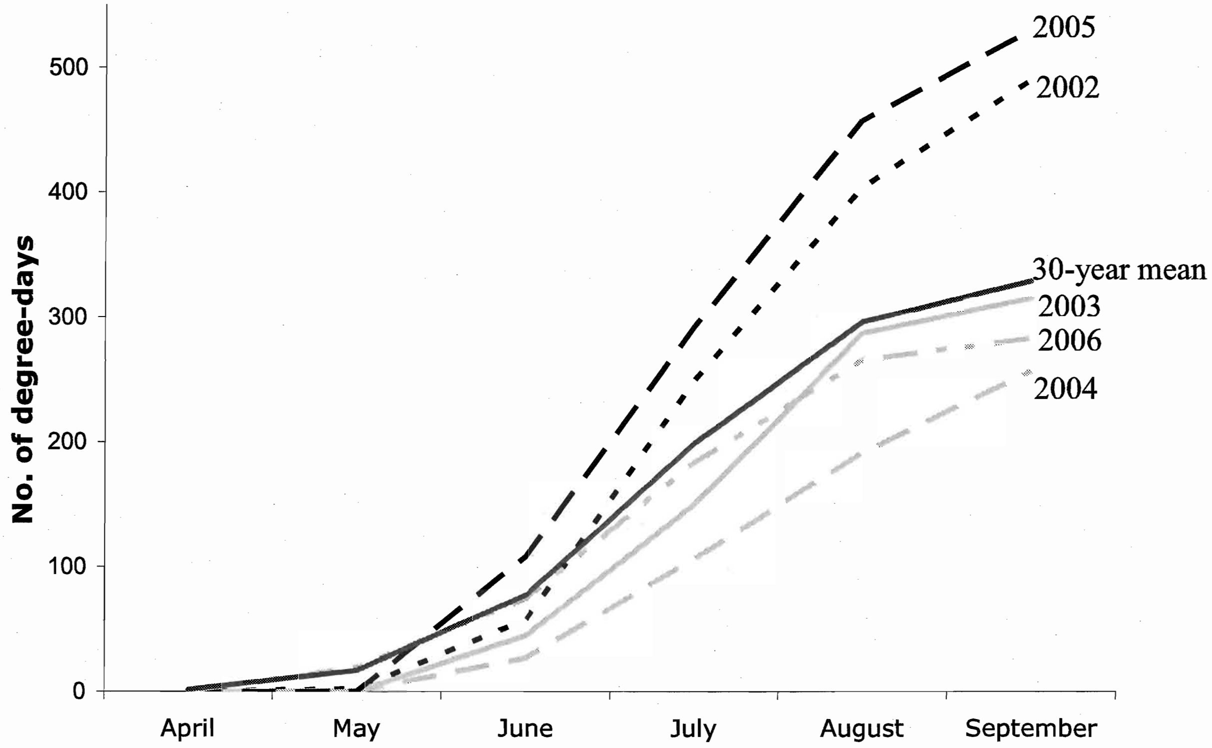


Figure 3.2: Cooling degree-day accumulation from 1 April to 30 September for the five years of this study and the 30-year mean (solid black line). Degree-days were calculated from base 18°C. Grey lines indicate years colder than the 30-year mean, broken black lines indicate years warmer than the 30-year mean. The coolest year of this study was 2004; the warmest year was 2005.

Table 3.2: Factor loading values for the first two principal components of variables related to weather. Loading factors scores ≥ 0.70 were considered significant for defining a principal component. Analysis is based on entire bee season (April-September). Bolded values indicate those treated as statistically significant.

Variable	**Factor value** **1st principal component**	**Factor value** **2nd principal component**
Total precipitation	-0.88413	-0.26794
No. rainy days	-0.81033	0.56649
Maximum temperature	0.84131	0.24562
Minimum temperature	0.95039	-0.53866
Cooling degree days	0.74281	0.59491
Eigenvalue	**3.75259903**	0.9711696
Percent Explained	**72.0**	21.0
Cumulative Percent	**72.0**	94.0

PCA reduces all the correlations among many variables into fewer variables known as principal components (PC or factors) each of which is independent and explains some percentage of overall variability. Usually, a PC is considered significant if it has an Eigenvalue greater than 1 (Zar, 1999). The extent to which a single variable contributes to a PC is represented by the factor loading score of that variable (Zar, 1999); usually a score greater than 0.70 is considered significant (Dillon and Goldstein, 1984). In the weather analyses, a single PC was found to explain 72% of all variability and so this single variable (PC1) was used to represent weather (climate) in all further analyses (Table 3.2). Plotting the value of the first principal component against year demonstrated that the years 2003 and 2004 grouped together as wet and cool, 2005 and 2006 were hot and dry, and 2002 was slightly warm and dry (Figure 3.3). Results were similar for a PCA that included all years of this study plus the years 1971-2000 (Appendix 5).

Relationships between weather and bee phenology

In order to test for relationships between weather and life history events, a series of linear regressions was conducted. Each model tested whether the date on which an event occurred was associated with weather. In each model, PC1 (weather) was entered as a predictor variable. The date of the event being examined was the dependent variable and was measured as the number of days since January 1 of the same year. January was chosen as it was unequivocally outside the "bee season" and was intuitive as the start of a year. These analyses were also performed replacing date with the ranked order in which events occurred among years; these analyses gave similar results. Events were only examined if at least three years of data were available.

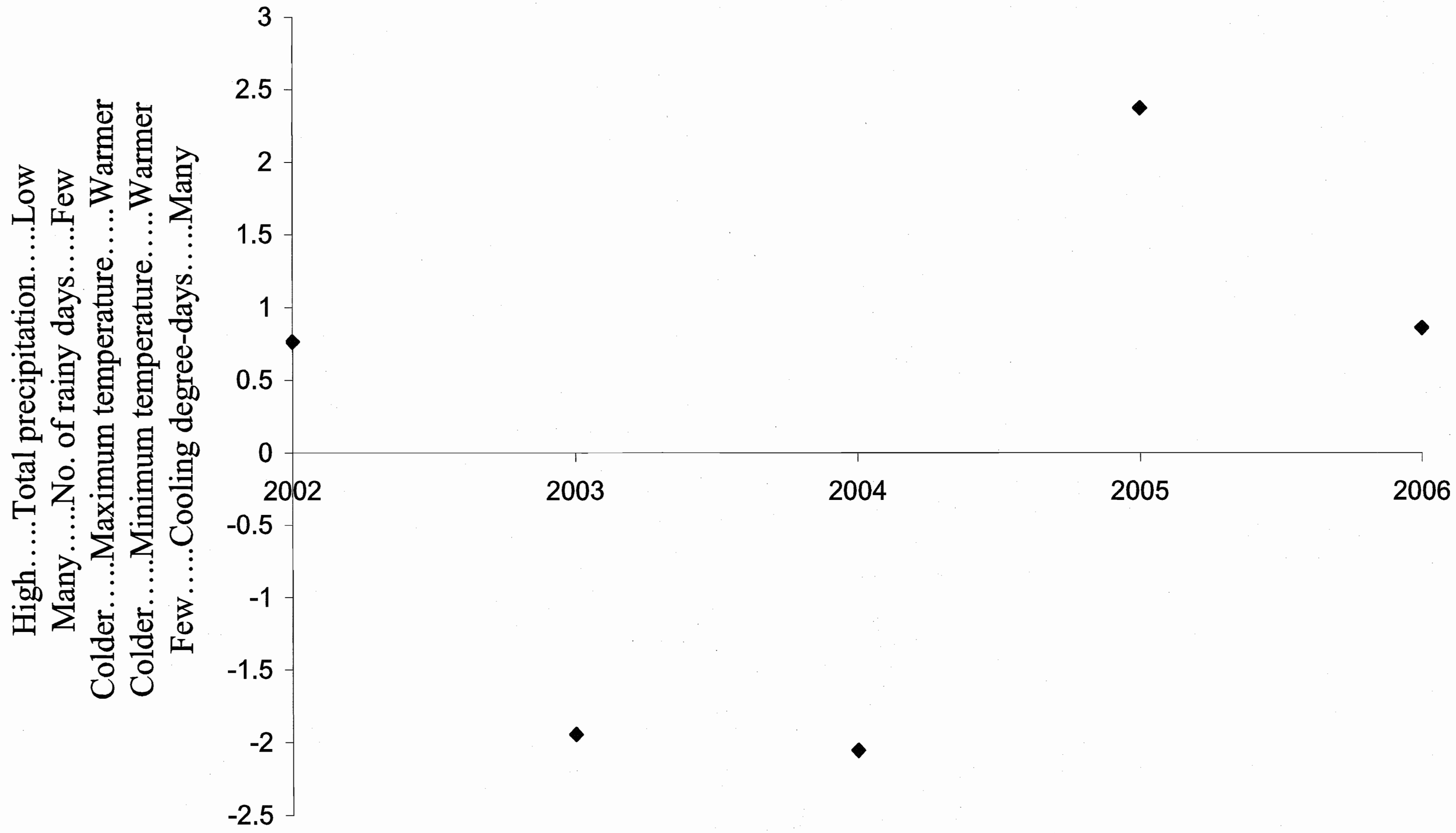


Figure 3.3: Positioning of the first principal for the entire bee season (April-September) in each of 2002 through 2006.

Evaluation of female foraging effort

In order to examine variation in foraging effort for *X. virginica* females, I observed females entering and exiting nests at the Courtyard site in 2003, 2004 and 2005. Preliminary observations suggested that females rarely begin foraging prior to 0930 h (observed twice during all applicable observations in three field seasons) and rarely foraged after 1700 h (3 observations in 3 field seasons). Observations were therefore limited to 6-hour periods, which should be representative of a full day's activity while keeping observation time consistent. In 2003, observations were made on 21 days, in 2004 on 11 days, and in 2005 on 16 days; for a total of 288 hours of observation.

During observations at nest entrances, the identities of all females entering and exiting a nest were noted. In addition, the presence of pollen on the female's legs and the time she entered or exited the nest was also recorded. If a female carried pollen on any return trip for a particular day, she was behaviourally like a forager for analyses for that occurred on that day. Females that entered or exited a nest but did not return with pollen on any trip for that day's observations were excluded from analyses. The following measures of foraging effort were calculated for each forager observed on each day: the number of foraging trips, mean flight time per trip (average time between departure and arrival), mean turnaround time per trip (time between arrival and departure), total flight time per day (mean flight time multiplied by the number of trips), total turnaround time per day (mean turnaround time multiplied by number of trips), and total provisioning time per day (time from first sighting to last sighting). Number of trips equalled one with only a single sighting; all other foraging data calculations required at least two sightings in a single day. The flight time variable assumed that females returning with pollen had spent most of their time away from the nest collecting nectar, pollen or both, so flight time should reflect time spent foraging. Similarly, the turnaround time variable assumed that a female spent some or all of the time in a nest between trips depositing pollen; turnaround time thus includes handling time.

Statistical analyses of foraging effort

Foraging effort was tested using a General Linear Model (GLM) in SAS 9.1.3 with the model: foraging effort = Date + Year + Date*Year. In this model, the date effect described variation within the season while the year term represented variation among years. Data were adjusted to account for differences in the start of the foraging season among years by adding nine days to date values in 2003 and 29 days to 2005. The values used in adjustments reflect the difference in the number of days between the first observations of a female in each year. Inspection of the data revealed one instance in each of 2003 and 2004 in which a female was observed, and suitable data were collected, on multiple days. In 2005, there were three confirmed observations and one possible observation of the same female. In handling these repeat observations, the 1st day of observations was retained and observations on subsequent days were discarded. Thus, each female is represented in analyses only once. Since some violations in assumptions of normality were found, nonparametric analyses based on ranks were also performed; however, most variables were normally distributed and all methods gave similar results, and so parametric data are presented. Analyses are reported as type I (sequential) sums of squares, as it was desirable to examine seasonal effect prior to annual effects. To adjust for potential non-independence, all p-values were corrected using the conservative Bonferroni method.

Annual variation in morphological size

The size of bees is known to be associated with the amount of provisions provided to brood: offspring provided larger pollen balls will become larger adults. Weather conditions may influence foraging, pollen availability and the quality of available pollen. Therefore, I compared physical size of both males and females among years, to look for effects of weather on foraging in the previous year. Size comparisons were based on head capsule width (HW) as it was the only morphological variable measured for marked bees in all years of this study (see general methods). I tested differences in size relative to year via ANOVA with a model that treated year as a categorical variable and ranked HW as the dependent variable. Ranks were used because HW was not normally distributed for either sex. Differences among years were further examined using Scheffé post-hoc tests.

Results

Phenology and life cycle of *Xylocopa virginica*

The annual life cycle for *X. virginica* in southern Ontario is summarized in Table 3.3. The season typically began mid-April after two to three consecutive days of temperatures greater than 14°C. The initial indication of seasonal activity was when debris began to appear below the entrances of nests; debris consisted of old brood partitions, dead adults and pupae and occasionally mouldy pollen balls. Nest renovation and construction activities began 1 to 4 weeks after nest debris appeared. This was easily recognized as sawdust began to appear below nest entrances and could be seen in observations with the videoscope. Activity was first observed outside the nest as a female crawling out of a nest and walking on the wooden surface for a short time (approximately 5-15 minutes); no flight occurred.

Following the start of nest construction, males were seen flying around nesting aggregations, and shortly after, they established territories (Table 3.3). Males maintained territories for three to four weeks (Chapter 4), and usually disappeared after females began provisioning brood cells (Pollen Foraging I in Table 3.3). Mating attempts began within a few days of the first female flights and continued until males disappeared from the study site.

The first females were observed flying in late April or early May; however, females did not appear in sweep samples from fields near Brock University until June. Females were first observed carrying pollen in mid-to late May, and generally appeared to have finished provisioning brood by early July. Following the first foraging and provisioning period, activity decreased and remained low until late July or August. After the lull of activity, a second period of pollen collection began (these patterns can be seen in Figure 1.17); this period followed observations of larvae and pupae in nests, and coincided with video observations of teneral individuals. All nest construction, mating and foraging activity usually concluded in August or September by which time all broods had eclosed. Old and new females, in addition to new males, overwintered together in the same nests. The number of females (overwintered foundresses and newly emerged females) in a nest varied over a year; however, the median number of females per nest remained above one individual (Figure 3.4).

Table 3.3: The date of important events in the *X. virginica* life cycle in 2002-2006. All dates are based on behavioural observations, and recordings of the presence of nest debris. Data from 2003 also include nest dissection data. Data from 2004, 2005 and 2006 are also based on videoscope observations. Question marks indicate no data available for that year.

Event	2002	2003	2004	2005	2006
First day above 14 °C	12 April	17 March	5 March	31 March	10 March
Appearance of dead bees and nest debris	?	20 April	17 April	10 April	19 April
Sawdust appeared below nests	?	14 May	12 May	15 April	28 April
First female flight observed	6 May	20 April	30 April	19 April	10 April
Male emergence	19 April	18 April	30 April	19 April	19 April
Male territories established	?	18 May	10 May	10 May	1 May
First mating attempted	28 May	23 May	10 May	10 May	8 May
First female collected in fields	?	12 June	14 June	21 June	?[a]
Pollen foraging I	28 May to 25 June	26 May to 7 July	20 May to 8 July	10 May to 18 Aug	?[b]
Last males guarding territories	18 June	30 June	21 June	20 June	13 June[c]
First larvae found[d]	?	20 July	?	22 July	6 July
First pupa found[d]	?	8 Aug	?	22 July	19 July
Pollen foraging II[e]	?	30 July to 25 Sept	? to 15 Sept	? to 29 Aug	?[b]
Last female observed near courtyard	5 July	15 July	15 Sept	22 Sept	8 Sept
Last female in pan and sweep samples	?	16 Sept	16 Sept	29 Aug	?[a]

---

[a] Collections were only via pan traps, and no *Xylocopa virginica* were caught

[b] Individuals were trapped at nest entrances preventing determination of a date

[c] Male was captured at nest entrance so territoriality cannot be confirmed

[d] Nest dissection or videoscope

[e] Sweeps and flower associations

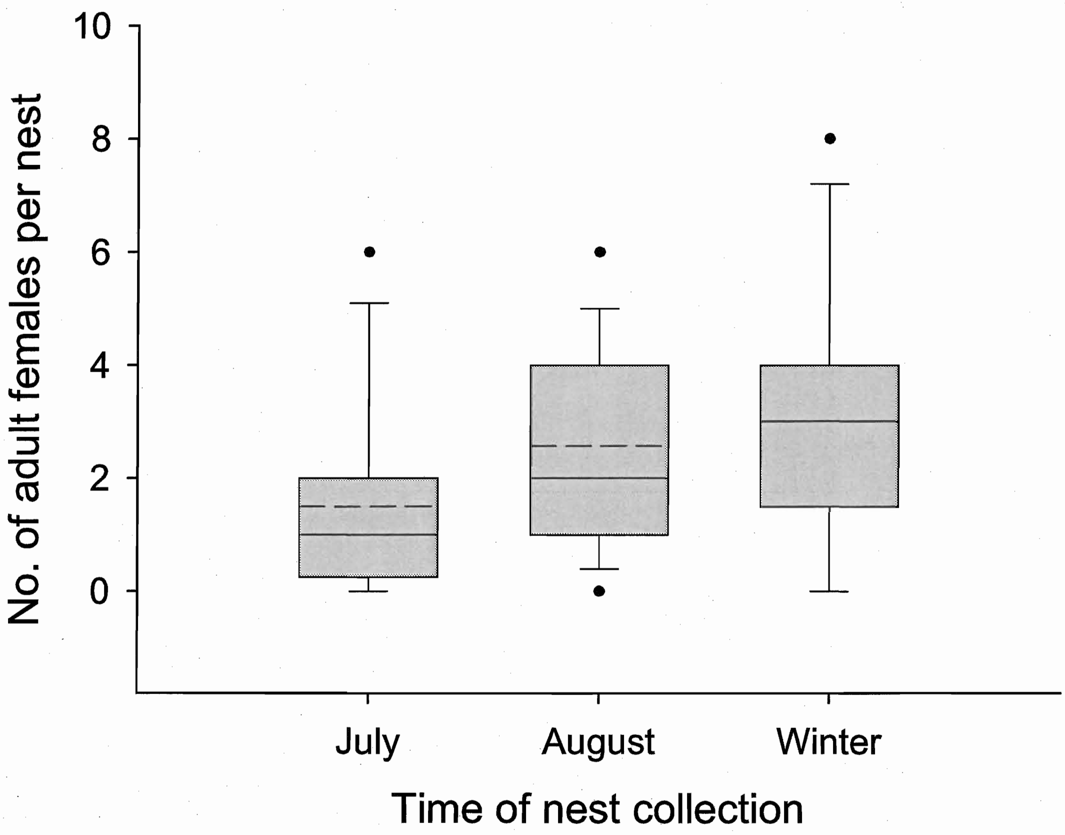


Figure 3.4: Seasonal variation in the number of adult females per nest in destructively sampled nests. Boxes indicate first and third quartiles, tails indicate 90th and 10th percentiles, dots indicate maximum and minimum values, dashed lines indicate median and solid lines indicate the mean. Missing points indicate not enough values to calculate. Values are for all years combined. The winter category includes one nest collected in December 12, nests collected in January, and one nest collected in March. July and August females are overwintered foundresses, mainly born the previous year. Data are based on all nests collected at Farmhouse and Burgoyne Woods, and 15 additional nests collecting in winter in various locations in St. Catharines, Ontario.

Demographic indicators of phenology

I also found variation in demographic indicators of timing. Brood developmental stage and the timing of emergence reflect phenology and timing of life cycle events. As can be seen in Table 3.3, the timing of many events in the phenology of *X. virginica* varied among years, although the order in which events occurred generally did not.

I compared the developmental stages of broods from nests collected on the same date (19 July) in 2003 and 2006 to determine if broods were at similar developmental stages. If the developmental rates and the start of provisioning were similar in 2003 and 2006 then developmental stages would have been similar between these two years. In 2003 development was significantly delayed compared to 2006 (Figure 3.5); nests collected on 19 July 2003 contained fewer pupae and more pollen balls that those collected on 19 July 2006 (Chi-square: $\chi^2_1=21.88$, $p<0.01$). These results suggest an effect of weather (rainfall and possibly temperature) on developmental time or the start of provisioning.

Since an individual can only be marked if it leaves a nest, and since bees were marked at the first opportunity, the number of bees marked in a year reflects the timing of some events in that year. The number of bees marked on a particular day reflects the number of new bees flying that day, while the first day on which bees were marked reflects when bees became active for the season. The point at which the cumulative number of bees reached an asymptote reflects the point at which new bees were no longer becoming active. The 2006 season was the earliest of the years studied; females were marked earlier (30 April) and the cumulative number of females reached an asymptote earliest (Figure 3.6). The 2003 season started the latest of all years with no females marked until after 20 May and new females appearing until late July. Male activity also began earliest in 2006 (3 April) and latest in 2003 (13 May) (Figure 3.6).

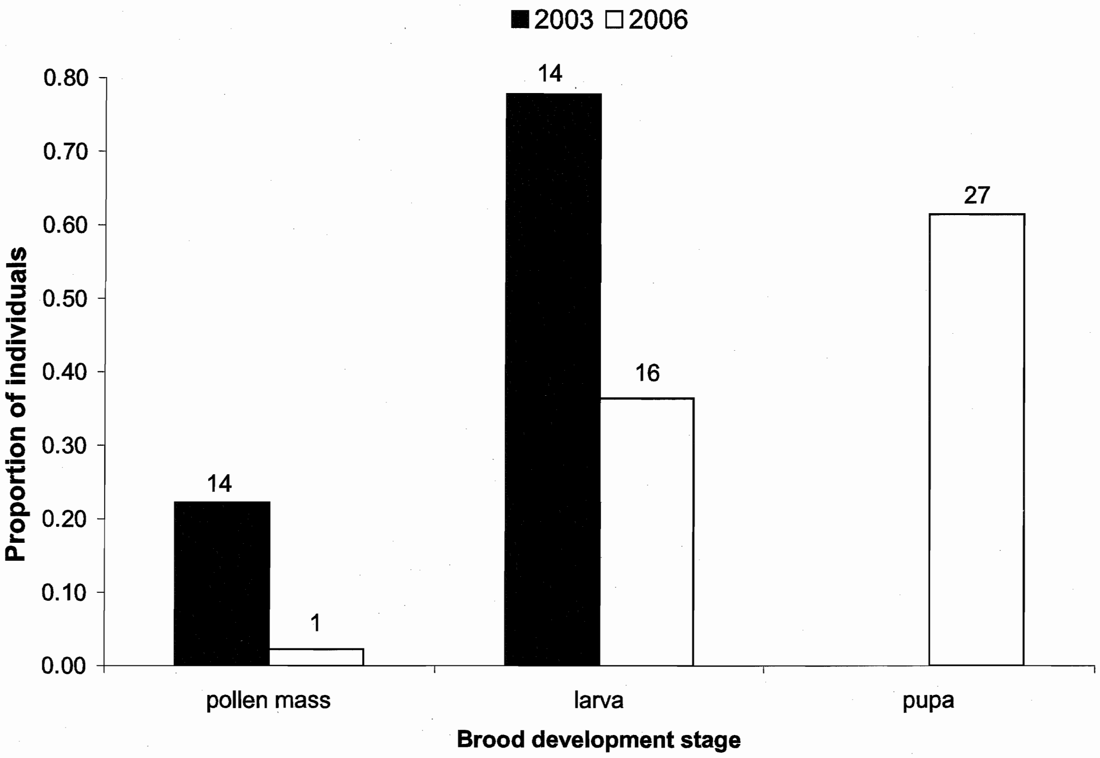


Figure 3.5: The developmental stage of individuals from nests collected on 19 July 2003 (black bars, n=4) and 19 July 2006 (white bars, n=3) in St. Catharines, Ontario. Numbers above bars indicate sample size (number of individuals). The distribution of individuals from 2003 was significantly different from 2006 (Chi-square: $\chi^2_1=21.88$, $p<0.01$). Despite collection at identical points in their respective seasons (chronologically), individuals from the 2006 brood were generally older.

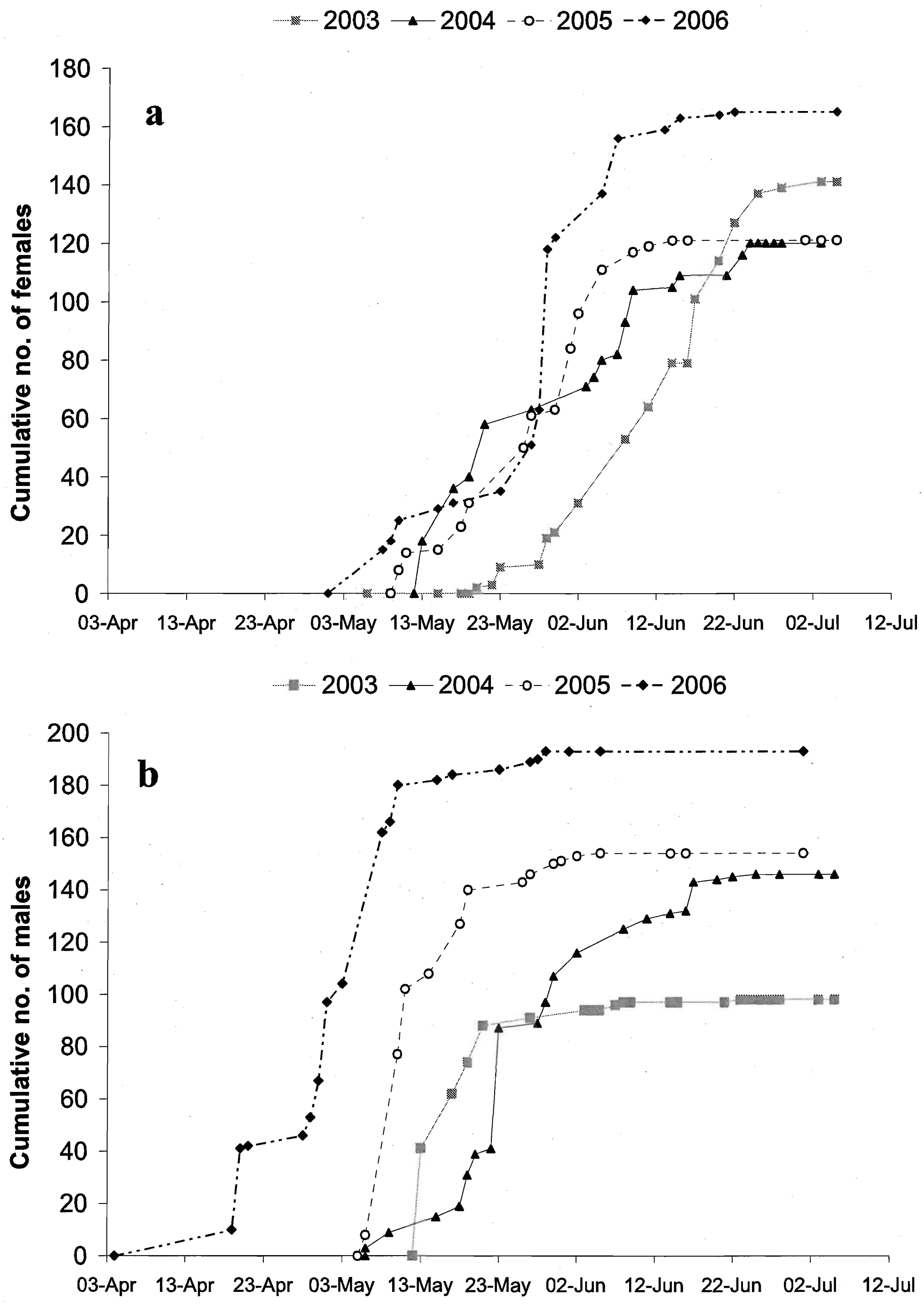


Figure 3.6: The cumulative number of females **(a)** and males **(b)** in the courtyard site for the years 2003 through 2006.

Weather conditions can influence the number of days a female can forage and consequently the amount of pollen she can collect and the size of her brood. If weather conditions are poor early in a season, a female may begin foraging later, which could also result in smaller broods. Since broods laid in one summer will become the individuals marked in the next summer, the number of marked individuals should reflect a proportion brood size the previous summer (when individuals were laid). These patterns were examined in the context of weather patterns as explained by the PCA (Figure 3.3), which indicated that 2002 was average (warm in 30-year PCA, Appendix 5) temperature and slightly wet, 2005 and 2006 were warm and dry, and 2003 and 2004 were cool and wet. Thus, the population abundance appeared greatest in 2003, even though it was a cold and wet year (Figure 3.3), but 2002 was an average year and only slightly wet. Conversely, the fewest individuals were marked in 2004, which followed the cool, wet 2003 season (Figure 3.3). These patterns suggest that brood size may be influenced by weather and that poor weather conditions lead to smaller broods (in number of individuals).

Spring timing and weather may be associated with nest construction and re-use. A female must potentially choose between using an old nest (if available), constructing a new nest, joining another nest or waiting until the next season. This decision likely reflects a trade off between nest construction and foraging. Good weather conditions will result in more time for foraging and nest renovation than in years with poor weather conditions. Thus, in good years more new nests should be constructed as the time expenditure is less costly. In the Courtyard site, both the number of new nests constructed (Figure 3.7) and the number of existing nests that were unused (Figure 3.7) varied among years. The most new nests were constructed in the 2004 season (19 nests); however, in 2003 a greater proportion of active nests were newly constructed. Linear

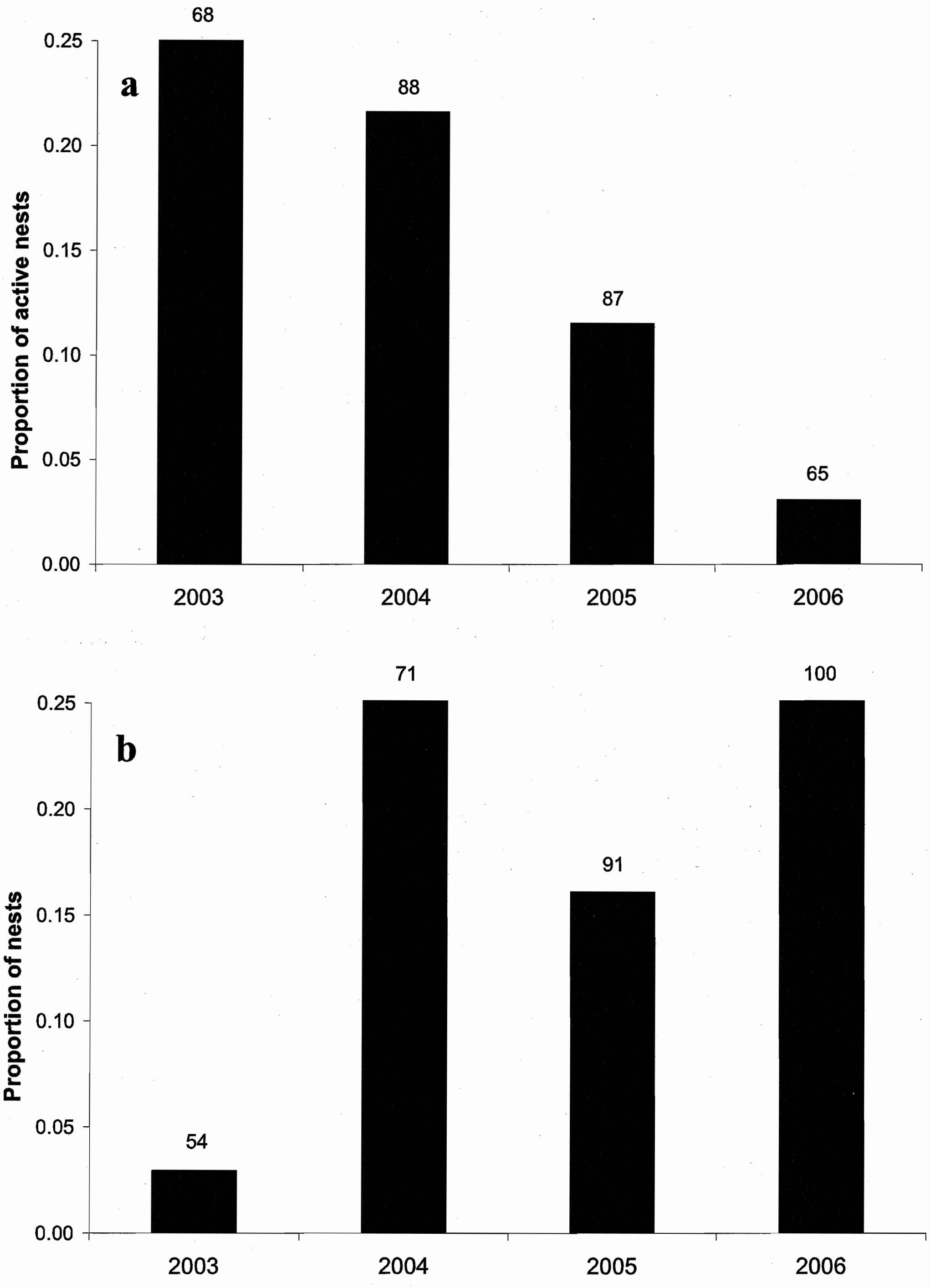


Figure 3.7: The proportion of active nests (those inhabited by *X. virginica*) at Courtyard that were newly constructed **(a)**. The total active nests are given above each bar. The proportion of existing (newly constructed excluded) nests that were unused **(b)**. The total nests minus new nests are given above each bar. For both a and b, numbers above bars give denominator of proportion.

regression of weather data (PC1) versus the number of newly constructed nests in a year (Regression: $F_{1,3}$=5.69, p=0.14) or the proportion of newly constructed active nests (Regression: $F_{1,3}$=12.93, p<0.07), demonstrate no significant relationships between weather and nest construction.

Relationships between weather and bee phenology

Weather factors have the potential to influence the order and timing of events in a species' life cycle. Temperature is likely to influence when a bee starts activity and both temperature and precipitation affect the probability of a female foraging. Therefore, if a given year is warm earlier than usual, bees may begin activity earlier than normal. Conversely, if a season is particularly cold or rainy, some activities may be delayed or last for shorter durations. I examined potential relationships between weather and phenology via linear regression of the date on which an event occurred and PC1. Each life cycle event (those in Table 3.3) was examined in a separate model. The start of nest construction was the only event significantly related to PC1, and occurred earlier in the warm dry years of 2005 and 2006 (Table 3.4).

Evaluation of female foraging effort

I compared variation in foraging effort within years (seasonal differences) and among years (annual differences) to determine if weather patterns influenced foraging behaviour. I calculated various measures of foraging effort including flying time, turnaround time and number of trips (Appendices 7-9).

Table 3.4: The results of linear regressions examining the relationship between the dates (no. of days since 1 January) on which each phenological event occurred and weather for that year (PC1). Each event was examined in a separate analysis. Significant effects are in bold.

Event	F	df	p-value
First nest debris found	4.96	3	0.16
Nest construction begins	181.87	3	**0.0055**
First male territories established	0.74	3	0.48
First males emerge	0.31	5	0.36
First female observed	1.16	4	0.36
First mating attempt observed	0.19	4	0.69
Last female observed	0.44	4	0.56
Last male observed	0.97	3	0.43

Total provisioning time varied significantly both within and between years (Table 3.5). Turnaround time showed a significant effect between years. However, the model was not significant when the p-value was adjusted. All significant trends were due to 2004, which showed longer turnaround times, and longer (although not statistically significant) average flight times. Scheffé's post hoc analyses demonstrated that differences in turnaround time were significant only between 2003 and 2004. Total provisioning time also differed only between 2003 and 2004

Annual variation in morphological size

Both female ($F_{3,\,695}$=53.35, $p<0.0001$) and male ($F_{3,\,708}$=40.02, $p<0.0001$) HW varied significantly among years (Figure 3.8), and pairwise comparisons with Scheffé's post-hoc test indicated that HW in males and females differed significantly between 2004 and all other years. Female HW also differed between 2005 and 2006; females marked in 2006 were larger than females marked in 2005. Since broods overwinter as adults, the weather in the previous spring should represent the weather conditions under which marked individuals were provisioned. For example, individuals marked in 2005 would have been provisioned in the summer of 2004. Figure 3.8 shows both male and female head capsule width as it relates to the previous spring's weather. Females measured in 2004 were significantly smaller than females from all other years, females provisioned in 2005 (those marked in 2006) were larger than the females provisioned in the 2004 season. Interestingly, the opposite pattern is seen among males. Males marked in 2004 (provisioned in 2003) were larger than those from all other years.

Table 3.5: Annual and seasonal variation in measures of foraging effort for *X. virginica*, in southern Ontario for the years 2003, 2004 and 2005. Means and standard deviation are reported with sample sizes (number of females) in parentheses. Analyses used the model effort = date + year + date*year. Each independent variable is reported as the partial effect (F-value) based on the Type I sum of squares. All times are in minutes. Boldface indicates significance at α=0.05 and a Bonferroni correction for the overall model. NS indicates no significance based on a Bonferroni corrected p-value.

Variable	2003	2004	2005	Between years	Within years	Date * Year
No. foraging trips per day	2.8 ± 1.8 (111)	2.3 ± 0.8 (22)	2.9 ± 1.9 (78)	F=0.13, n.s.	F=3.95, p=0.05	F=0.40, n.s.
Flight time per trip	42.3 ± 41.8 (65)	65.1 ± 33.1 (7)	29.6 ± 21.6 (24)	F=3.25, n.s.	F=0.67 p=0.04	F=0.31, n.s.
Turnaround time per trip	66.2 ± 67.2 (54)	83.0 ± 27.8 (5)	49.4 ± 29.0 (25)	F=2.32, n.s.	F=0.77, n.s.	F=0.47, n.s.
Total flight time	135.1 ± 147.7 (65)	193.8 ± 98.3 (5)	112.5 ± 58.4 (27)	F=0.79, n.s.	F=0.74, n.s.	F=3.05, n.s.
Total turnaround time	75.9 ± 157.7 (19)	91.0 ± 31.5 (8)	76.8 ± 100.3 (44)	F=4.65, p=0.01	F=0.14, n.s.	F=1.17, n.s.
Total provisioning time	203.0 ± 143.3 (58)	276.4 ± 151.2 (5)	193.8 ± 88.0 (23)	**F=10.59, p<0.001**	**F=4.58, p=0.04**	F=0.57, n.s.

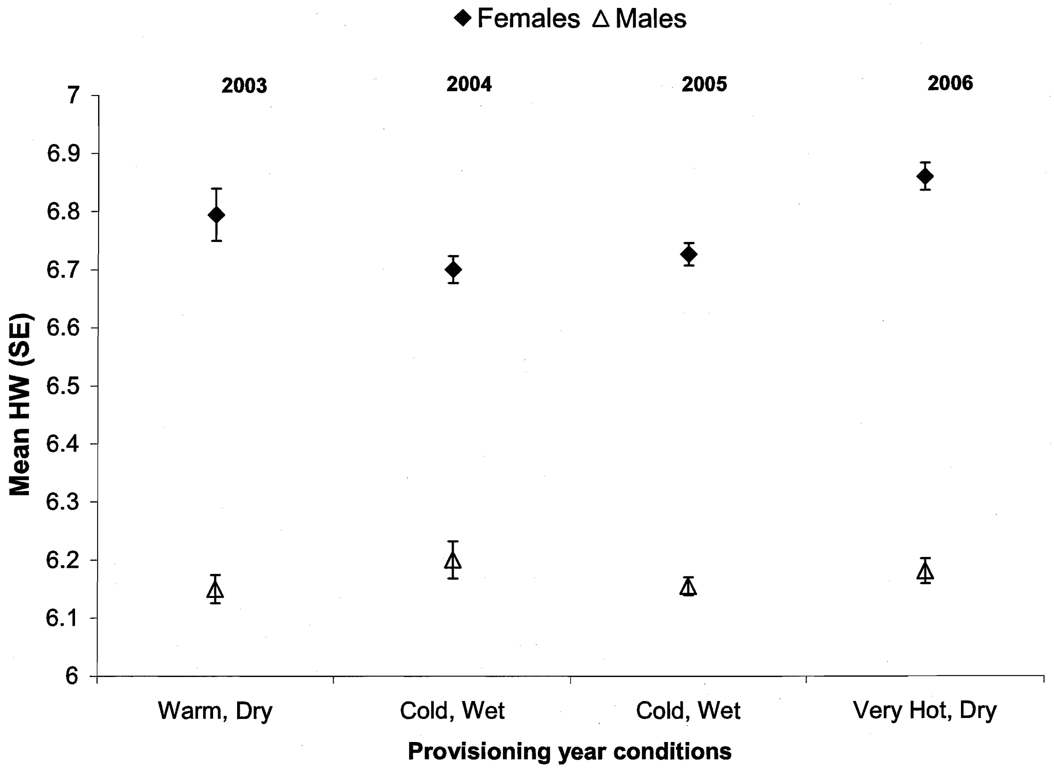


Figure 3.8: The mean head capsule width in millimetres (HW) for males (open triangles) and females (closed diamonds) marked in the courtyard site in the years 2003 through 2006. The x-axis gives the weather conditions for the summer in which most individuals were laid and represents the conditions under which they were provisioned. The year that the individuals were measured is given at the top. Size data from 2006 were collected as part of another study (M. Peso, MSc). Both female ($F_{3,\ 695}$=53.35, p<0.0001) and male ($F_{3,\ 708}$=40.02, p<0.0001) HW varied among years.

Discussion

Annual variation in phenology

In the five years of this study, the life cycle of *X. virginica* did not vary with respect to the type, or the order in which phenological events occurred. However, the dates on which events occurred varied among years; some events varied by up to a month. Some of the variation in timing may be an artefact of using different indicators to infer dates in different years when some data were not available; videoscope data were not available for 2003 and nests were not collected in 2004 or 2005. Nevertheless, given that some events shifted dates in a uniform manner, it is more likely that the timing of events changes as a function of ecological factors.

I found no significant relationship between most life cycle events and annual weather patterns, but these analyses had limited statistical power, as data were often available for only three years. The only event that showed a significant relationship to weather was the timing of nest construction. This is an important event, as it precedes most other activities in the life cycle and may determine season length. In addition, because of the linear nature of *X. virginica* nests, a female must complete most nest construction and renovation activities before she can begin to provision brood cells (Chapter 2). Further, while foraging may depend on factors such as nest flower phenology, nest construction is seemingly dependent only on the start of female activity. Thus, a female may begin nest construction as soon as weather permits, but may still have to wait to conduct other tasks. In 2003 nest construction began later than in 2006, and broods were older (at more advanced developmental stages) for equivalent dates of the respective years. I was unable to determine a date for pollen foraging in 2006 but it appears that this too started late.

Geographic variation in life cycle and phenology

The life cycle presented here indicates geographical variation in the timing of life cycle events relative to dates presented by Gerling and Hermann (1976) and by Rau (1933) for *X. virginica* in more southern environments. Specifically, *X. virginica* in the southern environment of Georgia first flies in March, almost a full month earlier than in Ontario. Further, Gerling and Hermann (1976) reported that 23 °C is required to induce activity, while I frequently observed activity at or around 14 °C. Similar to southern Ontario, in Georgia once brood eclose there is a reduction in female flight activity followed by a second round of pollen foraging. In Georgia, this second round of foraging occurs in October, versus late August or September in Ontario. Interestingly, Gerling and Hermann report that nesting activity in 1973 ended on August 25; it is not clear if this included the second foraging event, but if it is only referring to cell provisioning, then this event occurs at about the same time as in cold wet years in southern Ontario. As in my study, Rau (1933) and Gerling and Hermann (1976) found males overwintering in their natal nests. Overall, *X. virginica's* life cycle appears to be similar in all latitudes, with the timing of events changing, but not the order. This suggests that *X. virginica* has a single brood (univoltine) across its range, and thus cannot produce separate worker and gyne broods.

Analyses of foraging effort

In light of my findings on variation in phenology, one might also expect variation in foraging effort across the season. Females might maximize foraging effort in response to different weather conditions in different years, foraging longer per day to compensate for fewer suitable days. The data show longer total provisioning times in the bad year

(2004), but this increased time was seemingly due to longer turnarounds (the effect was significant but the Bonferonni corrected model was not). All variation between years was due to the 2004 season and not due to differences with the exceptionally warm and dry 2005 season, suggesting a negative influence of poor weather, rather than an advantage to good weather. This might result from females coping with poor weather by working longer, but not as efficiently. Females do not adjust their foraging effort during the season.

There are no published studies with *Xylocopa* foraging data similar to those presented here; some authors have reported observations on foraging in *Xylocopa* and studies have been conducted on foraging in halictid bees (Minckley et al., 1994; Richards, 2004; Richards and Packer, 1995). Gerling and Hermann reported on foraging of *X. virginica* in Georgia where 54 of 89 (61%) observed arrivals were by females with pollen versus 35 (39%) without. Gerling and Hermann (1976) further reported that trips without pollen usually resulted in less time spent in the nest (lower handling time), but that in all trips the time spent in the nest was usually less than that spent in the field. In this study, foraging *X. virginica* females spent more time flying than in the nest; the total flying time was greater than the total turnaround time. This pattern was reversed when mean times were examined and I occasionally observed turnarounds that well exceeded time away from the nest. It is possible that during any one round trip, time in the nest is spent on tasks aside from depositing pollen and turnaround time reflects more than just handling time. Velthuis and Gerling studied foraging in *X. sulcatipes* and *X. pubescens* (Gerling et al., 1983; Velthuis et al., 1984), and found that females in these species predominantly foraged in early morning. *X. sulcatipes* reportedly foraged for pollen every day, making an average of 10 pollen flights per day (Gerling et al., 1983; Velthuis et al., 1984), while, *X. pubescens* made only 2.8 flights per day and took less time per trip (Gerling et al., 1983; Velthuis et al., 1984). The behaviour of *X. virginica* is more similar to that of *X. pubescens*; on average bees made less than 3 trips per day, never made more than 11 trips in any a six hour period and rarely foraged on consecutive days. Finally, *X. pubescens* reportedly ends the day with a nectar foraging trip (a trip with no pollen); *X. virginica* may occasionally do this also, but it does not seem a consistent trend, as some females were never observed returning to a nest without pollen. *X. virginica* probably collects pollen and nectar in the same trip, as in *X. pubescens*. Variation in *X. virginica* foraging behaviour is likely due to differences in pollen availability but might also result from the time required to perform other tasks such as nest repair or from variation in the degree of help in social species.

Richards (Richards, 2004; Richards and Packer, 1995) conducted studies on the sweat bee *Halictus ligatus* with similar measures to those presented here. Contrary to my findings, both studies found significant annual and seasonal effects in foraging. Richards concluded that pollen gathering ability served to connect climate to colony development. Since *X. virginica* is univoltine, metasocial and has a short foraging season, it probably cannot adjust foraging for weather. A single brood and no workers means that broods are likely best compared to the first brood in eusocial *Halictus ligatus*, which is also provisioned by a single foundress. However, unlike the eusocial sweat bee, all *Xylocopa* individuals of a brood are potentially reproductive and so the consequences on brood size and the size of individuals might differ. Richards (2004) found that poor weather resulted in smaller first broods and smaller individuals. This coincides with the finding that following poor years, population size was smaller and females were smaller. As with Richards' study, it appears that even if foraging can change, *X. virginica* may not be adaptable enough to completely compensate for poor weather conditions.

Weather and nest re-use

I found no relationship between nest re-use and weather. This contrasts with findings for *Halictus ligatus*, where increased colony failure was observed in years of high precipitation (Richards and Packer, 1995; Richards and Packer, 1996), which was attributed to the effects of mould and nest damage from precipitation. It is likely that in *Xylocopa,* which nest in wood, these effects are reduced because water does not penetrate the nests, limiting both mould and nest damage. Most failed *X. virginica* nests were unused from the start of the season, suggesting that unused nests were abandoned rather than failed. It is also of note that more nests were unused in the dry and warm year of 2006 than in any other year. This is a bit surprising, as one would expect that good conditions would allow females more time to locate nests and thus few nests would be unused. It would also be reasonable to find a large number of new nests as good conditions would allow more time for building nests, since presumably time spent building new nests is time that cannot be spent foraging. However, 2006 did not have a large number of nests and so I suggest that nest re-use is dependent on a factor or factors other than weather.

Taxonomic variation in phenology

Xylocopa phenology varies among species. Phenology is available for numerous species of *Xylocopa*, but few of these studies span more than a single year, or cover multiple populations. Many of these studies provide only limited data on number of generations and order of life cycle events. For instance, Watmough (Watmough, 1974) reports that some species (*X. caffra, X, apicalis, X. falvobicincta, X. capitata, X. lugubris, X. rufitarsis, X. hottentotta, X. fraudulenta*) are obligately univoltine exhibiting full "diapause" and reproducing in spring or summer. In Africa, high rainfall regions contain exclusively univoltine species (Watmough, 1974). Watmough (1974) suggests that univoltine life cycles are due to temperature and precipitation limits on food availability and the ability to use wood. Multivoltine species are apparently less limited and breed any time that conditions permit (Watmough, 1974). Steen and Schwarz (1998) observed that *X. bombylans* in Australia varied in voltinism with geography, but did not observe such variation in *X. aeratus* (Steen and Schwarz, 2000). This suggests that some species of *Xylocopa* can take advantage of climatic variability as is seen in sweat bees (Packer, 1986; Richards, 2000; Richards, 2001; Yanega, 1989; Yanega, 1997) and proposed for allodapine bees (Cronin and Schwarz, 1999c; Cronin and Schwarz, 2001), but data presented here suggest *X. virginica* cannot. *X. virginica* simply "finishes early" in good years without translating good weather into larger or additional broods, or into reduced foraging effort. Thus, *X. virginica* is univoltine in southern Ontario as it reportedly is in other regions (Gerling and Hermann, 1976). As seen in Appendix 4, this makes *X. virginica* one of only two species known to have multi-female nests and be univoltine.

Consequences of poor weather

I found that head capsule width varied significantly between years and it is seemingly associated with the weather in the year that individuals were provisioned. This relationship is logical and expected. Since females do not change the order in which many life cycle events are conducted, and since foraging effort did not vary much between years, females are forced to make decisions about pollen allocation. As females do not make more or longer trips in bad years, they will have less pollen with which to provision broods. The pollen that is collected can thus be used to make smaller overall broods, smaller individuals, or can be disproportionately allocated among sexes. The 2003 season was wet and cold, and in the following 2004 season, fewer and smaller individuals were observed. This may indicate that weather prevented females from foraging as efficiently in 2003 as they would in drier and warm years. As suggested in other chapters, and in Appendix 3, brood sex ratios are likely even. This suggests that when faced with less available pollen, females produce broods of fewer and smaller individuals.

The 2004 season stands out as the most anomalous of those studied here. The 2004 season was the coldest year of this study, and was the second consecutive cold wet season. This may explain the reduced population sizes and greater number of unused nests in 2004. In addition, females marked in 2004 were smaller and 2004 showed variation in foraging with longer turnaround (handling) times and total provisioning times relative to other years. I propose that many of these factors are related. Smaller population size is probably associated with fewer active nests. Nest use decisions probably involve multiple factors, but greater re-use may reflect increased competition for nests due to more females in the population.

Conclusions

Here I have illustrated the life cycle of *X. virginica* in southern Ontario, the northern extent of its range. This species has a similar life cycle at most latitudes, although the chronology of the events varies annually in southern Ontario. There are some connections between climate and phenology, and these are generally related to when nest construction and renovation begins. With little exception, foraging effort does not differ within a season or between years. Variation that exists is apparently not related to a particular climate factor. Neither nest re-use nor population size is related to bee season weather, but bee season weather does influence when brood provisioning is completed. Finally, the univoltine nature and fixed order of phenology in *X. virginica* likely precludes the types of social flexibility seen in halictid and allodapine bees.

Chapter 4: Male behaviour, mating tactics and aggression in association with female traits in the large carpenter bee, *Xylocopa virginica*

Introduction

In the Hymenoptera, especially the bees, behavioural research tends to focus on females. This is largely because studies focus on aspects of sociality and all worker bees are female. Further, males are often represented as simple drones that do little more than donate sperm (Paxton, 2005). However, there is a considerable body of literature that suggests diversity and complexity in the mating systems and strategies of many bee species. Diversity has been demonstrated with respect to number of mates (Moritz, 1985; Tarpy and Page, 2001), mating tactics (Abrams and Eickwort, 1981; Alcock, 1978; Alcock, 1991; Barrows, 1976; Barrows, 1983; Paxton et al., 1999; Plowright and Pallett, 1979; Velthuis and Gerling, 1980) and courtship behaviour (Eickwort and Ginsberg, 1980). These studies have shed light on distinct behavioural patterns in the mate finding strategies of male bees, while addressing the adaptive significance of various mating tactics (Alcock et al., 1977).

Emlen and Oring (1977), Thornhill and Alcock (1983) and Shuster and Wade (2003) all define mating systems based on the relative number of mates a male or female has. Monogamy is a situation where there is one male to one female, while polygyny is the situation in which one male mates with multiple females. Polygynous systems are usually further subdivided based on the strategy that males use to locate and obtain mates. Resource defence polygyny is the tactic in which a male establishes a territory, or otherwise defends an item of value to females in order to intercept females as they seek that resource. Female defence or harem polygyny is a scenario where males directly guard a group of females. Defence tactics are often associated with interloping, which is an alternate tactic where males attempt to "sneak" past dominants and obtain copulations. Polygynous strategies such as leks involve males and females gathering at seemingly arbitrary landmarks, which the males guard, for the sole purpose of mating (Emlen and Oring, 1977; Shuster and Wade, 2003). Scramble competition is a term for polygynous mating systems in which males compete directly to mate with females, typically during a very short breeding season. Recent reviews of mating behaviour in Hymenoptera have developed frameworks to explain the evolution or choice of various mating strategies (Boomsma et al., 2005; Paxton, 2005). These studies make predictions about what strategy a male should use given a particular set of these conditions. Many of these conditions are related to traits associated with females including the location and proximity of nests to each other, female foraging habits, and the physical size of males or females.

Bees in the genus *Xylocopa* are considered ideal candidates for studying bee mating systems (Barthell and Baird, 2004). These bees are large and conspicuous which allows for easy observation and they exhibit a variety of mating systems allowing for comparative studies. Studies in *Xylocopa* have shown that males may exhibit female defence polygyny, resource defence polygyny, scramble competition, and a system that resembles a mammalian lek (Alcock, 1991; Alcock and Johnson, 1990; Barrows, 1983; Barthell and Baird, 2004; Frankie et al., 1977; Leys, 2000a; Minckley and Buchmann, 1990; Minckley et al., 1991; Vinson and Frankie, 1990). Further, an association has been proposed between chemical attractants, morphology and mating system: males that exhibit lek systems often have large metasomal glands that may be associated with the production of a chemical attractant (Minckley, 1994; Minckley et al., 1991).

Three previous studies of male *X. virginica* have demonstrated territoriality in males. Gerling and Hermann (1976) suggested that males in a Georgia population are territorial around active nest entrances and grab females as they make trips to or from the nest. Barrows (1983) examined male behaviour in a Washington, DC population and interpreted the repeated observation of males near a nesting site on multiple days as evidence of territoriality. Barrows further suggested that territoriality was one of multiple mating tactics (also including interloping and scramble competition) exhibited by males within the same populations. Finally, Barthell and Baird (2004) studied the subspecies *X. v. texana*. In their study, they used a similar approach to Barrows in defining territoriality and then examined size variation and aggression between males identified at the "nesting site" and the "nesting periphery and flower." Barthell and Baird (2004) concluded that the mating strategy, aggression and dominance are correlated with size (head capsule width). All three studies of male *X. virginica* have stressed that to confirm mating strategies, behaviours should be examined in populations with known and varying female nest densities.

In this study, I aim to accomplish three goals. First, I examine the behaviour of male *X. virginica* in the northern extreme of its range, concentrating on males within territories. Second, I examine the factors that determine male mating tactics. Finally, I examine male *Xylocopa* behaviour in the context of female life-history traits such as nest and female densities and compare it to predictions from the theoretical work such as that of Paxton (2005), and Baer (2003).

Methods

Study Site

All research was conducted at the previously described Brock University Courtyard site (Figure I.1) from 2003 through 2005. The site remained the same in all years with the exception that bench 7 was moved closer to other benches prior to the 2005 season (Figure I.1).

Marking for identification

Males and females were marked in every year of this study, according to the protocol described in the general introduction. Marking bees made it possible to identify individuals in observational studies. Since all males and females that were observed in the Courtyard site were marked (some females do not leave nest, see Chapter 1), the number of marked individuals approximates the active adult population size (see Figure 3.6 and Chapter 3).

Behavioural observations and censuses

In 2003-2005, I conducted behavioural observations at benches to determine rates of male-male and male-female interactions. Each year, behavioural observations began shortly after the first male of the season was observed hovering near a bench and continued daily (weather permitting) until males were no longer observed at the study site (approximately late May through June). In some rare instances, multiple males were seen hovering above opposite ends of the same bench, but usually a single male was observed above a bench. The areas of observation were defined as the three-dimensional space surrounding a bench, and were divided into perimeters of 0.5 m and 1 m from the bench (Figure I.1, Figure 4.1); these distances were easily identified as the walkway was constructed of 0.5-metre cement squares.

I observed the behaviour of males in a series of 15-minute observation periods carried out throughout the day at each bench in turn. The order of observations was randomized with respect to bench to avoid bias that might be associated with daily variability in male activity patterns. In instances where no activity was observed for five full minutes, observations at the bench were suspended until later in the day. If no activity was observed in two attempts, the bench was declared inactive for that day. *X. virginica* was not active when it rained so observations were not made on days with precipitation. Complete sets of observations (i.e. those uninterrupted by rain) were conducted on 18 days in 2003, seven days in 2004, and nine days in 2005, for a total of 122.5 hours of observations.

The following behaviours were recorded during observations: loop (L), chase (C), chase and touch (CT), chase and fight (CF), chase female (CFEM), and mating attempt (MA). Loop was defined as a previously hovering male briefly leaving a territory and flying in a circular motion not directed at an intruder or object before returning to his original position. Looping behaviour was conspicuous and may be an advertisement or represent a male searching for other males. Chase (C) was defined as a hovering male flying towards an object (other than a female), but not making contact with it. The chase and touch behaviour (CT) was defined as a chase that was followed by light contact

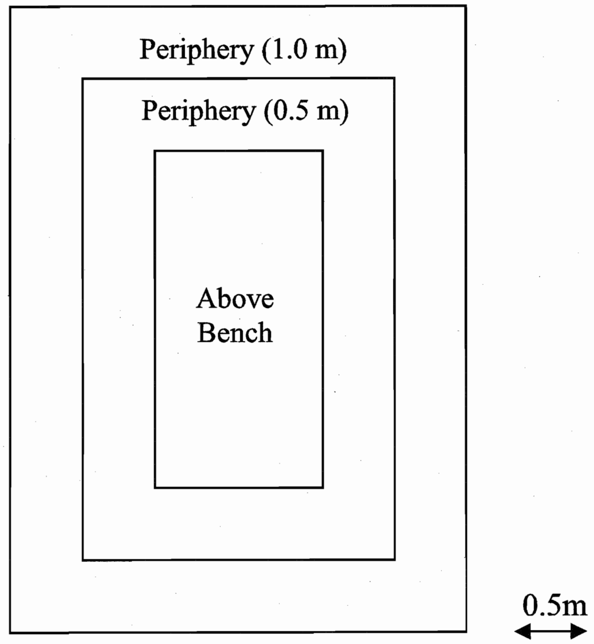


Figure 4.1: Scale diagram of a “territory” showing bench, and periphery divided into areas 0-0.5 and 0.5-1.0 metres from the bench.

between the male and the object of pursuit. A chase and fight (CF) was defined as a chase followed by prolonged contact, grappling, biting, or the individuals falling to the ground; this is roughly equivalent to the "pouncing" described by Barrows (1983).

Chase female (CFEM) was defined as a hovering male flying towards a female but not contacting her. A mating attempt (MA) was defined as a male chasing and grabbing a female with the result that the pair landed on a surface or flew out of the territory together. This is distinguished from CFEM where no contact was observed. MA events follow CFEM events; however, not all CFEM events result in mating attempts. It was not possible to observe copulation in most instances but MA behaviours are consistent with the precopulatory behaviour described by Barrows (1983). Behaviours were treated as single events, regardless of duration, and were recorded relative to a focal individual (male of interest), defined as the male that initiated the behaviour. All behaviours performed by all males within 1m of the bench were recorded.

In order to quantify patterns of residency throughout the day and season and in order to determine each male's positions relative to benches, I censused the bees near each bench throughout the day. A census consisted of a one-minute acclimation period (males often initially react to humans) followed by a 10-minute census of the identity and position of all individuals within 1m of a bench. Five censuses of each bench were carried out each day; censuses were conducted in the periods between 15-minute behavioural observations. These data were used to calculate the time spent in the study site, the number of days a male was observed, and to determine resident status.

Analytical Methods

Evidence for polygyny

I examined the ratio of males to females marked in the courtyard site in each year for evidence of polygyny. Specifically, I compared cumulative number of males and females marked in each week of the bee season of each year. In each year, weeks were designated a numerical value based on the start and end of each season. Comparisons were performed using one-way chi-square goodness of fit tests with the calculated expectation of a 1F:1M sex ratio (50% of individuals in each category). In instances where sample sizes were less than those required for chi-square analyses, Fisher's exact test was used. Chi-square tests were performed using Microsoft Excel; Fisher's exact tests were performed by hand using Excel. The ratio of marked males to females is an "operational sex ratio" (Kvarnemoa and Ahnesjo, 1996) and represents the numbers of males and females that had been documented outside a nest. This number differs from the population sex ratio as many females rarely (or never) leave the nest and are likely unmarked (Chapter 1).

Evidence of territoriality, resident and satellite males

In his study, Barrows (1983) interpreted the repeated observation of males near a nesting site on multiple days as evidence of territoriality. Similarly, Barthell and Baird (2004) designated two classes of male based on position within a territory. They referred to males near a nest as "nest males" and contrasted them to "periphery males" which were located farther away from a nest. I looked for "nest males" and "periphery males" (sensu Barthell and Baird (2004)) at the Brock Courtyard site. I refer to males near benches as "residents" and to those on the periphery as "satellites", as these terms better reflect common terminology. It is assumed that a bench and its surrounding area reflect a territory. Using data from 10-minute censuses, I tested whether males were randomly distributed among benches. I compared the proportion of individually censused males above a bench and on the periphery of a territory via chi-square goodness of fit tests. I tested the hypothesis that certain males would be repeatedly censused either on the periphery or above a bench, within or among days, using the average proportion of censuses in which a male was observed above a bench across days. The resulting values ranged from zero, which indicated a male was never above a bench on any day, to one, which indicated a male was always censused above a bench. Data from males not censused on two or more occasions were discarded.

I examined whether satellite males changed territories (moved between benches) more often than resident males. For each year, I identified males censused on multiple days and determined which males were censused exclusively at one bench, and those censused at multiple benches. Finally, I tested the hypothesis that resident males would remain in the study site longer than satellite males, because resident males live longer or are less likely to change tactics. I compared time spent at the study site [the number of days from marking (the first time a male was seen) until the last time the male was seen in a 10-minute census] for satellite and resident males using a Wilcoxon 2-sample test.

Interactions between satellite and resident males

In order to classify behaviours, and to reduce the number of variables, I performed principal components analysis (PCA) on the covariance matrix of all behaviours recorded during 15 minute observation periods (C, CT, CF, Loop, MA and CTF). To determine if resident males maintain status through greater aggression, or engage females more often, I compared the significant principal components (PCs) between satellite and resident males. Males censused above a bench in the multitude (most instances but not necessarily half) of the two to five 10-minute censuses conducted on the day that the observations were made were categorized as residents. All other males were categorized as satellites. Since analyses are unique to a day, a male may be a resident on one day, and a satellite on another day. I compared each PC individually using General Linear Models conducted in SAS 9.1.3. Each model used the PC as a dependent variable and the predictors: male identity nested within year, resident or satellite status (hereafter "status"), and days since the first territory was established in that year (hereafter "days"). The ID(days) term was included to account for repeat observations of a male on multiple days, while identity was included to account for individual variation between males and repeated use of male IDs in different years. There was no effect of days and this variable was subsequently excluded from analyses. Although not explicitly a male-male interaction, loop was also examined using this model.

I examined the rates of both chase-female (CFEM) and mating attempt (MA) events between resident and satellite males to determine if resident males had greater access to females. CFEM events were compared via ANCOVA using the number of events per male per 15-minute observation period as the dependent variable and the terms: satellite or resident (status), male identity nested within year, the number of days since males established territories in a given year, and bench. Bench was included to account for potential differences in encounter rate associated with territory quality. I also performed these analyses using the number of nests in a bench and number of females marked in proximity of the bench with no increase in the variation explained (r^2). I removed non-significant terms from the model and the final model included only the predictors male ID nested in year and days since territories were established. Mating attempts were compared between resident and satellite males using chi-square goodness of fit tests, because too few attempts were observed to apply more complex models.

Size relationships between males of different mating strategies

I compared males to determine if resident and satellite males differed in size. Head capsule width (HW) was recorded for every marked male; therefore I was able to compare these values among all censused and observed males. Since a male's position in a territory reflects its status, I compared HW among males above benches, between 0 and 0.5 m from a bench and more than 0.5 m from a bench. I performed ANCOVA with a model that included the predictors: year, days since 1-May of each year, bench, and position. Year was included to account for size differences among years, date to account for variation within the season, and bench to account for potential relationships between size and territory quality. The HW data were not distributed normally; thus, ranked HW data were used. I also tested HW as a function of status and date. Comparisons were performed via ANCOVA on ranks with the following predictors: status (resident or satellite) and date marked (days since 1 May) as a nested variable within the variable year. For this analysis resident was defined as a male that was a resident on any day, all others were "satellite". Finally, to determine if size influenced site fidelity, I compared HW between males censused within the study site and males that disappeared after marking using ANOVA. It was assumed that if males disappeared (were not censused) it was because they had adopted an alternate mating strategy outside the study site. The ANOVA model included a year variable to account for annual variation in HW.

Characteristics of territory quality

Benches vary in the number of nests they contain and in the number of females that visit them. This might result in varying territory quality from a male's perspective. I compared the average number of males censused at a bench among benches, which should reflect differences in males' perception of a territory's quality. Analyses were made using ANOVA with a model that included the categorical variables bench and year; year accounted for annual variation in population size and number of nests between years. I also examined whether equal numbers of males were marked near each bench. Comparisons were made via chi-square goodness of fit tests with an expected distribution of marked males equally distributed among benches. Patterns were examined separately for each year. I examined potential relationships between the number of males censused near a bench and both the number of nests in that bench and the number of females marked near that bench. Each factor (nests and females) was examined in a separate regression model and factors were nested within year to account for variation among years. Finally, I ranked bench quality to reflect territory quality as perceived by males. Since, more males should visit higher quality territories, ranks were based on the mean number of males censused at a bench per day. Lower scores were assigned to more frequently visited benches so a lower rank indicated a higher perceived quality (e.g. best=1, worst=7). Annual ranks reflected quality in a particular year, the mean rank of all years represents the overall trend.

Results

Evidence for polygyny

I examined the timing of male and female emergence and the number of marked individuals of each sex to confirm polygyny in the courtyard population. Overwintered males and females were first observed at about the same time (Table 3.3), but males tended to become active before females (Figure 4.2). At this time, males were aggregating around benches and were presumably establishing territories. The total number of males in the population increased for a period of about six weeks after which it reached an asymptote; during this time, there were more active males than females (Figure 4.2, Table 4.1). The number of marked individuals may underestimate the sex ratio bias, however, because in each year, the final number of males marked was greater than the number of males observed at benches. On average, 60% (38% in 2003; 70% in 2004; 58% in 2005) of males left the study site after they were marked.

Evidence of territoriality, resident and satellite males

In this study, after emergence some males remained in the courtyard and aggregated around the benches. These males usually hovered in place above and central to a bench, or hovered in a similar manner up to 1 m from a bench. When males above a bench were removed for marking, it was common to see one of the peripheral

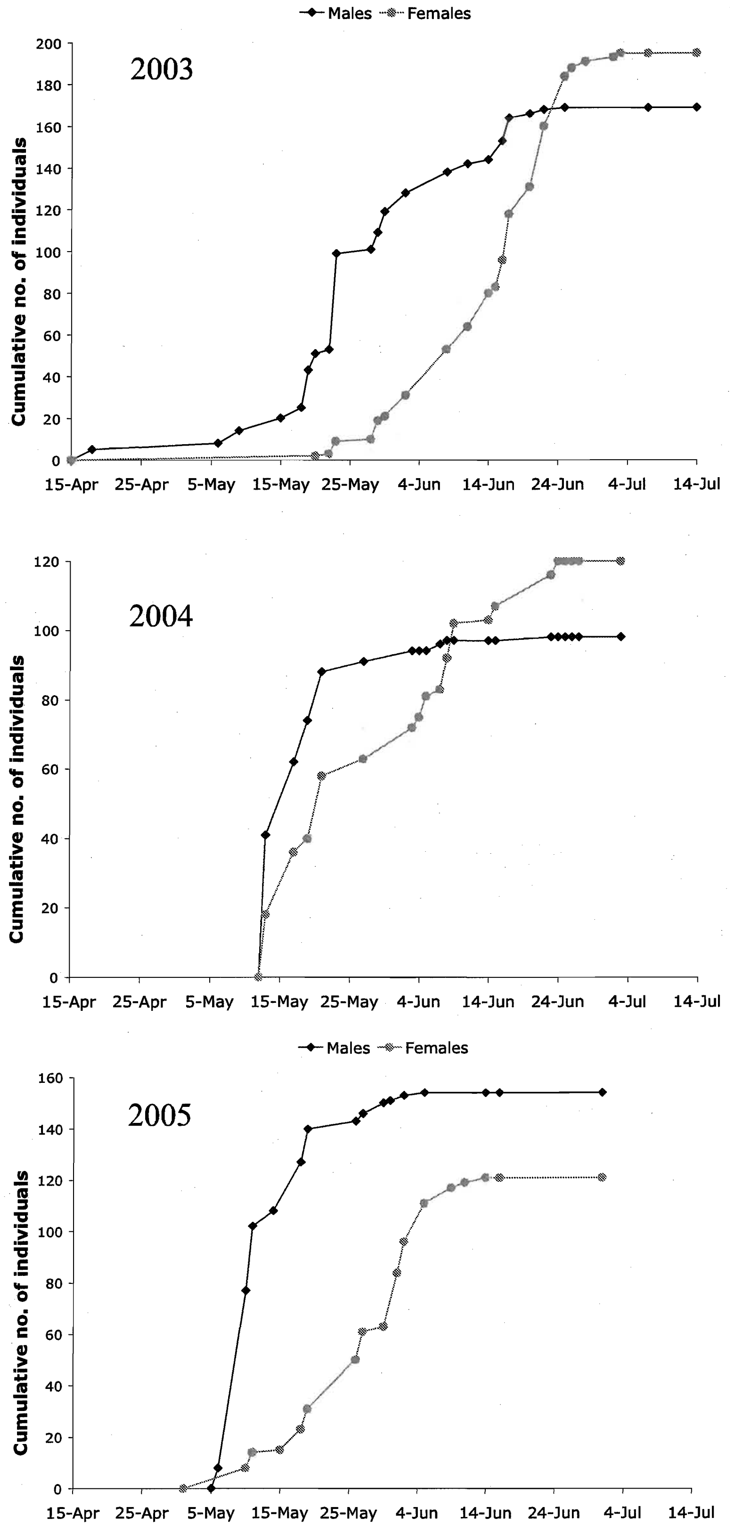


Figure 4.2: The cumulative number of marked males (black diamonds) and females (grey circles) at the Courtyard site for each year of this study.

Table 4.1: The cumulative number of males and females by week for the 2003, 2004, and 2005 seasons. Week 1 began with the first marked bee of each year and weeks continued until no new individuals were marked. Weeks are not equivalent between years. Week 7 is the total number of males and females marked in 2003; this is shown in week 6 for 2004 and 2005. Significant results are in bold. ns indicates p>0.05.

Week	Females	Males	χ2	p-value	Females	Males	χ2	p-value	Females	Males	χ2	p-value
	2003				2004				2005			
1	9	14	1.09	ns	18	41	8.96	**< 0.01**	0	8		**0.038** [a]
2	31	51	4.88	**< 0.05**	40	74	9.56	**< 0.05**	15	108	44.02	**< 0.001**
3	53	53	0	ns	63	91	4.74	**< 0.05**	31	140	69.22	**< 0.001**
4	83	109	3.26	ns	104	97	0.18	ns	96	153	13.05	**< 0.001**
5	160	142	0.96	ns	109	97	0.58	ns	119	154	4.24	**< 0.05**
6	195	168	1.86	ns	123	98	2.6	ns	121	154	3.72	0.053
7	195	169	1.86	ns								

---

[a] Fisher's exact test due to limited sample size

males (those within the 1 m area surrounding but not above a bench) assume the vacated position. Removed males often re-established their positions when released; the occupying male(s) returned to the periphery. The observation that males replace each other suggests that territoriality exists; the fact that males are differentially distributed on the periphery suggests the presence of both resident males (nest males in Barthell and Baird (2004)) and periphery males of Barthell and Baird (2004).

I used 10-minute census data to confirm whether males consistently reside over benches or on the periphery as did the nest and periphery males described by Barthell and Baird (2004). More censuses contained males only above benches in every year of this study (Table 4.2). In 2003 and 2005, there were also censuses with a male above a bench and on the periphery at the same time, but this did not occur in 2004 (Table 4.2). Less frequently, a bench was censused that had males only on the periphery (Table 4.2). Males that are above benches are better positioned to encounter females than males on the periphery. Males above benches should therefore be seen repeatedly above benches both within and among days. I examined this expectation using the average proportion of censuses in which a particular male was censused above a bench and found that most censused males were always located above a bench (mean proportion equals 1), some were always on the periphery, and some males moved between a bench and the periphery (Figure 4.3).

I examined 10-minute census data to determine if resident males (those usually above a bench) retained their status across days (Table 4.3). Within each year, males observed more than one day were more frequently resident males, suggesting males retain their status across days. Since a resident male may be displaced (or die) or a satellite

Table 4.2: The number of censuses in which males were located above a bench, on the periphery, and in both positions. Data are presented for each year of this study.

	Only above bench	Only periphery	Above and periphery
2003	240 (67 %)	32 (9 %)	87 (24 %)
2004	87 (91 %)	9 (9 %)	0 (0 %)
2005	57 (45 %)	16 (13 %)	53 (42 %)
Mean	128 (66%)	19 (10%)	47 (24%)

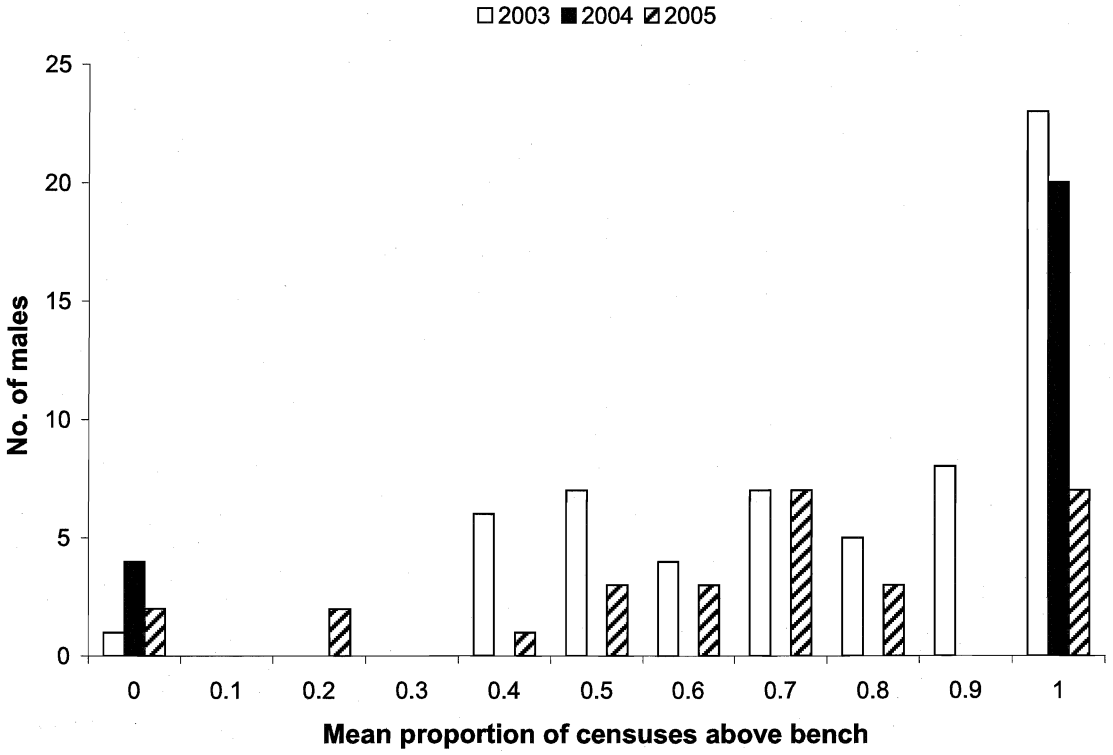


Figure 4.3: The number of males that on average were never above a bench (mean=0), were sometimes above a bench, and were always censused above a bench (mean=1). Data are based on five censuses per day for 18 days in 2003, 7 days in 2004, and 9 days in 2005.

Table 4.3: The mean number of days ± SD between marking and final census for resident males and satellite males. Test statistics are results of Wilcoxon 2-sample tests.

	Resident male	Satellite male	test-stat	p-value
2003	31 ± 19.5	22.9 ± 15.4	1326	0.11
2004	27 ± 9.7	33.8 ± 0.5	46.0	0.17
2005	16 ± 8.6	21.0 ± 11.6	158	0.18

male may assume a better position in another territory, some males should move between benches. In 10-minute censuses in 2003, 42% (30/54) of satellite males and 56% (13/31) of resident males were observed at the same bench each time the male was censused, and this was true for 100% of males censused in 2005 (n=16 residents, 12 satellites) (Table 4.3). This suggests that most residents maintain control of the territory through the season.

Territorial vs. satellite male behaviour

Interactions between satellite and resident males

In order to quantify male aggression, and determine if behavioural rates vary between territory holding and satellite males, I measured a series of male behaviours (chase, CT, CF, L) in addition to two behaviours that involve male-female interactions (chase female and mating attempts). Since these behaviours are potentially associated, and to reduce the number of variables, I performed principal components analysis (PCA) on all the behaviours recorded during 15-minute behavioural observations (Table 4.4). Four principal components were significant in explaining variation in male aggression (Table 4.4). PC1 explained the most variation and represents most male behaviours except mating attempts. PC2 primarily explains variation in the male behaviours that involve contact (CT and CF). PC3 explains variation in mating attempts. While no variable was significant, the chase-female behaviour loaded strongest onto PC4. While not significant, chase-female loaded most strongly onto PC4. In order to determine whether rates of male behaviour vary between territory-holder and satellite males, I performed ANCOVA on each of the first four PC scores (Table 4.4). PC1 differed significantly for satellite and residents, suggesting that territorial males are more aggressive than satellite males; no other PCs showed significant differences.

Table 4.4: Factor loading values for the first four principal components (PC) of variables related to male behaviour, and results of statistical comparisons between resident and satellite males. PCA analysis is based on covariance matrix. PCs were examined in seperately using the model: PC = ID(year) status [days]. Partial F and p-values are based on the type III sums of squares for the status term. Bold indicates model and effect were both significant Sample size is 363 for all analyses.

Behaviour	**Eigenvector PC1**	**Eigenvector PC2**	**Eigenvector PC3**	**Eigenvector PC4**
Chase (C)	0.778	0.042	0.012	-0.431
Chase-Touch (CT)	0.695	-0.433	0.063	0.215
Chase-Fight (CF)	0.518	-0.676	0.120	0.055
Loop (L)	0.688	0.435	-0.199	-0.274
Chase-Female (CFEM)	0.625	0.505	-0.008	0.555
Mating attempt	0.021	0.200	0.975	-0.064
Eigenvalue	18.55	6.60	4.61	1.63
Percent Explained	57.0	20.3	14.2	5.0
Cumulative Percent	57.0	77.3	91.5	96.5
Partial F	37.89	0.00	0.05	0.42
p-value	**P<0.0001**	0.97	0.82	0.5
df	1	1	1	1
Mean (SD)-Satellites	-1.30 ± 3.36	-0.15 ± 1.98	-0.17 ± 1.49	0.03 ± 1.15
Mean (SD)-Residents	3.95 ± 4.40	0.43 ± 3.78	0.49 ± 3.38	-0.09 ± 1.60

Male-female interactions

A male should adopt the mating strategy that provides him with the most opportunities to interact with females and thus the most mating opportunities. I examined two types of male-female interactions, chase-females (CFEM) and mating attempts (MA) with the prediction that resident males were in a preferred position and would gain more opportunities to interact with females. Specifically, residents should chase females more often than satellite males and should engage in more mating attempts. I compared the rate of chase-female events between resident and satellite males via ANCOVA. The model used included male identity nested within year to account for potential repeat observations of males, and days since males established territories to control for changes in behaviour over the season. The overall model was significant (ANCOVA: $F_{218, 145}=1.45$, $p<0.008$), and resident males chased females more frequently than satellite males (Residents: 3.7 ± 3.6 per 15 minute period, Satellites: 0.8 ± 1.7).

Males were occasionally observed "grabbing" females and engaging in what was presumably a mating flight. These flights involved a male mounting a female, followed by the individuals flying together, often out of view. In other instances, the pair crashed to the ground. Since these events usually took the pair out of the territory being observed, it was difficult to estimate the length of the events, but males often returned during the same 15-minute observation period. I observed few mating attempts in any year. In 2003, I observed seven attempts by resident males and 11 by satellites, which is not a significant difference (Chi-square goodness of fit: $\chi^2_1=0.5$, $p=0.48$). The sole MA observed in 2004 was performed by a resident. In 2005, all five observed attempts were by resident males. Summed across all years, residents did not perform significantly more mating attempts than satellites (13-resident, 11 satellite) (Chi-square goodness of fit: $\chi^2_1=0.04$, p=0.8).

Characteristics of territory quality

Territories containing more nests or that are visited by more females will provide males increased access to females. These differences in territory quality will result in variation with respect to both the average number of males censused per day (Figure 4.4a) and the total number of males marked in a territory (Figure 4.4b). I compared the average number of males censused per bench using an ANOVA model that included the categorical variables bench and year. The overall model was significant (ANOVA: $F_{8, 208}=8.18$, $p<0.0001$) as were the differences between benches ($F_{6, 202}=9.44$, $p<0.0001$). I also examined the total number of males marked at benches in each year to determine if equal numbers of males were marked at each bench. The number of males marked was not evenly distributed in any year (Chi-square goodness of fit: 2003: $\chi^2_6=76.5$, $p<0.001$; 2004: $\chi^2_6=62.6$, $p<0.001$; 2005: $\chi^2_6=24.2$, $p<0.001$). Both results suggest variation in territory quality.

A male's perception of a territory's quality should reflect the number of mating opportunities he can obtain within that territory. This can be examined as the number of males that visit a bench relative to the numbers of females marked near the bench (Figure 4.5a) or the number of nests in the bench (Figure 4.5b). I examined this relationship using the model (the total number of males marked at a bench in a given year) = number of nests in a bench, number of females marked near a bench, and an

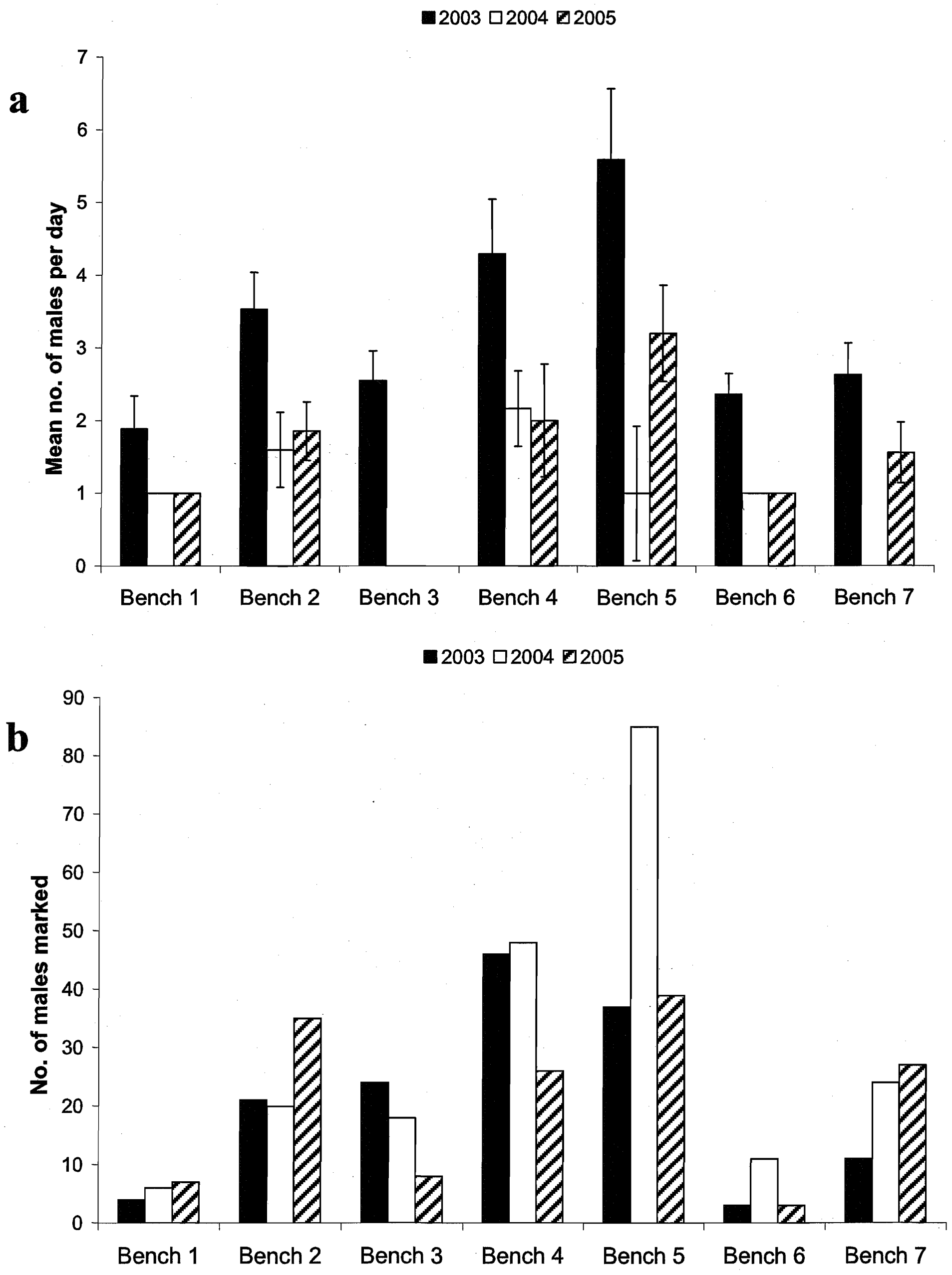


Figure 4.4: The mean number of unique males (SE) censused per day by bench for 2003 (solid bars) (n=18 days), 2004 (white bars) (n=7) and 2005 (hatched bars) (n=9). In 2004 and 2005, no males were censused near Bench 3. In each of 2004 and 2005 bench six was active on a single day, and only one male was censused on that day. No standard error indicates a sample size of one for that year. **b.** The number of males marked by each bench for 2003, 2004, and 2005.

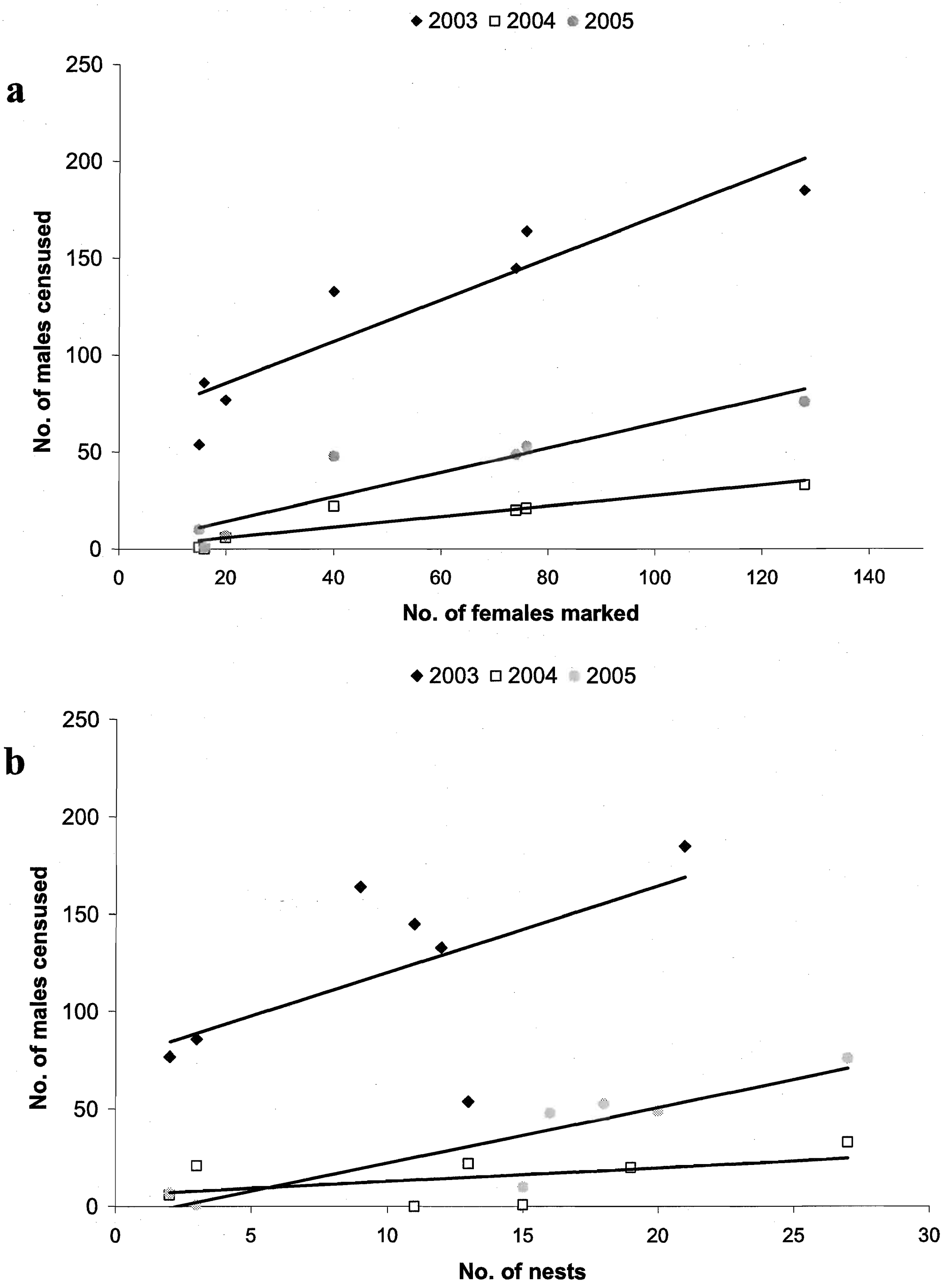


Figure 4.5: The total number of males observed in censuses relative to **(a)** female density (females marked in that territory) and **(b)** the number of nests in a particular bench in a particular year. Sample sizes are 21 for both panels.

interaction term for nests by females. The overall model was significant (Regression: $F_{5, 15}=11.19$, $p<0.0001$), as was the effect of females (Regression: $F_{1, 15}=12.09$, $p<0.004$). The effect of nests was not significant (Regression: $F_{1, 15}=1.78$, $p<0.2$).

Finally, to quantify male response to territory quality, I ranked benches based on the mean number of males censused near them (Table 4.5). Ranks varied between years, however, benches five and four were visited the most in each year, and benches three and one were visited the least. Similarly, when averaged across years, benches five and four were visited most often, and benches one and three were least visited. This suggests that benches four and five were high quality while three and one were low quality. Bench five contained the most nests in every year while benches three and six contained the least (Figure I.1).

Table 4.5: Ranked territory quality for each bench in the 2003, 2004 and 2005 seasons. Ranks are based on the average number of males censused per day. Lower ranks indicate higher territory quality. Mean is the average of ranks across all three years.

	Bench 1	Bench 2	Bench 3	Bench 4	Bench 5	Bench 6	Bench 7
2003	7	3	5	2	1	6	4
2004	3	2	4	1	3	3	4
2005	5	3	6	2	1	5	4
MEAN	5	2.7	5	1.7	1.7	4.7	4

Discussion

Mating system in *Xylocopa virginica*

While mating behaviour has been studied in *X. virginica*, previous studies did not provide detailed information on sex ratio, or associations with factors such as nests and females. In this study, I examined mating behaviour, and present findings in the context of these additional factors. Past studies of male *X. virginica* note as many as five mating tactics, including female defence polygyny, territoriality at plants, scramble competition, symbolic territories and interloping (Barrows, 1983; Barthell and Baird, 2004). While Barrows did not distinguish between female defence and resource defence polygyny, Barthell and Baird suggested that female defence polygyny is the primary strategy in *X. v. texana*, and the current study shows similar results for *X. v. virginica* in southern Ontario. I have shown that males emerged prior to females, and are significantly outnumbered by females during the mating period, strongly supporting polygyny. Further, males aggregated around nesting substrates, showed aggression towards conspecific males and assumed vacated positions above benches; these behaviours are all common indicators of territoriality in insects (Baker, 1972; Baker, 1983). As in *X. v. texana* (Barthell and Baird, 2004), males aggregated both near the centre (near nests) and periphery of territories. Some males were exclusive to one position while others moved between positions. These males constitute residents and satellites respectively, and are likely equivalent to the nest and periphery males of *X. v. texana* of Barthell and Baird (2004). Also, similar to *X. v. texana*, resident and satellite males did not differ in head capsule width.

Behavioural differences between residents and satellites

Since size does not vary between satellite and resident males, I propose that roles are determined via males' willingness to chase competitors and an advantage to initially holding a territory. Resident males were more aggressive than satellite males even though aggressive behaviours involving contact have been shown to cause physical damage in other species of bee (Jaycox, 1967). No difference was detected in the time between marking date and final census observation date for satellite and resident males. This suggests that satellite and resident males have similar lifespans, and that consequently that the risks from aggressive interactions are minimal in *X. virginica*.

Numerous studies of male territoriality in insects have demonstrated that resident males have an advantage in male-male contests (Kelly, 2006a; Kelly, 2006b; Kemp and Wiklund, 2001; Kemp and Wiklund, 2004; Olsson and Shine, 2000), although no such phenomenon has been confirmed in bees. These studies suggest that the resident will be more aggressive in chasing away competitors. When the increased aggression leads to the resident winning the encounter, the resident receives positive feedback, which results in increased aggression in future encounters. Therefore, winning is expected to result in an advantage for the resident. Such scenarios require residents to be more aggressive than satellites or interlopers, but do not require a size dimorphism between types of male. While I did not explicitly test these scenarios, I found that in *X. virginica* males there was no size difference between satellite and resident males, but overall residents were more aggressive and mostly retained their positions. Anecdotally, males appear to become more aggressive over time; however, the data do not allow for this observation to be empirically tested. These findings resemble those expected if these bees show resident male advantage. This is similar to the findings of Barthell and Baird (2004) who found increased aggression in males near nesting sites relative to those on the periphery.

Male-female interactions

Resident males pursued females with a greater frequency than did satellite males, but did not engage in significantly more mating attempts. Possibly, this is due to the increased potential for interactions associated with holding a territory, but it may also indicate less risk of injury or loss of status to residents if they chase unreceptive females. *X. virginica* flights often take the pair out of a territory (Gerling and Hermann, 1976), and so resident males which can regain their territories may be more likely to pursue females. Interestingly, Barthell and Baird (2004) did not detect a difference in male-female interactions between nest and periphery males of *X. v. texana*, but did find that males in nesting sites interacted with females more often than males at flowers.

Neither the rate of male-female chases nor the number of mating attempts varied with territory. This is somewhat surprising as the number of females varied between territories, and increased with the number of nests, and thus it would have been expected that the number of receptive females also varied. However, since the rates of male-female interactions may be influenced by female activity rates, particularly with respect to leaving and arriving on foraging trips, 15 minutes of observation may not have been sufficient to detect trends. Overall, the rate of male-female encounters was much greater than the actual number of mating attempts; I recorded over 700 male-female chases between 2003 and 2005 but less than 50 mating attempts; of these, only 14 were by resident males. This is similar to what was found in *Anthidium septemspinosum* (Sugiura, 1991), in *X. fimbriata* (six copulations and 14 male-female interactions in 38 hours)

(Vinson and Frankie, 1990) and in *X. californica* (no copulations in 158 male-female encounters) (Alcock, 1991). However, more frequent mating has been observed in *X. virginica texana* (16 contacts per 15 minute observation period near nests versus 13.05 per 15 minute period on the periphery) (Barthell and Baird, 2004) and in *X. varipuncta* (7.35 per 15 minute observation period) (Alcock and Smith, 1987). Alcock speculates that the limited copulations in *X. californica* was because most females were already mated (Alcock, 1991) and that females mate a single time, a notion that is proposed in other studies of *Xylocopa* (Barrows, 1983; Barthell and Baird, 2004; Gerling and Hermann, 1976).

It is not clear if females in these populations mate more than once. However, I never observed the same female engage in multiple mating flights in an observation period, and often saw females acting aggressively towards males. Velthuis and Gerling (1980) suggest that in species where males wait near nests, some mating attempts will not result in insemination because females will not be receptive. Genetic evidence of multiple mating is limited in *Xylocopa,* but Steen demonstrated multiple patrilines in the subgenus *Xylocopa Lestis* via allozymes (Steen, 2000). In Ontario, I found that at the end of the season, more than 50% of nests contained at least a single mated female (Chapter 1). Also, I found that multiple females may enter or exit the same nest entrance (Chapter 1 and 3). However, I also found that many unmated females were unworn (performed limited flying) and undeveloped (not reproductive), suggesting they were not reproductive. Therefore, if males pursue every female that passes through a territory, some interactions may be between males and non-reproductive females.

Territory quality

While behavioural rates did not vary with bench, the number of males visiting and defending territories did. For instance, multiple males frequently aggregated around bench five while bench three rarely had more than a single resident male and no satellites. Similarly, the number of unique males marked varied among benches. Most importantly, benches without active nests often had no resident males and usually were not guarded at all. Therefore, territories appear to vary in quality as perceived by males. Further, males seem to prefer guarding the far periphery of a higher quality territory where many females will pass through, than to be central in a very poor quality territory in which few females are present. Thus many males will be satellites in good territories rather than hold poor territories.

Michener (2000) has shown that some bees may construct nests that are particularly visible to males, while Barthell and Baird (2004) have suggested that *X. v. texana* males might cue in on particularly visible substrates. If every nest has at least one female associated with it, then by defending resources (nests), a male can also defend mates (the female(s) residing within the nests) and thus maximize the number of mating opportunities. While I found that males are associated with both the number of nests and the number of females in a territory, since males establish territories prior to female activity, it is likely that males in this population are cuing on nests.

Mating strategies in Hymenoptera

The literature on male behaviour in the Hymenoptera tends to cover three general topics. Many studies focus on mating behaviour in mostly solitary species (Alcock et al., 1977; Alcock et al., 1976; Danforth and Desjardins, 1999; Danforth and Neff, 1992). A second body of work examines conflict over sex allocation in social species (Boomsma and Eickwort, 1993; Boomsma and Ratnieks, 1996). The remaining literature is devoted to developing and reviewing general frameworks for the evolution of male mating tactics, and systems in the Hymenoptera (Baer, 2003; Barrows, 1976; Boomsma et al., 2005; Eickwort and Ginsberg, 1980; Willmer and Stone, 2005). In one such review, Paxton (2005) presented a conceptual framework for examining male mating behaviour in bees. Paxton suggested an association between the location and density of nests and male mating behaviour. Territorial males were predicted when females are gregarious and when nests are densely aggregated, but males should adopt an alternative strategy when nests are dispersed. These predictions are echoed in other literature (Boomsma et al., 2005). Paxton's (2005) framework also suggests that foraging patterns can influence male mating strategy. Specifically, it is suggested that polylectic species should be territorial at nesting sites, while oligolectic species should defend at flowers (foraging sites) since females are very likely to visit them. This paper provides the data necessary to examine these frameworks for *X. virginica*.

I have demonstrated that *X. virginica* displays female defence polygyny where territorial males guard sites containing nests and consequently females. These nesting sites are typically dense and are highly aggregated. *Xylocopa virginica* is polylectic (Hurd, 1978; Hurd and Moure, 1963; Rutgers-Kelly, 2005) and while evidence suggests alternative mating tactics, this is expected when some males have significant mating advantages. In this study, residents did not mate more often, but had more interactions with females, which may represent such an advantage. In addition, territoriality appears to be the primary tactic and also the most common, as it is *X. fimbriata* and *X. gualanensis,* which also have dense nesting aggregations (Vinson and Frankie, 1990). In contrast, males in species with less gregarious nesting, such as *X. sulcatipes*, guard foraging sites (Velthuis and Gerling, 1980) or adopt a strategy similar to a mammalian lek (Minckley and Buchmann, 1990; Minckley et al., 1991).

While the predictive frameworks presented seem to conform to *Xylocopa* behaviour, this is not true for all species. *Anthidium manicatum* (Jaycox, 1967; Nachtigall, 1997) and *Osmia rufa* (Seidelmann, 1999), for example, are polylectic but are not territorial. This might indicate that other factors are involved with mating tactic choice, but there may also be taxonomic complications. Paxton, Baer and Boomsma have all stressed the potential benefits of examining male hymenopteran behaviour in taxa with multiple tactics and in the context of phylogeny (Baer, 2003; Boomsma et al., 2005; Paxton, 2005). I propose that the genus *Xylocopa* would be well suited to these studies because individuals are large, there is enormous variation in mating system both within and between species, individuals of both sexes have been studied and because factors such as nest density can easily be quantified. In addition, while no species level phylogeny exists, there are phylogenies of the subgenera and Velthuis and Gerling (1980) have speculated that male behaviour is consistent within a subgenus.

Finally, theoretical and conceptual studies of mating in bees make predictions about size relative to mating strategy or system. Paxton (2005) notes that in solitary species which are not territorial, males should show greater size dimorphism than in territorial species. Alcock et al. (1977) suggests that if males are larger than females they would be difficult to repel resulting in polyandry. Size has been shown to influence mating system in some species of bee. For instance, in communal *Perdita* that exhibit mixed mating systems (mating inside and outside nests), large males monopolize mating within nests while smaller males obtain mating opportunities at flowers (Danforth, 1991).

Alcock et al (1976) found that size differences between males with different mating tactics in *Centris pallida*; larger males searched for females at nesting sites (Alcock et al., 1976).

My data suggest that *X. virginica* males are smaller than females (Figure 3.8) but still territorial and polygynous; so size dimorphism may result from differential sex and resource allocation decisions rather than mating biology.

Conclusions

Previous studies of *Xylocopa* mating behaviour have cited a need for data on male mating tactics with respect to female densities and male size. Here I present such data for *X. virginica*. My analyses show that male *X. virginica* exhibit female defence polygyny, but probably have alternate mating tactics such as resource defence polygyny at flowers. Males appear to prefer to defend “high quality” territories with more nests and females. While size does not differ between satellite and resident males, residents are more aggressive and may have an inherent advantage.

General Discussion and Conclusions

Associations between males and females

In this thesis, I examine the behaviour of both males and females from the same population. This is rather unusual in social insect biology where most studies concentrate on females, and in which studies of males rarely consider social organization. *Xylocopa* is a particularly good group in which to conduct integrative studies of male and female behaviour because there is variation in both social organization and male mating strategy. Many theoretical frameworks that describe evolution of male mating tactics are based on ecological and life-history traits realized via females, such as nesting site selection, sex allocation and choice of foraging patches. I found that males of *X. virginica* are territorial, in accordance with many predictions. I also found evidence that many instances of males pursuing females do not result in mating. Further, females are often not receptive to mating attempts, suggesting that females may only mate once. These factors may greatly influence relatedness and in turn elements related to sociality in these bees.

Anomalous 2004 season

A reoccurring trend in this thesis is that the 2004 season was anomalous. In almost every comparison among years, significant variation among years was due to differences in 2004. In 2004, the population size was smaller (as it was in 2005, which also followed a cold and wet season), females had smaller head widths, males had larger head widths, and females spent more time in nests between foraging trips and exhibited longer foraging times. Further, both males and females were active on fewer days and the density of males near territories was lower. It is likely that many of these aspects are related and associated with weather (2004 was unusually cold and wet). However, I hypothesize that some factors such as the size individuals reach, and the size of a population are associated with the provisions they are provided as brood, and thus the weather in the previous bee season. This would mean that the size of individuals in 2004, and the small population size in 2004 would be related to the weather in 2003, which was both wet and cold. It appears that various elements of life-history are influenced by weather in this species.

Sociality in *Xylocopa*

In this study, I provide evidence that *Xylocopa* (Xylocopoides) *virginica virginica* is social. Sociality was supported by a combination of behavioural observations at nest entrances, video observation of behaviour within nests, and dissections and analyses of wear and ovarian development in females. Elements of nest architecture and phenology also support these conclusions.

Early studies of Xylocopini suggested that the tribe contained either solitary, social or in some instances communal species. Instances of multiple females within a nest were attributed to a mistake or some variety of coincidence (Michener, 1974; Wilson, 1971). These findings are being overturned as detailed reports of multi-female colonies of *Xylocopa* become available (Sabrosky, 1962; Stark, 1992a; Steen, 2000; Watmough, 1974). Studies suggest that species of *Xylocopa* may be communal, quasi- or semi-social and perhaps primitively eusocial. Gerling at al. (1983) have proposed the term metasocial to describe a form of social organization in *Xylocopa* where there is cooperation between females of more than one generation but no matrifilial associations. Detailed studies have demonstrated both division of labour and reproductive skew in the *Xylocopa*, among both relatives and unrelated individuals (Gerling et al., 1983; Gerling et al., 1989; Hogendoorn and Leys, 1993; Stark, 1992a; Wcislo, 1996). These relationships are often based on complex tradeoffs involving guarding and pollen robbery (Dunn and Richards, 2003; Hogendoorn, 1996; Hogendoorn and Leys, 1993), but may also be related to nest inheritance (Stark, 1992a; Steen, 2000).

In *X. virginica*, it appears that social nests are either aggregations of sisters, or nieces and aunts. Since these bees are univoltine, it is unlikely that there are any significant matrifilial associations. As in other social *Xylocopa*, social nests demonstrate a division of labour and reproductive skew. In most instances, a single female is reproducing and performing most work, but there are instances when multiple females show physical signs of flying or nest construction. Division of labour is seemingly more common in nests with more foundresses, as nests with more foundresses contain more foundresses that demonstrate some wear or OD. However, since brood evidence suggests that cells are provisioned one at a time, these bees are not communal. Most appropriately this species should be classified as either semi-social or metasocial.

Social *X. virginica* probably result from females remaining within the natal nest. In some instances, these females may have attempted to found nests in spring and failed. These failed females may function as guards, but there are no apparent benefits to guards in the populations studied. It is possible that this behaviour is ancestral to the group and is exhibited even when it is unnecessary. It is also possible that guards protect against nest usurpation or pollen robbery, but evidence of such behaviours is limited. I propose that social nesting did not evolve uniquely in *X. virginica,* but instead is more likely inherited from a common ancestor in the *Xylocopa* or perhaps the Xylocopini. The fact that social nesting remains in this species likely results from multiple factors including the benefit of a guard in some populations (although not in the northerly extreme of the range studied here) and, more importantly, the fitness gains associated with nest inheritance as suggested by Ragsdale (1999). One topic that deserves further investigation is the mechanism that determines nest inheritance, as this may greatly affect such a system.

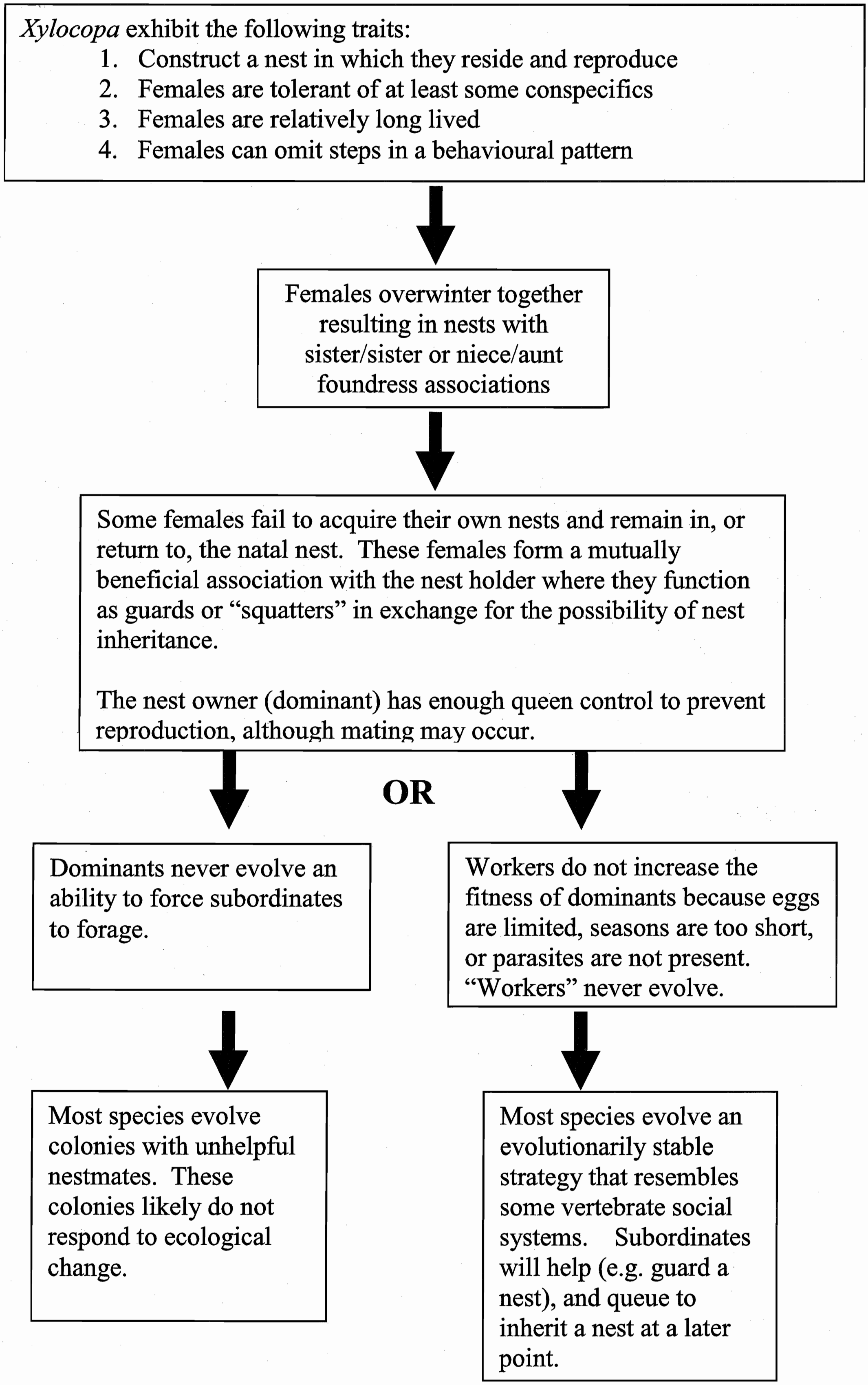
In an attempt to explain the evolution of sociality, Michener (1974) proposed a series of factors that may function as pre-adaptations to cooperation. These factors include construction of a nest that houses the reproductive female and brood, long reproductive life of adult females, tolerance of bees of the same species and the ability to omit parts of stereotyped behaviours such as brood cell construction. These factors resemble traits considered pertinent to social evolution in birds (Bull and Schwarz, 1996). In birds, and in allodapine bees, two additional factors, staggered opportunities for reproduction and progressive rearing, are also considered relevant to evolution of cooperation. In this thesis, I present data necessary to evaluate most of these factors in *X. virginica*, while published data can be used for comparisons with other species. I suggest that while *Xylocopa* and perhaps all Xylocopinae have pre-adaptations to cooperation, ecological conditions were not appropriate for these factors to result in the evolution of advanced sociality in most species. Further, I propose that the form of sociality exhibited by *X. virginica* is evolutionarily stable.

X. virginica, as with some other species of *Xylocopa,* exhibits a form of sociality where a single dominant female performs most tasks associated with reproduction. Inside females (presumed subordinates) compose the higher percentage of unworn females, which suggests that inside females do little work and may wait to inherit nests in the following season. These behaviours are similar to those observed in birds such as scrub and grey jays, which have helpers that perform some work, do not reproduce, and later inherit a territory (Cockburn, 1998; Emlen, 1982; Stacey and Ligon, 1987; Waite and Strickland, 1997). In many of these scenarios, the "helper" has much to gain (experience, a territory, a nest etc.), but there is little cost to the individual receiving the assistance, who may also receive some added fitness. In the *Xylocopa* studied here, the additional females inherit a nest, do not cost the nest holder, and offer some benefit (possible indirect fitness and perhaps some protection for the nest). It is interesting, though, that this behavioural pattern is considered evolutionarily stable (an evolutionary stable strategy) in birds, but in insects, it is considered unusual or even maladaptive. This attitude is present despite the occurrence of similar behaviour in species of *Xylocopa* (Gerling et al., 1983; Hogendoorn and Velthuis, 1995; Michener, 1990)*, Exoneura* (Bull and Schwarz, 1996), the sweat bee *Pseudaugochloropsis spp.* (Michener, 1974), and in wasps (Strassmann and Queller, 1989). I suggest that cooperation in birds and carpenter bees may have evolved due to similar ecological pressures acting on similar pre-adaptations; although, carpenter bees likely had more pre-adaptations than jays.

It is of note that in a few instances, when foraging females were removed, an additional unmarked female was seen returning to the nest with pollen. These females may either be newly emerged females that are returning with pollen for all their siblings, or inside females that have replaced the original forager in feeding the brood. Both of these explanations are possible; however, my data cannot distinguish between them.

A proposed sequence for the evolution of sociality in *Xylocopa*

Given that *Xylocopa,* and possibly all the Xylocopinae exhibit many of the proposed pre-adaptations for cooperation, I hypothesize the sequence of events outlined in Figure D.1 to explain the evolution of sociality in some species of *Xylocopa.* Much of



Xylocopa exhibit the following traits:

1. Construct a nest in which they reside and reproduce
2. Females are tolerant of at least some conspecifics
3. Females are relatively long lived
4. Females can omit steps in a behavioural pattern

Females overwinter together resulting in nests with sister/sister or niece/aunt foundress associations

Some females fail to acquire their own nests and remain in, or return to, the natal nest. These females form a mutually beneficial association with the nest holder where they function as guards or "squatters" in exchange for the possibility of nest inheritance.

The nest owner (dominant) has enough queen control to prevent reproduction, although mating may occur.

Dominants never evolve an ability to force subordinates to forage.

Workers do not increase the fitness of dominants because eggs are limited, seasons are too short, or parasites are not present. "Workers" never evolve.

Most species evolve colonies with unhelpful nestmates. These colonies likely do not respond to ecological change.

Most species evolve an evolutionarily stable strategy that resembles some vertebrate social systems. Subordinates will help (e.g. guard a nest), and queue to inherit a nest at a later point.

Figure D.1: A hypothetical scenario for the evolution of sociality in *Xylocopa* spp.

what is presented here is speculation; however, these steps can be examined with data provided in this thesis, published studies, or in future work.

I suggest that, since *Xylocopa* are long-lived (Michener, 1974) and reproduce within nests (that can typically be re-used for many seasons), there is a high probability that generations will overlap, or that female brood will reside together for an extended period. Matrifilial associations might thus be rare, since foraging females do not usually live multiple seasons, they appear to die during their second winter, but aunt-niece and associations between sisters would be common.

Once multiple female nesting had evolved, it would have only have taken a few simple steps to result in dominant and subordinate roles. If, at the start of a season, some females failed to found nests, then they may have returned to, or remained in, the natal nest. While these returning females probably did not reproduce that season, they would have been in a position to inherit the nest the following season. A queuing female would have been in an inferior position relative to the female that retained the nest, but would have experienced a delayed fitness benefit, since she would not have reproduced otherwise. The nest-holder would benefit if the queuing female guarded against predation, parasitism, pollen robbery or nest usurpation as observed in other species of *Xylocopa* (Dunn and Richards, 2003; Gadagkar, 1985; Gerling et al., 1983; Michener, 1990; Stark, 1992a; Watmough, 1974; Watmough, 1983). Over time, this mutually beneficial situation probably evolved so that the nest-holder gained more control over the queuing female. Eventually dominants evolved enough control to prevent the subordinate from reproducing, but could not force them to perform significant work.

If the social structure I describe is stable, as in birds or some allodapine bees (Bull and Schwarz, 1996), then it can sufficiently explain *Xylocopa* sociality. In these bees, workers may not result in greater fitness, reduced risk to foraging individuals, or lower brood mortality, and so selection for workers to evolve never existed. Many species of *Xylocopa* are univoltine, have short seasons, relatively long development times and must feed juveniles. Together, these factors may limit the fitness benefit of workers. Further, *Xylocopa* have very large oocytes (Iwata, 1964; Michener, 1974), which may take a long time to develop, resulting in a limit to the number of offspring that a female can produce. Such a limit reduces the utility of workers because the collection of pollen would likely outpace the development of oocytes. Finally, workers are beneficial if they result in longer-lived dominants that receive more opportunity to reproduce. However, evidence suggests low mortality in *Xylocopa* during foraging (Watmough, 1983) so workers are unlikely to increase lifespan of dominants. This scenario would favour guarding subordinates, but not workers. On the other hand, queen control may be limited in *Xylocopa.* A dominant may prevent other females from reproduction but may be unable to coerce any to perform helping tasks. If dominants cannot force others to work, a colony structure with subordinate females that do not forage but remain in nests will once again result. This, however, would be less a stable strategy than an evolutionary "dead end". Specifically, in the guard scenario different colony structures may evolve in response to different ecological conditions, while a "dead end" scenario implies an inability to evolve alternative colony structures. In the proposed guarding scenario, advanced sociality may arise; however, in the proposed "dead end" scenario advanced sociality cannot.

In summary, I suggest that meta/semi sociality as observed in *Xylocopa* is a stable strategy. Specifically, I propose that while these bees have most of the prerequisites for advanced forms of sociality, they are not in a position to benefit from workers. Instead, mutual benefits from guarding and nest inheritance have encouraged multi-female associations. These associations were acted upon by one or both of two pathways, which are not mutually exclusive. Since females did not benefit from workers, and dominants could not force subordinates to forage, *X. virginica*, and perhaps other species of *Xylocopa* did not evolve advanced sociality. However, the benefits of having nest inheritance to subordinates, or from reduced risk from a guard, makes this workerless association evolutionarily stable.

Further Research

Relatedness information would contribute significantly to further understanding of *Xylocopa* behaviour. Specifically, knowing whether a single female lays all eggs within a nest would allow examination of reproductive skew in full. Relatedness information would also allow evaluation of Hamilton's equation (Hamilton, 1964) in this species. Genetic evidence from microsatellite markers would also aid in quantifying male reproductive success. Unfortunately, because of time and cost constraints attempts to develop such markers were abandoned. While allozymes may be effective for some such analyses, preliminary screens did not reveal allelic variation, and these data were not robust enough for all analyses. In addition, many individuals were dead when nests were collected or died during development, making them unsuitable for allozyme analyses. Therefore future work should attempt to develop robust microsatellite markers for *X. virginica*, and ideally these markers will function across many species of *Xylocopa*.

A robust species-level phylogeny is essential to future work on *Xylocopa*. Currently the only phylogenies for the tribe (genus) are on the level of subgenus. With more than five hundred species and 30 subgenera, the relationships between many species must be inferred based on inconsistent taxonomic placements. One approach would be to create a phylogeny of the New World species first, as these appear to be a distinct clade within *Xylocopa.* This would allow further comparative studies to stem from this work on *X. virginica.* Future work on *Xylocopa* should concentrate on behavioural and genetic studies of species chosen based on phylogeny or specific ecological factors.

Literature Cited

Abrams J, Eickwort GC, 1981. Nest switching and guarding by the communal sweat bee *Agapostemon virescens* (Hymenoptera, Halictidae). Insectes Sociaux 28(2):105-116.

Abrol DP, 1992. Oxygen consumption of foraging bees. Journal of Animal Morphology and Physiology 39:27-42.

Alcock J, 1978. The ecology and evolution of male reproductive behavior in the bees and wasps. Zoological Journal of the Linnean Society 64:293-326.

Alcock J, 1991. Mate-locating behavior of *Xylocopa californica arizonensis* Cresson (Hymenoptera: Anthophoridae). Journal of the Kansas Entomological Society 64:349-356.

Alcock J, Eickwort GC, Eickwort KR, 1977. The reproductive behavior of *Anthidium maculsum* (Hymenoptera: Megachilidae) and the evolutionary significance of multiple copulations by females. Behavioral Ecology and Sociobiology 2:385-396.

Alcock J, Johnson MD, 1990. Female mate choice in the carpenter bee *Xylocopa varipuncta* (Hymenoptera: Anthophoridae). Journal of Zoology 221:195-204.

Alcock J, Smith AP, 1987. Hilltopping, leks and female choice in the carpenter bee *Xylocopa* (*Neoxylocopa*) *varipuncta*. Journal of Zoology 211:1-10.

Alcock JP, Jones CE, Buchmann SL, 1976. Location before emergence of the female bee, *Centris pallida* by its male (Hymenoptera: Anthophoridae). Journal Zoology London 179:189-199.

Alexander RD, 1974. The evolution of social behaviour. Annual Review of Ecological Systems 5:325-383.

Baer B, 2003. Bumblebees as model organisms to study male sexual selection in social insects. Behavioral Ecology and Sociobiology 54:521-533.

Baker RR, 1972. Territorial behavior of the nymphalid butterflies *Agalis urticae* (L) and *Inachis io* (L). Journal of Animal Ecology 41:453-69.

Baker RR, 1983. Insect Territoriality. Annual Review of Entomology 28:65-89.

Balduf WV, 1962. Life of the carpenter bee, *Xylocopa virginica* (Linn.) (Xylocopidae, Hymenoptera). Annals of the Entomological Society of America 55:263-271.

Barrows EM, 1976. Mating Behavior in Halictine Bees (Hymenoptera: Halictidae): II. Microterritorial and Patrolling Behavior in Males of *Lasioglossum rohweri*. Zeitschrift fur Tierpsychologie 40:377-389.

Barrows EM, 1983. Male Territoriality in the Carpenter Bee *Xylocopa virginica*. Animal Behaviour 31:806-813.

Barthell JF, Baird TA, 2004. Size variation and aggression among male *Xylocopa virginica* (L.) (Hymenoptera: Apidae) at a nesting site in central Oklahoma. Journal of the Kansas Entomological Society 77:10-20.

Bonelli B, 1976. Observatione etoecologiche sugli Imenotteri aculeati dell'Etopia. VIII. *Xylocopa* (*Mesotrichia) combusta* Smith (Hymenoptera-Anthophoridae). Bollettino dell'Istituto di entomologia della Università di Bologna 32:1412-1422.

Boomsma JJ, Baer B, Heinze J, 2005. The Evolution of Male Traits in Social Insects. Annual Review of Entomology 50:395-420.

Boomsma JJ, Eickwort GC, 1993. Colony structure, provisioning and sex allocations in the sweat bee *Halictus ligatus* (Hymenoptera: Halictidae). Biological Journal of the Linnaean Society 48:355-377.

Boomsma JJ, Ratnieks FLW, 1996. Paternity in eusocial Hymenoptera. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 351:947-975.

Bosch J, Kemp WP, 2004. Effect of pre-wintering and wintering temperature regimes on weight loss, survival, and emergence time in the mason bee *Osmia cornuta* (Hymenoptera: Megachilidae). Apidologie 35:469-479.

Bosch J, Vicens N, 2006. Relationship between body size, provisioning rate, longevity and reproductive success in females of the solitary bee *Osmia cornuta*. Behavioral Ecology and Sociobiology 60:26-33.

Bull NJ, Mibus AC, Norimatsu Y, Jarmyn BL, Schwarz MP, 1998. Giving your daughters the edge: bequeathing reproductive dominance in a primitively social bee. Proceedings of the Royal Society of London Series B-Biological Sciences 265:1411-1415.

Bull NJ, Schwarz M, 1996. The habitat saturation hypothesis and sociality in an allodapine bee: cooperative nesting is not "making the best of a bad situation". Behavioral Ecology and Sociobiology 39:267-274.

Camillo E, Garofalo CA, 1989. Social organization in reactivated nests of three species of *Xylocopa* (Hymenoptera, Anthophoridae) in southeastern Brazil. Insectes Sociaux 36:92-105.

Camillo E, Garofalo CA, 1982. On the bionomics of *Xylocopa frontalis* (Oliver) and *Xylocopa grisescens* (Lepeletier) in southern Brazil. I. Nest construction and biological cycle. Revista Brasileira de Biologia 42:571-582.

Cartar R, 1992. Morphological senescence and longevity: an experiment relating wing wear and life span in foraging wild bumble bees. Journal of Animal Ecology 61:225-231.

Chapman GB, 1999. Some cytological features of follicle cells in an ovariole of the carpenter bee (*Xylocopa virginica virginica*) (L) (Hymenoptera: Apidae). Tissue & Cell 31:421-427.

Chapman GB, Abu-Eid C, 2001. The proventriculus of the carpenter bee, *Xylocopa virginica virginica* (L.), with special reference to bacteria-containing luminal tunnels. Invertebrate Biology 120:78-87.

Chapman GB, 1986. Ultrastructural Features of the Cells of Dufor's Gland and Associated Structures in the Carpenter Bee, *Xylocopa virginica virginica* (L.) (Hymenoptera: Anthrophoridae). Annals of the Entomological Society of America 79:1008-1018.

Chapman TW, Kranz BD, Bejah K-L, Morris D, schwarz M, Crespi B, 1998. The evolution of soldier reproduction in social thrips. Behavioral Ecology 13:519-525.

Clutton-Brock T, Brotherton PNM, Russell AF, O'Rain MJ, Gaynor D, Kansky R, Griffin A, Manser M, Sharpe L, McIlrath GM, Small T, Moss A, Monfort S, 2001. Cooperation, Control and Concession in Meerkat Groups. Science 291:478-481.

Cockburn A, 1998. Evolution of Helping Behavior in Cooperatively Breeding Birds. Annual Review of Ecology and Systematics 29:141-177.

Cooper PD, Schaffer WM, Buchmann SL, 1985. Temperature Regulation of Honey Bees (*Apis melliferai*) Foraging in the Sonoran Desert. Journal of Experimental Biology 114:1-15.

Cronin AL, 2001. Social flexibility in a primitively social allodapine bee (Hymenoptera: Apidae): results of a translocation experiment. Oikos 94:337-343.

Cronin AL, Schwarz MP, 1999a. Latitudinal variation in the life cycle of allodapine bees (Hymenoptera; Apidae). Canadian Journal of Zoology-Revue Canadienne De Zoologie 77:857-864.

Cronin AL, Schwarz MP, 1999b. Latitudinal variation in the life cycle of allodapine bees (Hymenoptera; Apidae). Canadian Journal of Zoology 77:857-864.

Cronin AL, Schwarz MP, 1999c. Life cycle and social behavior in a heathland population of *Exoneura robusta* (Hymenoptera: Apidae): Habitat influences opportunities for sib rearing in a primitively social bee. Annals of the Entomological Society of America 92:707-716.

Cronin AL, Schwarz MP, 2001. Latitudinal variation in the sociality of allodapine bees (Hymenoptera: Apidae): sex ratios, relatedness and reproductive differentiation. Australian Journal of Zoology 49:1-16.

Crozier R, Pamilo P, 1996. Evolution of social insect colonies, sex allocation and kin selection. New York: Oxford University Press.

Da Silva MM, Bruckner CH, Picanco M, Molina-Rugama AJ, 1999. *Passiflora edulis* f. flavicarpa pollination, floral number, climate and population density of *Xylocopa spp*. (Hymenoptera: Anthophoridae). Revista De Biologia Tropical 47:711-718.

Daly HV, 1966. Biological Studies on *Ceratina dallatorreana,* an Alien Bee in California Which Reproduces by Parthenogenesis (Hymenoptera: Apoidea). Annals of the Entomological Society of America 59:1138-1154.

Daly HV, Michener CD, Moure JS, Sakagami SF, 1987. The relictual bee genus Manuelia and its relation to other Xylocopinae (Hymenoptera: Apoidea). Pan-Pacific Entomologist 63:102-124.

Danforth BN, 1991. The morphology and behavior of dimorphic males in *Perdita portalis* (Hymenoptera: Andrenidae). Behavioral Ecology and Sociobiology 29:235-247.

Danforth BN, Desjardins CA, 1999. Male dimorphism in *Perdita portalis* (Hymenoptera, Andrenidae) has arisen from preexisting allometric patterns. Insectes Sociaux 46:18-28.

Danforth BN, Neff JL, 1992. Male polymorphism and polyethism in *Perdita texana* (Hymenoptera: Andrenidae). Annals of the Entomological Society of America 85:616-626.

Dhaliwal HS, Kapil RP, 1968. Defence of nest by the female of *Xylocopa fenestrata* Fab. (Xylocopinae, Hymenoptera). Insectes Sociaux 15:419-422.

Dillon W, Goldstein M, 1984. Multivariate analysis: Methods and applications. New York: John Wiley and Sons.

Doolan SP, Macdonald DW, 1999. Co-operative Rearing by Slender-tailed Meerkats (Suricata suricatta) in the Southern Kalahari. Ethology. Berlin, Hamburg 105:851-866.

Duchateau MJ, Velthuis HHW, 1988. Development and Reproductive Strategies in *Bombus terrestris* Colonies.

Duffy J, 1996. Eusociality in a coral-reef shrimp. Nature 381:512-514.

Duffy J, Macdonald KS, 2000. Multiple origins of eusociality among sponge-dwelling shrimps (*Synalpheus*). Evolution 54:503-516.

Dugatkin LA, Reeve HK, 1998. Game Theory and Animal Behavior. New York, Oxford: Oxford University Press; 319.

Dukas R, Real LA, 1991. Learning foraging tasks by bees: A comparison between social and solitary species. Animal Behaviour 42:269-276.

Dunn T, Richards MH, 2003. When to bee social: interactions among environmental constraints, incentives, guarding, and relatedness in a facultatively social carpenter bee. Behavioral Ecology 14:417-424.

Eardley CD, 1983. A taxonomic revision of the genus *Xylocopa* Latreille (Hymenoptera: Anthophoridae) in southern Africa.

Eickwort GC, Eickwort JM, Gordon J, Eickwort MA, 1996. Solitary behavior in a high-altitude population of the social sweat bee *Halictus rubicundus* (Hymenoptera: Halictidae). Behavioral Ecology and Sociobiology 38:227-233.

Eickwort GC, Ginsberg HS, 1980. Foraging and mating behavior in Apoidea. Annals of the Entomological Society of America 25:421-446.

Emlen S, 1982. The evolution of helping. I. An ecological constraints model. American Naturalist 119:29-39.

Emlen S, Oring LW, 1977. Ecology, sexual selection and the evolution of mating systems. Science 198:215-233.

Franckie W, Vinson SB, Lewis LA, 1977. Territorial behvaior in male *Xylocopa micans* (Hymenoptera: Anthophoridae). Journal of the Kansas Entomological Society 52:313-323.

Frankie GW, and S. B. Vinson, 1977. Scent marking in Texas by Females of *Xylocopa virginica texana* (Hymenoptera: Anthophoridae). Journal of the Kansas Entomological Society 50:613-625.

Friese H, 1923. Die europaischen Bienen (Apidae). Berlin and Leipzig: W. de Cruyter.

Gadagkar R, 1985. Evolution of insect sociality: A review of some attempts to test modern theories. Proceedings of the Indian Academy Sciences (Animal Sciences) 94:309-324.

Gerling D, Hermann HR, 1976. Biology and Mating Behavior of *Xylocopa virginica* L. (Hymenoptera, Anthrophoridae). Behavioral Ecology and Sociobiology 3:99-111.

Gerling D, Hurd J, P.D., 1976. The Oviposition and Life Cyce of *Anthrax tigrinus*, *[DIPT.: BOMBYLIIDAE]* A Parasite of Carpenter Bees *[HYM.: XYLOCOPIDAE*]. Entomophaga 21:227-233.

Gerling D, Hurd PD, Jr., Hefetz A, 1981. In-Nest Behavior of the Carpenter Bee, *Xylocopa pubescens* Spinola (Hymenoptera: Anthophoridae). J. Kansas Entomological Society 54:209-218.

Gerling D, Hurd PD, Jr., Hefetz A, 1983. Comparative behavioral biology of two Middle East species of carpenter bees (*Xylocopa* Latreille) (Hymenoptera: Apoidea). Smithsonian Contributions to Zoology 369:1-33.

Gerling D, Velthuis HHW, Hefetz A, 1989. Bionomics of the large carpenter bees of the genus *Xylocopa*. Annual Review of Entomology 34:163-190.

Goodell K, 2003. Food availability affects *Osmia pumila* (Hymenoptera: Megachilidae) foraging, reproduction, and brood parasitism. Oecologia 134:518-527.

Guttman NB, M.S. Plantico, 1987. Climatic Temperature Normals. Journal of Climate and Applied Meteorology 26:1528-1435.

Hamilton WD, 1964. The genetical evolution of social behaviour. I. II. Journal of Theoretical Biology 7:1-52.

Hamilton WD, 1972. Altruism and related phenomena, mainly in social insects. Annual Review of Ecology and Systematics 3:193-232.

Hansell MH, 1993. The ecological impact of animal nests and burrows. Functional Ecology 7:5-12.

Hines HM, Hunt JH, O'Conner TK, Gillespie JJ, Cameron SA, 2007. Multigene phylogeny reveals eusociality evolved twice in vespid wasps. Proceedings of the National Academy of Science, USA 104:3295-3299.

Hogendoorn K, 1991. Intraspecific competition in the carpenter bee *Xylocopa pubescens* and its implications for the evolution of sociality. Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society 2:123-128.

Hogendoorn K, 1996. Socio-economics of brood destruction during supersedure in carpenter bee *Xylocopa pubescens*. Journal of Evolutionary Biology 9:931-952.

Hogendoorn K, Leys R, 1993. The superseded female's dilemma: Ultimate and proximate factors that influence guarding behaviour of the carpenter bee *Xylocopa pubescens*. Behavioral Ecology and Sociobiology 33:371-381.

Hogendoorn K, Velthuis HHW, 1993. The sociality of *Xylocopa pubescens*: Does a helper really help? Behavioral Ecology and Sociobiology 32:247-257.

Hogendoorn K, Velthuis HHW, 1995. The role of young guards in *Xylocopa pubescens*. Insectes Sociaux 42:427-448.

Hogendoorn K, Velthuis HHW, 1999. Task allocation and reproductive skew in social mass provisioning carpenter bees in relation to age and size. Insectes Sociaux 46:198-207.

Hurd J, P.D., 1958. Observations on the nesting habits of some new world carpenter bees with remarks on their importance in the problem of species formation. Annals of the Entomological Society of America 51:365-375.

Hurd J, P.D., 1978. An annotated catalogue of the carpenter bees (Genus *Xylocopa* Latreille) of the Western hemisphere (Hymenoptera: Anthophoridae). Washington, DC: Smithsonian Institute.

Hurd J, P.D., Moure JS, 1963. A classification of the large carpenter bees (Xylocopini) (Hymenoptera: Apoidea). University of Kansas Science Bulletin 29:4-16.

Iwata K, 1964. Egg giantism in subsocial Hymenoptera, with ethological discussion on tropical bamboo carpenter bees. Nature Life Southeast Asia 3:339-435.

Janzen DH, 1981. Bee Arrival at 2 Costa-Rican Female Catasetum Orchid Inflorescences, and a Hypothesis on Euglossine Population-Structure. Oikos 36:177-183.

Jarvis JUM, Bennett NC, 1993. Eusociality has evolved independently in two genera of bathyergid mole-rats - but occurs in no other subterranean mammal. Behavioral Ecology and Sociobiology 33:253-260.

Jaycox ER, 1967. Territorial behavior among males of *Anthidium bannigense* (Hymenoptera: Megachilidae). Journal of the Kansas Entomological Society 40:565-570.

Jeanne RL, 1975. The adaptiveness of social wasp nest architecture. The Quarterly Review of Biology 50:267-287.

Johnstone RA, 2000. Models of reproductive skew: a review and synthesis. Ethology 106:5-26.

Johnstone RA, Kokko H, 1999. Social queing in animal societies: a dynamic model of reproductive skew. Proceedings of the Royal Society of London Series B Biological Sciences 266:571-578.

Karsai I, Wenzel J, 1998. Productivity, individual-level and colony-level flexibility, and organization of work as consequences of colony size. Proceedings of the National Academy of Science, USA 95:8665-8669.

Keller L, Reeve HK, 1994. Partitioning of reproduction in animal societies. Trends in Ecology & Evolution 9:98-102.

Kelly CD, 2006a. Fighting or harems: assessment strategies during male-male contests in the sexually dimorphic Wellington tree weta. Animal Behaviour 72:727-736.

Kelly CD, 2006b. Resource quality or harem size: what influences male tenure at refuge sites in the tree weta (Orthoptera: Anostostomatidae)? Behavioral Ecology and Sociobiology 60:175-183.

Kemp DJ, Wiklund C, 2001. Fighting without weaponry: a review of male-male contest competition in butterflies. Behavioral Ecology and Sociobiology 49:429-442.

Kemp DJ, Wiklund C, 2004. Residency effects in animal contests. Proceedings of the Royal Society of London Series B Biological Sciences 271:1707-1711.

Kent D, Simpson J, 1992. Eusociality in the beetle *Austroplatypus incompertus* (Coleoptera: Platypodidae). Naturwissenschaften 79:86-87.

Kranz BD, Schwarz M, Morris D, Crespi B, 2002. Life History of *Kladothrips ellobus* and *Oncothrips rodwayi*: insight into the origin and loss of soldiers in gall-inducing thrips. Ecological Entomology 27.

Kranz BD, Schwarz MP, Giles LC, Crespi BJ, 2000. Split sex ratios and virginity in a gall-inducing thrips. Journal of Evolutionary Biology 13:700-706.

Kranz BD, Schwarz MP, Mound LA, Crespi BJ, 1999. Social biology and sex ratios of the eusocial gall-inducing thrips *Kladothrips hamiltoni*. Ecological Entomology 24:432-442.

Kukuk P, May B, 1991. Colony dynamics in a primitively eusocial halictine bee *Lasioglossum (Dialictus) zephyrum* (Hymenoptera: Halictidae). Insectes Sociaux 38:171-189.

Kvarnemoa C, Ahnesjo I, 1996. The dynamics of operational sex ratios and competition for mates. Trends in Ecology & Evolution 11:404-408.

Leys R, 2000a. Mate locating strategies of the green carpenter bees *Xylocopa* (Lestis) *aeratus* and *X.* (L.) *bombylans*. Journal of Zoology 252:453-462.

Leys R, 2000b. A revision of the Australian carpenter bees, genus *Xylocopa* Latreille, subgenera *Koptortosoma* Gribodo and Lestis Lepeletier & Serville (Hymenoptera: Apidae). Invertebrate Taxonomy 14:115-136.

Leys R, Cooper SJB, Schwarz MP, 2000. Molecular phylogeny of the large carpenter bees, genus *Xylocopa* (Hymenoptera: Apidae), based on mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 17:407-418.

Leys R, Cooper SJB, Schwarz MP, 2002. Molecular phylogeny and historical biogeography of the large carpenter bees, genus *Xylocopa* (Hymenoptera: Apidae). Biological Journal of the Linnean Society 77:249-266.

Lin N, 1964. Increased parasitic pressure as a major factor in the evolution of social behavior in Halictine bees. Insectes Sociaux 11:187-192.

Lin N, Michener C, 1972. Evolution and selection in social insects. Quarterly Review of Biology 47:131-159.

Maeta Y, Miyanaga R, Sugiura N, Lu SS, 1996. Additional notes on the nesting habits of the Taiwanese bamboo carpenter bee, *Xylocopa* (Biluna) *tranquebarorum tranquebarorum* (Hymenoptera, Anthophoridae). Japanese Journal of Entomology 64:669-680.

Maeta Y, Sakagami SF, 1995. Oophagy and Egg Replacement in Artificially Induced Colonies of a Basically Solitary Bee, *Ceratina* (Ceratinidia) *okinawana* (Hymenoptera, Anthophoridae, Xylocopinae), with a Comparison of Social Behavior among Ceratina, *Xylocopa* and the Halictine Bees. Japanese Journal of Entomology 63:347-375.

Maeta Y, Sakagami SF, Shiokawa M, 1985. Observations on a nest aggregation of the Taiwanese bamboo carpenter bee *Xylocopa* (Biluna) *tranquebarorum tranquebarorum* (Hymenoptera, Anthophoridae). Journal of the Kansas Entomological Society 58:36-41.

Michener CD, 1969. Comparative Social Behavior of Bees. Annual Review of Entomology 14:299-342.

Michener CD, 1974. The Social Behavior of the Bees, A Comparative Study. Cambridge, Massachusetts: Harvard University Press.

Michener CD, 1985. From Solitary to Eusocial - Need There Be a Series of Intervening Species. Fortschritte Der Zoologie 31:293-305.

Michener CD, 1990. Caste in Xylocopine bees. In: Social Insects: An Evolutionary Approach to Castes and Reproduction (Engels, W, ed). Berlin New York: Springer Verlag; 120-144.

Michener CD, 2000. The Bees of the World. Baltimore, MD: The Johns Hopkins University Press.

Michener CD, Brothers DJ, 1974. Were workers of eusocial Hymenoptera initially altruistic or oppressed? Proceedings of the National Academy of Sciences, USA 71:671-674.

Michener CD, Kerfoot WB, 1967. Nests and social behavior of three species of *Pseudaugochloropsis*. Journal of the Kansas Entomological Society 40:214-232.

Minckley RL, 1994. Comparative morphology of the mesosomal 'gland' in male carpenter bees (Apidae: Xylocopini). Biological Journal of the Linnean Society 53:291-308.

Minckley RL, 1998. A Cladistic Analysis and Classification of the Subgenera of the Large Carpenter Bees, Tribe Xylocopini (Hymenoptera: Apidae). Scientifc Papers, Natural History Museum, The University of Kansas 9:1-47.

Minckley RL, Buchmann SL, 1990. Territory site selection of male *Xylocopa* (Neoxylocopa) *varipuncta* Patton (Hymenoptera: Anthophoridae). Journal of the Kansas Entomological Society 63:329-339.

Minckley RL, Buchmann SL, Wcislo WT, 1991. Bioassay evidence for a sex attractant pheromone in the large carpenter bee, *Xylocopa varipuncta* (Anthophoridae: Hymenoptera). Journal of Zoology 224:285-291.

Minckley RL, Wcislo WT, Yanega D, Buchmann SL, 1994. Behavior and phenology of a specialist bee (*Dieunomia*) and sunflower (*Helianthus*) pollen availability. Ecology 75:1406-1419.

Moritz REA, 1985. The effects of multiple mating on the worker-queen conflict in *Apis mellifera* L. Behavioral Ecology and Sociobiology 16:375-377.

Nachtigall W, 1997. Territorial Defence by Male Wool Carder bees, *Anthidium septemdentatum* (Hymenoptera: Megachilidae), Directed towards Giant Carpenter bees, *Xylocopa violacea* (Hymenoptera: Megachilidae). Entomologia Generalis 22:119-127.

Nininger HH, 1916. Studies on the life histories of two carpenter bees of California, with notes on certain parasites. Journal of Entomological Zoology 8.

Olsson M, Shine R, 2000. Ownership influences the outcome of male-male contests in the scincid lizard, *Niveoscincus microlepidotus*. Behavioral Ecology 11:587-590.

Packer L, 1986. The biology of a subtropical population of *Halictus ligatus*. IV. A cuckoo-like caste. Journal of the New York Entomological Society 94:458-466.

Packer L, 1988. The effect of *Bombylius pulchellus* (Diptera; Bombyliidae) and other mortality factors upon the biology of *Halictus ligatus* (Hymenoptera; Halictidae) in southern Ontario. Canadian Journal of Zoology 66:611-616.

Packer L, 1990. Solitary and eusocial nests in a population of *Augochlorella striata* (Provancher) (Hymenoptera: Halictidae) at the northern edge of its range. Behavioral Ecology and Sociobiology 27:339-344.

Packer L, 1991. The evolution of social behavior and nest architecture in sweat bees of the subgenus *Evylaeus* (Hymenoptera: Halictidae): A phylogenetic approach. Behavioral Ecology and Sociobiology 29:153-160.

Packer L, 1993. Multiple-foundress associations in sweat bees. In: Queen Number and Sociality in Insects (Keller, L, ed): Oxford University Press; 215-233.

Packer L, Sampson B, Lockerbie C, Jessome V, 1989. Nest architecture and brood mortality in four species of sweat bee (Hymenoptera: Halictidae) from Cape Breton Island. Canadian Journal of Zoology 67:2864-2870.

Paini DR, Bailey W, 2002. Seasonal sex ratio and unbalanced investment sex ratio in the Banksia bee *Hylaeus alcyoneus*. Ecological Entomology 27:713-719.

Paxton J, 2005. Male mating behavior and mating systems of bees: an overview. Apidologie 36:145-156.

Paxton RJ, Giovanetti M, Andrietti F, Scamoni E, Scanni B, 1999. Mating in a communal bee, *Andrena agilissima* (Hymenoptera Andrenidae). Ethology Ecology & Evolution 11:371-382.

Peso, MP, 2008. Nestmate recognition in the Large Carpenter Bee *Xylocopa virginica.* (Msc). St. Catharines, Ontario: Brock University.

Plowright RC, Pallett MJ, 1979. Worker-male conflict and inbreeding in bumble bees (Hymenoptera: Apidae). Canadian Entomologist 111:289-294.

Ragsdale J, 1999. Reproductive skew theory extended: The effect of resource inheritance on social organization. Evolutionary Ecology Research 1:859-874.

Rau P, 1933. The Jungle Bees and Wasps of Barro Colorodo Island (With Notes on Other Insects). St Louis, Mo.: Kirkwood.

Reeve HK, and L. Keller, 1995. Partitioning of reproduction in mother-daughter versus sibling associations: a test of optimal skew theory. American Naturalist 150:119-132.

Reeve HK, Emlen S, Keller L, 1998. Reproductive sharing in animal societies: reproductive incentives or incomplete control by dominant breeders? Behavioral Ecology 9:267-278.

Reeve HK, Jeanne RL, 2003. From individual control to majority rule: extending transactional models of reproductive skew in animal societies. Proceedings of the Royal Society of London Series B-Biological Sciences 270:1041-1045.

Reeve HK, Keller L, 2001. Tests of reproductive-skew models in social insects. Annual Review of Entomology 46:347-385.

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

Richards MH, 2000. Evidence for geographic variation in colony social organization in an obligately social sweat bee, *Lasioglossum malachurum* Kirby (Hymenoptera; Halictidae). Canadian Journal of Zoology 78:1259-1266.

Richards MH, 2001. Nesting biology and social organization of *Halictus sexcinctus* (Fabricius) in southern Greece. Canadian Journal of Zoology 79:2210-2220.

Richards MH, 2003. Variable worker behaviour in the weakly eusocial sweat bee, *Halictus sexcinctus* Fabricius. Insectes Sociaux 50:361-364.

Richards MH, 2004. Annual and Social Variation in Foraging Effort of the Obligately Eusocial Sweat Bee, *Halictus ligatus* (Hymenoptera: Halictidae). Journal of the Kansas Entomological Society 77:484-502.

Richards MH, Packer L, 1994. Trophic aspects of caste determination in *Halictus ligatus*, a primitively eusocial sweat bee. Behavioral Ecology and Sociobiology 34:385-391.

Richards MH, Packer L, 1995. Annual variation in survival and reproduction of the primitively eusocial sweat bee *Halictus ligatus* (Hymenoptera: Halictidae). Canadian Journal of Zoology 73:933-941.

Richards MH, Packer L, 1996. The socioecology of body size variation in the primitively eusocial sweat bee, *Halictus ligatus* (Hymenoptera: Halictidae). Oikos 77:68-76.

Richards MH, Packer L, Seger J, 1995. Unexpected patterns of parentage and relatedness in a primitively eusocial bee. Nature 373:239-241.

Richards MH, von Wettberg EJ, Rutgers AC, 2003. A novel social polymorphism in a primitively eusocial bee. Proceedings of the National Academy of Sciences, USA 100:7175-7180.

Roberts SP, Harrison JF, 1998. Mechanisms of Thermoregulation in Flying Bees. American Zoologist 38:492-502.

Roig-Alsina A, Michener CD, 1993. Studies of the phylogeny and classification of long-tongued bees (Hymenoptera: Apoidea). University of Kansas Science Bulletin. 55:123-173.

Roulston TH, Cane JH, 2002. The effect of pollen protein on body size in the sweat bee *Lasioglossum zephyrum* (Hymenoptera: Apiformes). Evolutionary Ecology 16:49-65.

Rutgers-Kelly AC, 2005. The bees of Niagara: a Test of the Intermediate Disturbance Hypothesis (Msc). St. Catharines, Ontario: Brock University.

Sabrosky CW, 1962. Mating in *Xylocopa virginica*. Proceedings of the Entomological Society of Washington 64: 184.

Sakagami SF, Laroca S, 1971. Observations on the Bionomics of Some Neotropical Xylocopine Bees, with Comparative and Biofaunistic Notes (Hymenoptera, Anthophoridae). Journal of the Faculty of Science Hokkaido University, Series IV, Zoology 18(1).

Sakagami SF, Maeta Y, 1977. Some Presumably Presocial Habits of Japanese *Ceratina* Bees, With Notes On Various Social Types In Hymenoptera. Insectes Sociaux 24:319-344.

Sakagami SF, Maeta Y, 1995. Task allocation in artificially induced colonies of a basically solitary bee Ceratina (Ceratinidia) okinawana, with a comparison of sociality between *Ceratina* and *Xylocopa* (Hymenoptera, Anthophoridae, Xylocopinae). Japanese Journal of Entomology 63:115-150.

Sakagami SF, Michener CD, 1987. Tribes of Xylocopinae and Origin of the Apidae (Hymenoptera: Apoidea). Annals of the Entomological Society of America 80:439-450.

Sakagami SF, Munakata M, 1972. Distribution and bionomics of a transpalaeartic eusocial halictine bee, *Lasioglossum (Evylaeus) calceatum*, in northern Japan, with reference to its solitary life cycle at high altitude. Journal of the Faculty of Science, Hokkaido University, Series 6 {Zoology} 18:411-439.

Sakagami SF, Zucchi R, Araujo VP, 1977. Oviposition Behavior of an Aberrant African Stingless Bee *Meliponula bocandei*, with notes on the mechanism and evolution of Oviposition Behavior in Stingless Bees. Zoology 20:647-690.

Schwarz MP, 1986. Persistent multi-female nests in an Australian allodapine bee, *Exoneura bicolor*. Insectes Sociaux 33:258-277.

Schwarz MP, Bull NJ, Hogendoorn K, 1998. Evolution of sociality in the allodapine bees: a review of sex allocation, ecology and evolution. Insectes Sociaux 45:349-368.

Schwarz MP, Richards MH, Danforth B, 2006. Changing Paradigms in Insect Social Evolution: Insights from Halictine and Allodapine Bees. Annual Review of Entomology 52:127-150.

Schwarz MP, Silberbauer LX, Hurst PX, 1997. Intrinsic and extrinsic factors associated with social evolution in allodapine bees. In: Evolution Of Social Behaviour In Insects and Arachnids (Choe, J and Crespi, B eds): Cambridge University Press; 293-315.

Seidelmann K, 1999. The race for females: The mating system of the red mason bee, *Osmia rufa* (L.) (Hymenoptera: Megachilidae). Journal of Insect Behavior 12:13-25.

Sherman PW, 1977. Nepotism and the evolution of alarm calls. Science 197:1246-53.

Shuster SM, Wade MJ, 2003. Mating Systems and Strategies. Princeton and Oxford: Princeton University Press.

Skaife SH, 1952. The yellow-banded carpenter bee *Mesotrichia caffra* (Linn.) and its symbiotic mite, *Dinogamassus braunsi*. Journal of the Entomological Society of South Africa 15:61-76.

Stacey PB, Ligon D, 1987. Territory Quality and Dispersal Options In The Acorn Woodpecker, and a Challenge to the Habitat-Saturation Model of Cooperative Breeding. American Naturalist 130:654-676.

Stark RE, Hefetz A, Gerling D, Velthuis HHW, 1990. Reproductive competition involving oophagy in the socially nesting bee *Xylocopa sulcatipes*. Naturwissenschaften 77:38-40.

Stark RE, 1992a. Cooperative nesting in the multivoltine large carpenter bee *Xylocopa sulcatipes* Maa (Apoidea: Anthophoridae): Do helpers gain or lose to solitary females? Ethology 91:301-310.

Stark RE, 1992b. Sex ratio maternal investment in the multivoltine large carpenter bee *Xylocopa sulcatipes* (Apoidea: Anthophoridae). Ecological Entomology 17:160-166.

Stark RE, Hefetz A, Gerling D, Velthuis HHW, 1990. Reproductive competition involving oophagy in the socially nesting bee *Xylocopa sulcatipes*. Naturwissenschaften 77:38-40.

Steen Z, 2000. Social behavior in endemic Australian carpenter bees (PhD). Adelaide: Flinders University of South Australia.

Steen Z, Schwarz MP, 1998. Within-nest behaviour in a eusocial Australian allodapine bee *Exoneura (Exoneurella) tridentata* Houston (Apidae: Xylocopinae). Transactions of the Royal Society of South Australia 122:55-63.

Steen Z, Schwarz MP, 2000. Nesting and life cycle of the Australian green carpenter bees *Xylocopa (Lestis) aeratus* Smith and *Xylocopa (Lestis) bombylans* (Fabricius) (Hymenoptera: Apidae: Xylocopinae). Australian Journal of Entomology 39:291-300.

Stern D, Foster W, 1997. The evolution of sociality in aphids: A clone's-eye view. In: Social Behavior in Insects and Arachnids. Cambridge: Cambridge University Press; 150-165.

Stokes M, Davis CS, Koch GG, 2000. Categorical Data Analysis Using the SAS System, 2nd edition: SAS Press.

Strassmann JE, Queller DC, 1989. Ecological determinants of social evolution. In: The genetics of social evolution (Breed, MD and Page, RE eds). Boulder, Colorado: Westview Press.

Sugiura N, 1991. Male Territoriality and Mating Tactics in the Wool-Cader Bee, *Anthidium septemspinosum* Lepeletier (Hymenoptera: Megachilidae). Journal of Ethology 9:95-103.

Tarpy DR, Page RE, 2001. The curious promiscuity of queen honey bees (*Apis mellifera*): evolutionary and behavioral mechanisms. Annales Zoologici Fennici 38:255-265.

Tepedino VJ, Torchio PF, 1982. Phenotypic variability in the nesting success among *Osmia lignaria propinqua* females in a glasshouse environment (Hymenoptera: Megachilidae). Ecological Entomology 7:453-462.

Thoenes SC, Buchmann SL, 1994. Fan palm as an urban nesting substrate for *Xylocopa californica arizonensis* Cresson (Hymenoptera: Anthophoridae). Pan-Pacific Entomologist 70:330-332.

Thornhill R, Alcock J, 1983. The Evolution of Insect Mating Systems. Cambridge, MA: Harvard University Press.

Tóth E, Duffy J, 2005. Coordinated group response to nest intruders in social shrimp. Proceedings of the Royal Society of London: Biology Letters. 1:49-52.

Van Der Blom J, Velthuis HHW, 1988. Social behaviour of the carpenter bee *Xylocopa pubescens* (Spinola). Ethology 79:281-294.

Velthuis HHW, Gerling D, 1980. Observations on Territoriality and Mating Behaviour of the Carpenter Bee *Xylocopa sulcatipes*. Entomologia Experimentalis et Applicata 28:82-91.

Velthuis HHW, Gerling D, 1983. At the brink of sociality: Interactions between adults of the carpenter bee *Xylocopa pubescens* Spinola. Behavioral Ecology and Sociobiology 12:209-214.

Velthuis HHW, Wolf Y, Gerling D, 1984. Provisioning and preparation of the brood cell in two carpenter bees, *Xylocopa sulcatipes* Maa and *Xylocopa pubescens* Spinola (Hymenoptera: Anthophoridae). Israel Journal of Entomology 18:39-51.

Vicidomini S, 1996. Biology of *Xylocopa violacea* (Hymenoptera): In-nest ethology. Italian Journal of Zoology 63:237-242.

Vicidomini S, 1996. Biology of *Xylocopa violacea* (Hymenoptera): In-nest ethology. Italian Journal of Zoology 63:237-242.

Vicidomini S, 1998. Biology of *Xylocopa* (*Xylocopa*) *violacea* (Linnaeus, 1758) (Hymenoptera: Apidae): interesting observations and records in the Emerald Coast and neighbouring areas (Sassari, Sardinia). Giornale Italiano di Entomologia 9:155-158.

Vicidomini S, Priore R, 1999. Xylocopini (Hymenoptera: Apidae: Xylocopinae) contained in the Italian entomological collections: the Filippo Silvestri Entomological Institute of Portici.127-134.

Vinson SB, Frankie GW, 1990. Territorial and mating behavior of *Xylocopa fimbriata* F. and *Xylocopa gualanensis* Cockerell from Costa Rica. Journal of Insect Behavior 3:13-32.

Waite TA, Strickland D, 1997. Cooperative Breeding in Gray Jays: Philopatric Offspring Provision Juvenile Siblings. The Condor 99:523-525.

Ward P, Zahavi A, 1973. The importance of certain assemblages of birds as "information centres" for finding food. Ibis 115:517-534.

Waser PM, 1988. Resources, philopatry, and social interactions among mammals. In: The Ecology of Social Behavior (Slobodchikoff, CN ed). San Diego, CA: Academic Press; 109-130.

Watmough RH, 1974. Biology and behaviour of carpenter bees in southern Africa. Journal of the Entomological Society of South Africa 37:261-281.

Watmough RH, 1983. Mortality, sex ratio and fecundity in natural populations of large carpenter bees (*Xylocopa* spp.). Journal of Animal Ecology 52:111-125.

Wcislo WT, 1996. Commentary: Solitary behavior in social bees. Behavioral Ecology and Sociobiology 38:235-236.

Williams HJ, Elzen GW, Strand MR, Vinson SB, 1983. Chemistry of Dufour's gland secretions of *Xylocopa virginica texana* and *Xylocopa micans* (Hymenoptera: Anthophoridae)--a comparison and re-evaluation of previous work. Comparative Biochemistry and Physiology, B 74B:759-761.

Willmer P, Stone GN, 1997. Temperature and Water Relations in Desert Bees. Journal of Thermal Biology 22:453-465.

Willmer PG, Stone GN, 2005. Behavioral, ecological and physiological determinants of the activity patterns of bees. Advances in the Study of Behavior. 34:347-466.

Wilson EO, 1971. The *Insect Societies*. Cambridge, Massachusetts: Harvard University Press.

Wilson EO, 2000. Sociobiology: The New Synthesis, Twenty-fifth Anniversary edition. Cambridge: Harvard University Press.

Wyman LM, Richards MH, 2003. Colony social organization of *Lasioglossum malachurum* Kirby (Hymenoptera, Halictidae) in southern Greece. Insectes Sociaux 50:201-211.

Yanega D, 1989. Caste determination and differential diapause within the first brood of *Halictus rubicundus* in New York (Hymenoptera: Halictidae). Behavioral Ecology and Sociobiology 24:97-107.

Yanega D, 1993. Environmental influences on male production and social structure in *Halictus rubicundus* (Hymenoptera: Halictidae). Insectes Sociaux 40:169-180.

Yanega D, 1997. Demography and sociality in halictine bees (Hymenoptera: Halictidae). In: Evolution Of Social Behaviour In Insects And Arachnids (Choe, JC and Crespi, BJ, eds). Cambridge: Cambridge University Press; 293-315.

Zar JH, 1999. Biostatistical Analysis, Fourth edition. Upper Saddle River, NJ: Prentice Hall.

Appendices

Appendix 1: The contents of all nests collected and included in this study in Ontario. Brood cells refer to the total number of cells regardless of contents. Columns are not additive.

Nest ID	Source	Date opened	No. foundresses	No. brood cells	No. cells containing *Bombyliids*	No. cells containing larvae	No. cells containing pupae	No. cells with pollen ball, empty or dead
D	Farmhouse site	15 Jul 03	0	7	1	5	0	2
E	Farmhouse site	25 Jul 03	3	8	0	6	0	2
F	Farmhouse site	25 Jul 03	1	7	3	2	2	2
H	Farmhouse site	08 Aug 03	4	7	0	2	4	1
I	Farmhouse site	08 Aug 03	5	12	1	1	7	3
J	Farmhouse site	08 Aug 03	1	2	1	1	0	0
K	Farmhouse site	11 Aug 03	5	9	3	1	3	2
L	Farmhouse site	12 Aug 03	2	9	1	0	6	2
M	Farmhouse site	11 Aug 03	2	12	3	0	9	0
N	Farmhouse site	13 Aug 03	2	8	0	3	4	0
O	Farmhouse site	14 Aug 03	4	3	1	2	0	0
P[a]	Farmhouse site	14 Aug 03	0	0	0	0	0	4
Q	Farmhouse site	14 Aug 03	2	12	2	0	10	1
R	Farmhouse site	18 Aug 03	3	14	0	0	8	6
S	Farmhouse site	18 Aug 03	5	7	0	0	7	0
T	Farmhouse site	21 Aug 03	3	15	1	0	13	1
U	Farmhouse site	21 Aug 03	≥ 1[b]	6	0	0	4	1
V	Farmhouse site	21 Aug 03	≥ 1[b]	11	1	0	10	1
W	Farmhouse site	21 Aug 03	≥ 1[b]	10	1	0	8	0
X	Farmhouse site	21 Aug 03	1	5	1	0	4	0
Y	Farmhouse site	22 Aug 03	2	10	5	0	4	2
Z	Farmhouse site	22 Aug 03	3	15	5	0	7	4

Nest ID	**Source**	**Date opened**	**No. foundresses**	**No. brood cells**	**No. cells containing *Bombyliids***	**No. cells containing larvae**	**No. cells containing pupae**	**No. cells with pollen ball, empty or dead**
AA	Farmhouse site	26 Aug 03	4[c]	10	0	0	0	0
BB	Farmhouse site	26 Aug 03	2	9	2	0	7	0
CC	Farmhouse site	26 Aug 03	1	11	0	0	11	0
DD	Farmhouse site	26 Aug 03	1	14	3	0	9	0
EE	Farmhouse site	26 Aug 03	1	16	0	0	15	1
FF	Farmhouse site	26 Aug 03	1	10	0	0	7	3
GG	Farmhouse site	26 Aug 03	0	11	0	0	8	2
HH	Farmhouse site	10 Dec 03	1	0	0	0	0	0
AAAA[d]	Burgoyne Woods	19 Jul 06	0	0	0	0	0	0
BBBB	Burgoyne Woods	19 Jul 06	1	5	1	0	2	0
CCCC	Burgoyne Woods	19 Jul 06	3	13	0	9	3	1
DDDD	Burgoyne Woods	19 Jul 06	6	21	1	0	20	0
EEEE[d]	Burgoyne Woods	19 Jul 06	0	0	0	0	0	0

---

[a] Nest contained 4 empty brood cells and no foundress

[b] Five foundresses were found among nests U, V and W but they could not be assigned to a specific nest. Nest V also contained a dead, marked female.

[c] This nest had multiple termini when opened and brood could not be properly assigned

[d] Nest contained no brood or foundresses

Appendix 2: Destructively sampled nests used in each analysis in Chapters 1 and 2. Numbers in parentheses indicate the total number of nests collected from that site. X indicates that the set was not included. + indicates all nests from the set were included. Numbers indicate how many nests from the set were excluded.

Analyses-Chapter 1	Farmhouse (30)	Burgoyne Woods (5)
No. of foundresses	-1	X
Productivity (brood/foundress by foundress)	-5	X
Parasitism (all analyses)	-3	X
Foundress survival	-3	X
Mated status	-8	X

Analysis-Chapter 2	(31[a])	(5)
Brood laying order	-8	X
Sex by position	-3	X
Parasitism by position	-8	X
Parasitism and length	-4	X
Nest architecture and foundresses	-11	X

Analysis-Appendix 9	(31[a])	(5)
Nest measurements (non brood related)	+	+
Nest measurements (brood related)	-2	-2
Nest contents (brood cells)	-2	X

---

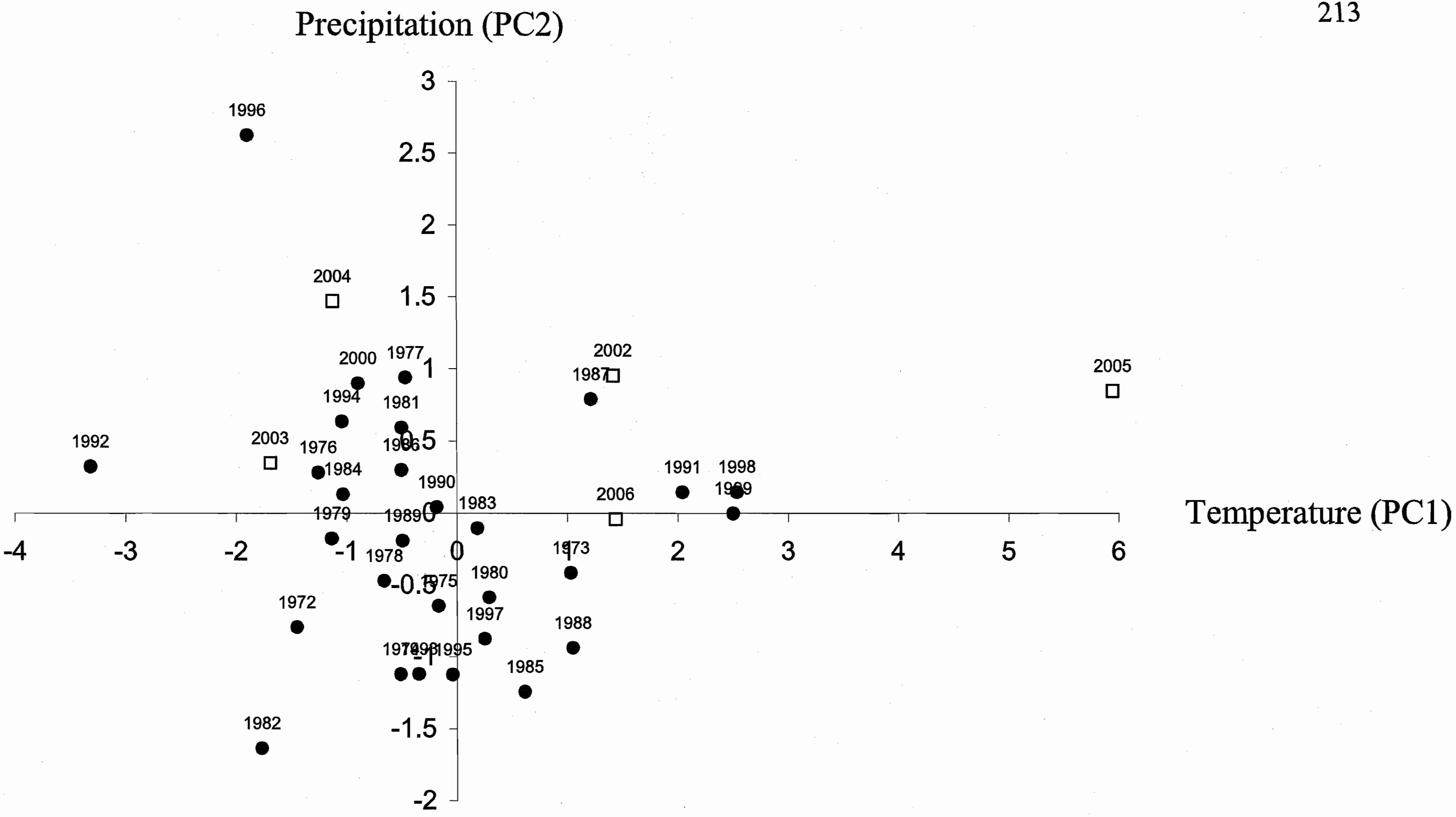
[a] Measurements also include a nest collected at the Farmhouse site in winter for a total of 31 nests

Appendix 3: The number of male, female and unknown sex offspring for active nests collected at Farmhouse in 2003 (n=27). Nests with no brood are excluded (n=3).

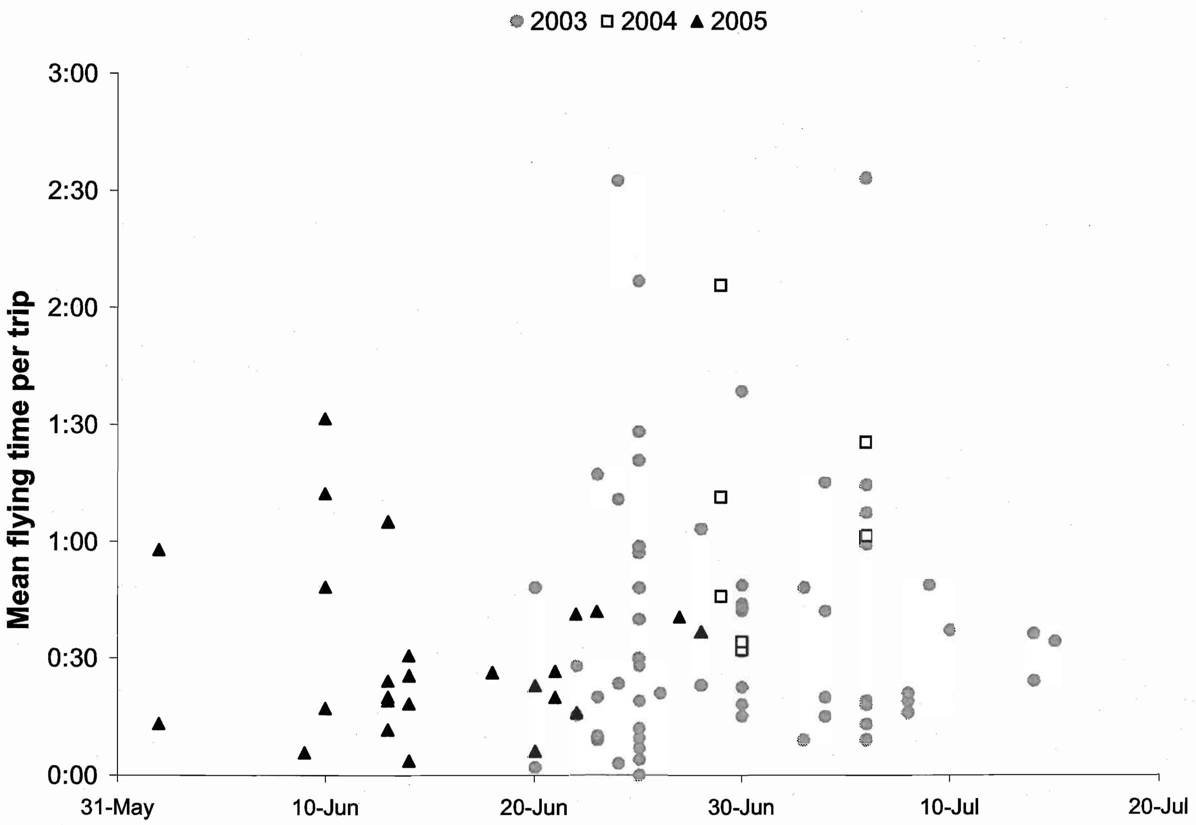
Nest ID	**Number of Females**	**Number of males**	**Number of unknowns**	**Total brood size**	**Proportion male**
A	0	2	6	8	0.25
C	0	0	1	1	0.00
D	0	0	7	7	0.00
E	0	1	4	5	0.20
F	0	0	2	2	0.00
H	2	3	0	5	0.60
I	4	4	1	9	0.44
J	1	0	0	1	0.00
K	3	2	0	5	0.40
L	4	2	0	6	0.33
M	5	4	0	9	0.44
N	3	4	0	7	0.57
O	1	0	2	3	0.00
Q	1	4	3	8	0.50
R	3	4	1	8	0.50
S	2	5	0	7	0.71
T	2	4	7	13	0.31
X	2	1	1	4	0.25
Y	2	2	0	4	0.50
Z	1	1	0	2	0.50
BB	3	1	3	7	0.14
DD	3	5	1	9	0.56
EE	1	9	5	15	0.60
FF	3	4	2	9	0.44
GG	5	0	3	8	0.00
U	3	0	1	4	0.00
V	5	3	2	10	0.30
W	1	5	2	8	0.63
Population	60	70	54	184	0.38

Appendix 4: Social organization and life history aspects for species of *Xylocopa*. – indicates no data available

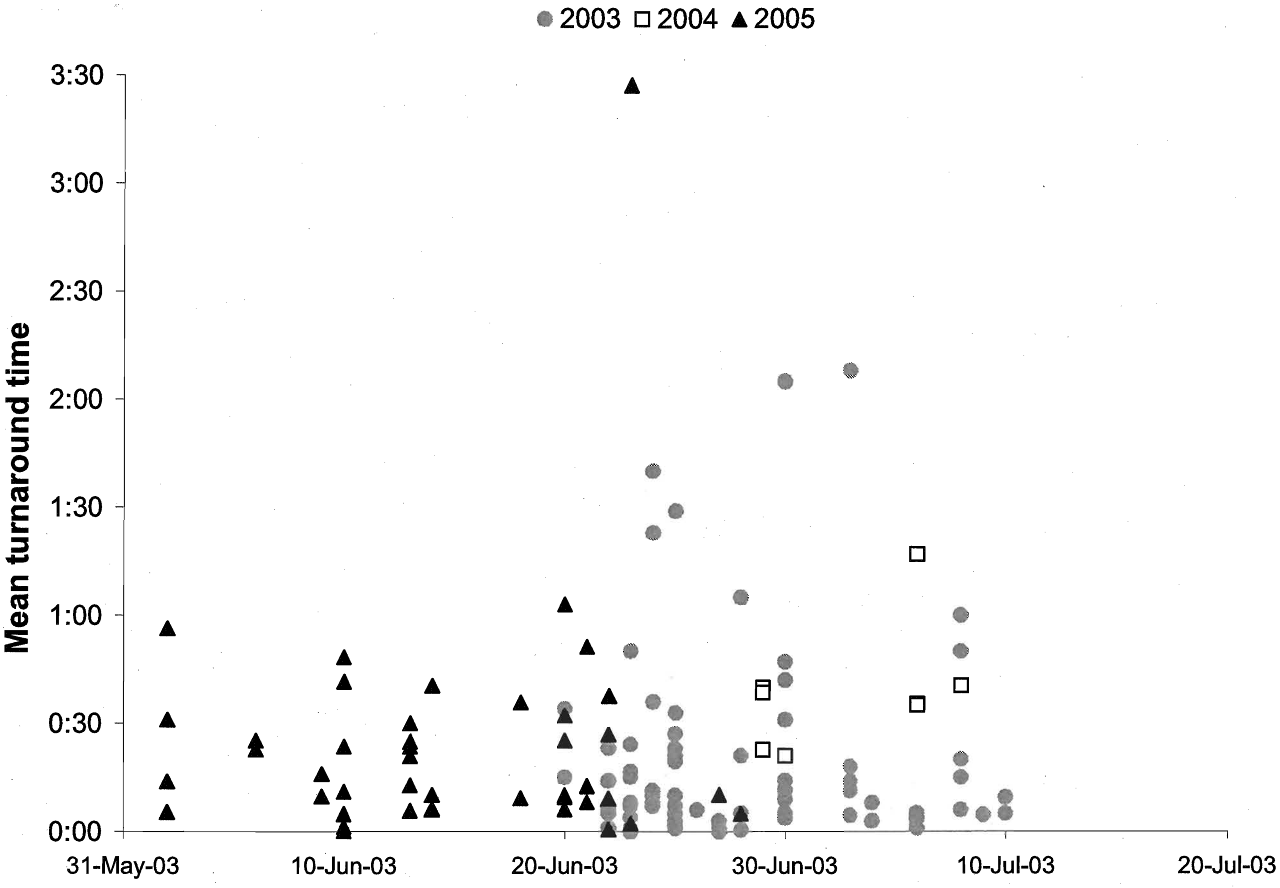
Species	Voltinism	Multi-females	Primary mortality	Division of labour among foundresses	Reference
aeratus	Univoltine	Yes	Huntsman spider, ants, mites	Single forager & guard	Steen, 2000
artifex	Multivoltine	Yes	-	-	Sakagami & Laroca, 1971
bombylans	Bivoltine	Yes	Huntsman spider, ants, mites	Single forager & guard	Steen 2000
capitata	Univoltine	No	-	-	Watmough, 1983
carinata	Multivoltine	Yes	-	-	Bonelli 1976
combusta	Multivoltine	Yes	-	Eusocial ?	Bonelli 1976
fimbriata	-	Yes	-	-	Vinson et al, 1986
flavicolis	Multivoltine	Yes	-	-	Watmough, 1983
flavorufa	Bivoltine	No	-	-	Watmough, 1983
frontalis	Multivoltine	Yes	-	-	Camillo & Garafolo, 1982
imitator	Bivoltine	Yes	-	-	Gerling 1989
inconstans	Multivoltine	Yes	-	-	Bonelli 1974, Watmough 1983
iris	Univoltine	No	-	-	Sakagami & Laroca 1971, Bonelli 1976
nigrita	Bivoltine	Yes	-	-	Watmough 1983, Watmough1974
nogueirai	Multivoltine	Yes	Nest usurpation	-	Sakagami & Laroca, 1971
pubescens	Multivoltine	Yes	*Coelopencyrtus* (wasp)	Single reproductive forager & guard	Hogendoorn 1995, , Hogendoorn, 1993, Velthuis 1988, Gerling 1981 Mordechi 1978
rufitaris	Univoltine	No	Bombyliids	-	Watmough, 1983
sonoria	Bivoltine	Yes	-	-	Gerling 1983
sulcatipes	Bivoltine	Yes	-	Single reproductive forager & guard, delayed	Stark 1990, Stark 1992,
tranquebarorum	Bivoltine	Yes	Ants, *Melittobia sousi*	-	Sakagami & Laroca 1971
varipuncta	Multivoltine	Yes	-	-	
violacea	Varies	No	-	-	Van Der Blom, 1988; Vicidomini, 1998; Vicidomini, 1998; Vicidomini, 1996
virginica	Univoltine	Yes	Bombyliids	Forager(s) & guard(s)	This study; Gerling 1978



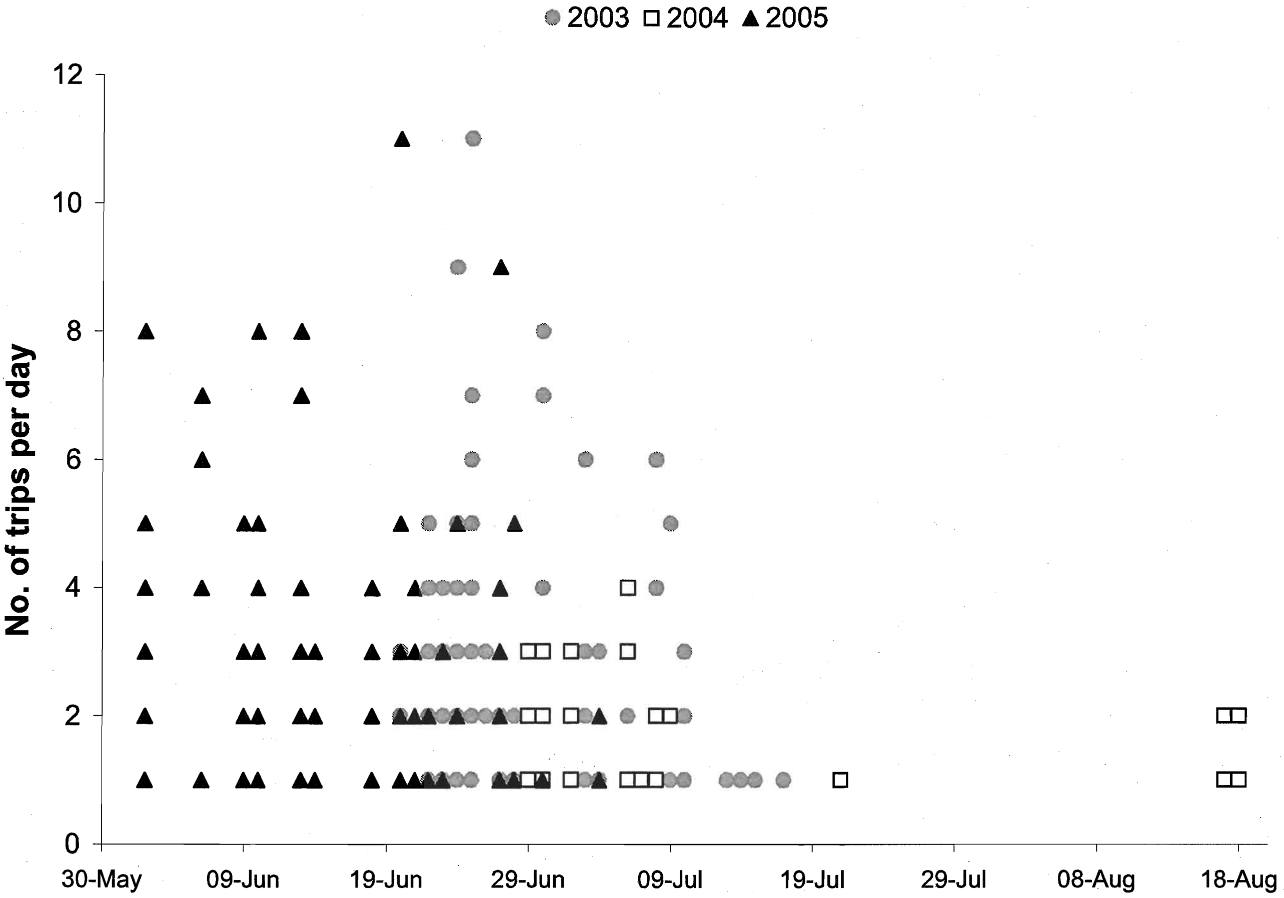
Appendix 5: Positioning of principal components 1 and 2 for 30-year climate data. PC1 accounts for 70% of variation and represents temperature. PC2 has a marginal eigenvalue of 0.78, and accounts for an additional 19% of variation. PC2 represents precipitation. The PC analysis was based on the months April to September and included the variables: total precipitation, mean temperature, mean maximum and mean minimum temperature. Open squares represent the years of this study.



Appendix 6: Annual and seasonal variation in mean flight time per trip (hours) for female *X. virginica* in 2003 (grey circles), 2004 (open squares), and 2005 (closed triangles). Each point represents a single female. Statistical analyses are available in Table 3.5.



Appendix 7: Mean turnaround (handling) time (hours) per trip for females in 2003 (grey circles), 2004 (open squares) and 2005 (closed triangles). Each point represents a single female. Statistical analyses are available in Table 3.5.



Appendix 8: Annual and seasonal variation in the number of trips per day per female for the years 2003 (circles), 2004 (squares), and 2005 (triangles). Statistical analyses are available in Table 3.5.

Appendix 9: Head capsule width for males of various conditions in the years in 2003, 2004 and 2005.

Condition	Mean ± SD (Range) (cm)			
	2003	2004	2005	All years
All marked males	6.2 ± 0.5 (5.0-7.7)	6.5 ± 0.4 (5.7-7.3)	6.2 ± 0.3 (5.1-6.7)	6.3 ± 0.4 (5.0-7.7)
All censused males	6.3 ± 0.4 (5.2-7.0)	6.2 ± 0.2 (5.7-6.5)	6.2 ± 0.2 (5.6-6.5)	6.3 ± 0.3 (5.2-7.0)
Satellite males	6.5 ± 0.5 (5.7-7.7)	NA[a]	6.2 ± 0.3 (5.1-6.4)	6.4 ±0.5 (5.0-7.7)
< 0.5 m from bench	6.4 ± 0.6 (5.7-7.7)	NA[a]	6.2 ± 0.3 (5.9-6.4)	6.3 ± 0.5 (5.8-7.5)
> 0.5 m from bench	6.5 ± 0.4 (5.8-7.5)	NA[a]	6.2 ± 0.2 (5.1-6.4)	6.5 ± 0.4 (5.8-7.5)
Resident males	6.4 ± 0.4 (5.7-7.7)	6.3 ± 0.1 (6.3-6.5)	6.2 ± 0.2 (5.1-6.4)	6.4 ± 0.4 (5.0-7.7)
Disappeared males	6.3 ± 0.7 (5.0-7.0)	6.2 ± 0.2 (5.7-6.5)	6.1 ± 0.2 (5.1-6.6)	6.2 ± 0.5 (5.0-6.5)

---

[a] No males were identified as satellites in 2004

Appendix 10: A descriptive and comparative study of nest architecture in *Xylocopa virginica*

Introduction

Nest architecture of *Xylocopa virginica* has been studied extensively only twice previously, by Rau (1993) in Missouri and later by Gerling and Hermann (1976) in Georgia. Here I describe the nest architecture of *X. virginica* in southern Ontario and Maryland, and compare these findings to previously published records of nest structure for more southern populations.

Methods

Study sites, nesting site and substrate selection

Destructive sampling of nests in Ontario and Maryland

In order to determine if climate or latitude influences nest architecture, I collected nests in two geographic locations. The first location was southern Ontario, primarily St. Catharines, Ontario. Nests were collected at the Brock Farmhouse site in 2003 (n=31) and Burgoyne Woods in 2006 (n=5). I also collected nests from the campus of the United States Department of Agriculture's Beltsville Agricultural Research Center Beltsville, Maryland, approximately 490 km south of St. Catharines. Nine nests were obtained on 15 March 2005 and six nests on 26 January 2006. These nests were from abandoned wood structures in fallow fields and grazing areas.

Destructively sampled nests from St. Catharines were dismantled according to the protocol in the general methods. Procedures varied slightly in Maryland as it was not necessary to seal the nests prior to collection because they were collected in winter and thus were inactive. The nests collected in 2005 were stored in a 4°C cold room overnight before being opened, while those collected in 2006, were opened on the day of collection. In some instances, not all variables could be measured. In particular, some nests were collected without foundresses (n=3) or brood (n=3); one nest contained neither. Nests collected in winter (n=20) or after brood had emerged do not contain brood cells and related measurements could not be made. Details for nests collected in Ontario are available in Appendix 1, while specific sample size information is provided in Appendix 2.

Observational and survey methods

In addition to using nests from known populations from St. Catharines, I also conducted surveys of various locations in search of nests. During surveys I examined older wooden structures for nest entrances and signs of nest use such as sawdust; I also looked for males, as males conspicuously hover around nests. I inspected potential nesting sites three times a season in each of 2003, 2004, and 2005. I used a similar approach in the Maryland location during my collecting trips (March 2005, January 2006). In addition, I inspected three wooded sites in St. Catharines for the presence of nests. In each site, I walked transects looking for hovering males, or wood such as logs and fallen trees; these were carefully inspected for nest entrances.

For nests in the Courtyard population, where bees were being observed for other studies, I calculated rates of nest re-use. The number of nests in the population was determined based on direct count at the start of each season. New nests were also identified by direct count; the appearance of a new nest entrance was obvious during my systematic surveys of benches for nests. Nest re-use was determined based on a combination of all available evidence including: observation at nest entrances, daily spot checks (inspections for debris on the pavement under entrances) and videoscope observations. Not all evidence was available in all years; specifically, videoscope data were not available in 2003.

Geographic comparisons

To test the hypotheses that nest architecture would vary with respect to latitude and climate, I compared nest measurement data from different geographic locations. In addition to the data I collected in Ontario, and Maryland, when possible, I also used published measurements of number of termini and total length from Missouri (Rau, 1933) and Georgia (Gerling and Hermann, 1976). This provided a range of latitudes with Ontario as the northernmost location and Athens, Georgia as the southernmost.

When suitable data were available (raw data were not available for Georgia), locations were compared using ANOVA on ranks which allows for post-hoc tests and gave similar results to non-parametric Kruskal-Wallis tests. Pairwise comparisons were made using Tukey's post hoc tests. Since the Georgia population was not included in ANOVA tests, where possible t-tests were used to allow analyses of the Georgia population.

Evaluation of nest architecture

Physical structure

I measured disassembled nests to describe and quantify nest architecture. Nests were measured using a standard tape measure and analog calipers. For all nests, I measured: number of termini; total length; width (diameter) of the beginning, middle and end of each branch; entrance diameter; and the length of the entrance (gallery) (Figure

A.10.1). These measurements were made for all nests collected in Ontario and all nests collected in Maryland.

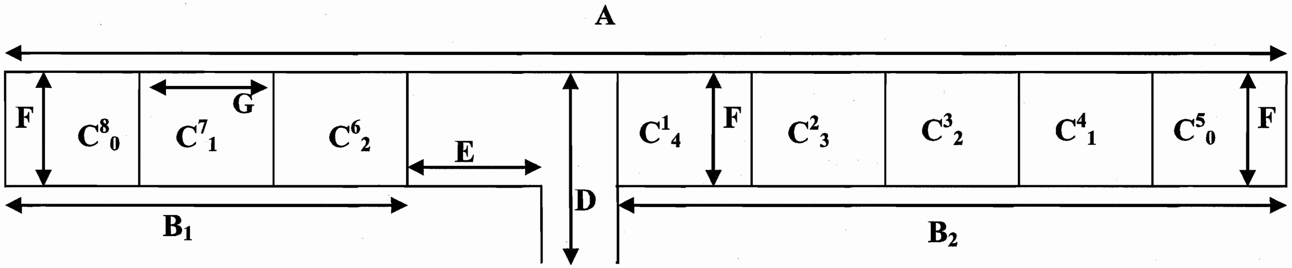
Measurements associated with brood

In disassembled nests that contained brood, additional measures were possible. These measures included brood cell length, the number of brood cells per nest, the space occupied by brood cells and the amount of empty space (Figure A.10.1). These data were used to test the hypothesis that branched nests would contain larger broods (see chapter 2), and to examine the relationship between nest architecture and space dedicated to brood or foundresses. Measures of cells per nest and brood cell length were also available in Gerling and Hermann (1976) for populations from Georgia.

Videoscope inspection of nest architecture

From 2004-2006, nests in the Brock Courtyard site were inspected using the videoscope. Video inspection followed the protocol in the general methods. Here I present data from video observations relevant to nest structure. Variables noted include: basic nest shape (linear vs. branched), number of termini and the position of nests within a board with respect to the grain and also the edges of the board. In addition to general description of nests, these data were used to test the hypothesis that nests will increase in the number of branches (termini) as they are resued.

a) Linear nest



b) Branched nest

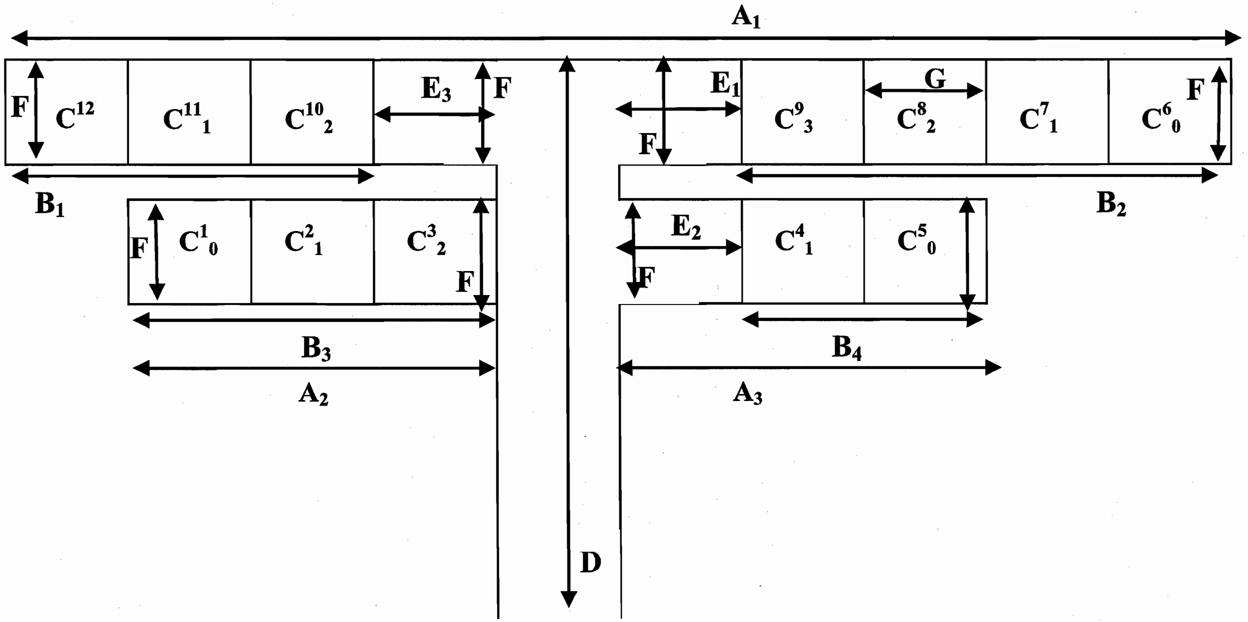


Figure A.10.1: Diagram illustrating the measurements used throughout this appendix, Chapter2 and quantified in Table A.10.2 for a linear nest with 2 termini (a), and a branched nest with 4 termini (b). The following measurements were taken for all benches: number of termini (=2 in **a**, =4 in **b**); total length (linear nest: **A**, branched nest: **A_1+A_2+A_3**); terminus (cell) width measured at the beginning and end of each terminus; **(F)** and entrance (gallery) length **(D)**. In nests containing brood, the following additional measures were taken: brood cell length **(G for each C)**; space occupied by brood cells (**sum of Bs**); brood cells per nest (**frequency of Cs**); empty space (sum of **Es**). Brood cells **(Cs)** are numbered with respect to the order exposed during excavation (**superscripts**) and assigned a value that reflects their distance (in number of cells) from the terminus (**subscripts**).

Results

Nesting site and substrate selection

X. virginica apparently prefers to construct nests in softwood structural lumber rather than natural materials. All of the nests I located or collected were contained in fabricated structures (Table A.10.1). Among all survey transects, I failed to locate a single nest in a natural substrate such a tree branch or stump. Nests were located in pressure treated, painted, old and new wood. Nests were significantly not randomly oriented (Chi-square goodness of fit: $\chi^2_3=52.6$, $p<0.001$), with more nests oriented North-South than East-West, and the fewest nests facing east (Table A.10.1). Anecdotally, nests were less common in shade; the shaded Farmhouse site had more nests than the courtyard site in 2003; and no nests were located in the shaded locations of Burgoyne Woods. Many seemingly suitable habitats, often within a few metres of inhabited nests, were unused. In Maryland, I conducted fewer surveys, and did not survey along transects, but I again only located nests only in man-made softwood structures. Almost all the nests collected in Maryland were located in abandoned pine animal pens (18 of 21); two were located in a redwood picnic table (Table A.10.1), and one was located in the fascia of a garage.

In every year of this study, new nests were constructed at the courtyard site (Figure A.10.2). In addition, some nests were abandoned and others were reused (Figure A.10.2). Moreover, the number of new nests and the rates of use and re-use varied among benches and among years. In every year except 2002, the distribution of nests among

Table A.10.1: Nesting substrates, frequencies and orientation of *X. virginica* nests in the Niagara region, Ontario and Beltsville. Compass direction refers to the outward facing side(s) of the structure on which nests are located. Number of nests refers to the number of nests observed; values in brackets indicate the number of nests collected from that location. - indicates data were not available.

Location	Type of structure	Properties	Compass direction(s)	No. of Nests
Brock University Jubilee Courtyard	Park benches	Untreated, unpainted cedar	N, S	100
Farmhouse site, Brock University Campus	Park benches	Untreated, unpainted cedar	N, S	34 [31]
Glenridge Quarry Naturalization site	Gazebo	Pressure treated pine	E	2
Symphony House, Brock University Campus	Eavestrough	Untreated and painted pine	N, W	6
Brock University Athletic fields	Equipment shed eaves	Painted wood	S, E	5
Brock University, South Campus	Support structures	Treated pine	S	3
Highway 55, Niagara on the Lake, Ontario	Barn eaves	Pine	E	2
Cat's Caboose, St. Catharines, Ontario	Deck	Treated pine	S	2
River Road, Niagara Falls	Eaves	Untreated cedar	-	3
North St. Catharines residential areas	Porch roof	Treated painted pine	N, W	4
Merriton, St. Catharines, Ontario	Garden shed	Treated, painted pine	W	2
South St. Catharines	Tool shed	Treated pine	S	1
Port Dalhousie, Ontario	Patio roof	Treated pine	N	3
Burgoyne Woods Park, St. Catharines, Ontario	Equipment sheds, picnic pavilion	Painted eaves	N, S, E, W	< 10 [5]
North St. Catharines	Garden ornament	Painted hardwood	-	2
USDA, Beltsville, Maryland	Abandoned animal pens	Painted pine	-	[18]
USDA, Beltsville, Maryland	Picnic table	Redwood	-	[2]
USDA, Beltsville, Maryland	Fascia of garage	Pine	S	[1]

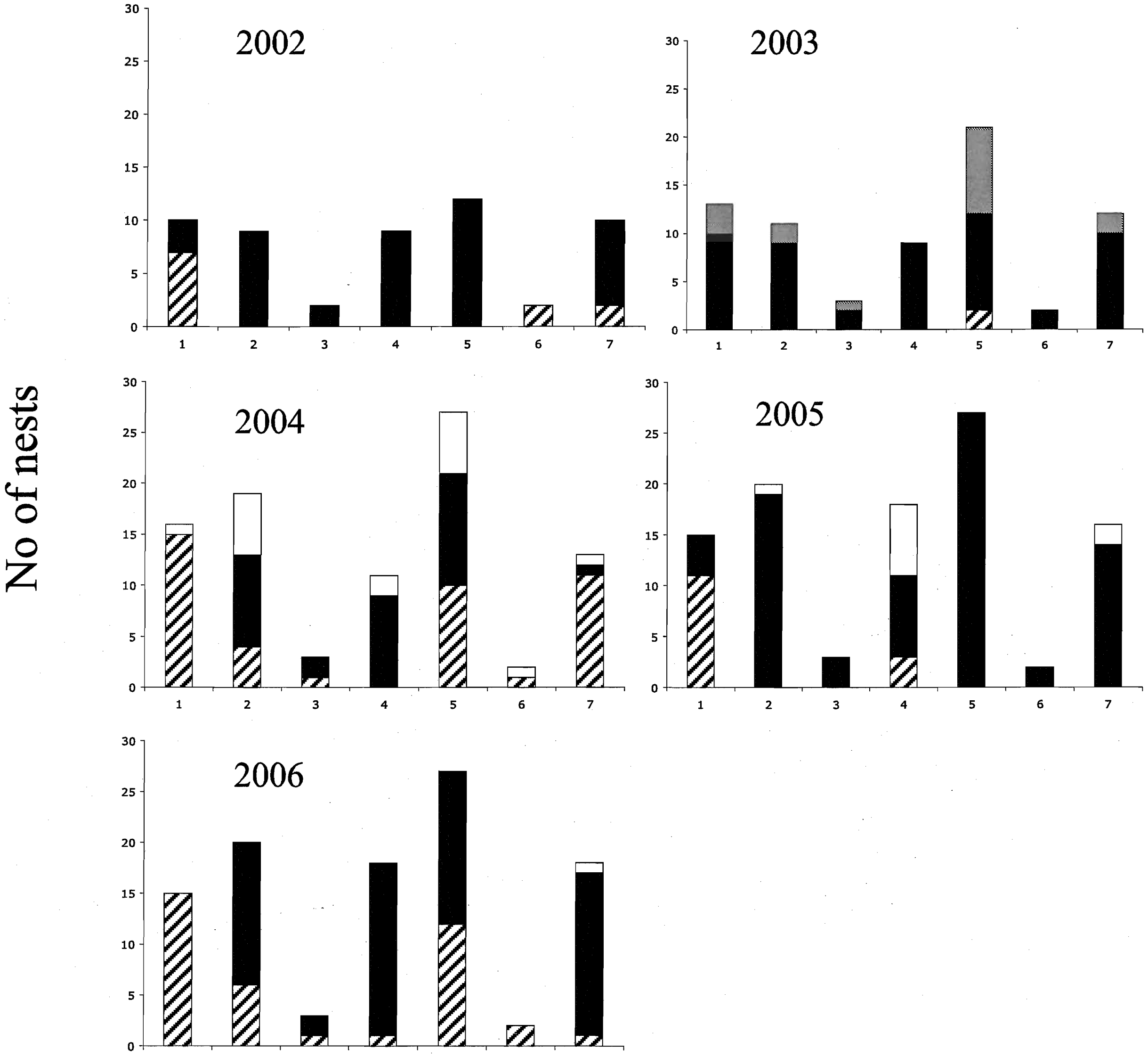


Figure A.10.2: The number of new (white), re-used (black), and unused (hatched) nests in each bench for 2002-2006. Occupancy in 2002 was determined from daily spot checks and presence of nest debris; occupancy in 2003 was determined from observations at nest entrances, debris and daily spot checks. In all other years nest occupancy was determined from daily spot checks, nest debris, observations at nest entrances, and videoscope observations. See text for details of patterns of nest use and reuse. In every year except 2002, the distribution of nests among benches was statistically different from random with $p<0.01$. In 2002, nests were marginally different from random with a p-value of 0.06.

benches differed from random (p-value<0.001 for each year); in 2002 there was marginal significance of p=0.06. Some benches appear to be preferred (e.g. bench 5 and bench 4); these are re-used more often and had more new nests than other benches in each year. Other benches are seemingly less suitable for re-use or construction of nests (e.g. bench 3 and bench 6) and had fewer new nests and were re-used less often. Finally, some benches, especially bench 1, appear to become permanently unsuitable and showed an overall increase in the number of unused nests; benches 1 and 3 were completely abandoned after the 2006 season.

Nest architecture

Nests collected at Farmhouse were constructed inside the same type of cedar benches as in Courtyard. Each bench consisted of 12 boards, 235 cm long, 11 cm wide and 4 cm thick (Figure I.2b). The boards were supported by two 37 cm by 25 cm concrete blocks and boards were arranged so that the widest part was vertical and the thinnest segment was horizontal (Figure I.2b). A space of 7.5 mm existed between boards, maintained by a thin cedar spacer (Figure I.2b). Bees oriented their nests within the boards so that nest entrances were vertical, and the majority of the nest was parallel to the grain of the nest. In 2003 at the Farmhouse site, 88% (30/34) of nests were located in the outside boards of a bench. In 2005 68% (27/71) of nests at the Courtyard site were in the outer boards of the bench.

All the nests examined contained a single round entrance, an average of 1.03 ± 0.3 cm (n=28) in diameter that led to a vertical gallery (Table A.10.2). The galleries terminated at a tunnel oriented perpendicular to them (Figures A.10.1 and A.10.3). Tunnels extended in both directions relative to the gallery and parallel to the grain of the wood; the extensions are referred to as 'termini'. No nests had termini perpendicular to the grain.

I found both simple linear nests with two branch termini (Figure A.10.3a) and multiply branched nests containing three or more termini (Figure A.10.3b); the latter are referred to as "branched nests". In branched nests, additional tunnels usually extended from the entrance gallery, and occasionally from other tunnels. In the Farmhouse site, 35% (12 of 34) of nests were branched (Table A.10.2). When the proportion of branched nests in the Courtyard site was calculated from videoscope observations, the proportion was found to increase each year. In 2004, 71% (19/41) of nests were branched, this increased to 80% (35/44) in 2005, and 82% (31/38) in 2006; no videoscope data exist for 2003. Half (10/21=50%) of all nests collected in Maryland were branched.

Nest tunnels from both Ontario and Maryland terminated with a convex end and were, on average, 1.8 ± 0.19 cm in diameter (n=11); this provided enough space for two bees to pass each other. Tunnel lengths varied from slightly larger than the length of a single bee (2 cm) to 93.5 cm in total length (Table A.10.2). *Xylocopa virginica* eggs were laid in separate elliptical brood cells, arranged end to end within tunnels, parallel to the grain of the wood. Figure A.10.4 shows a typical cell partition, which was bowl

Table A.10.2: Descriptive statistics for nest architecture in 31 (11 branched, 19 linear, one unknown[a]) *X. virginica* nests collected at the Farmhouse site 2003, and 5 nests collected at Burgoyne Woods in 2006. Two linear nests from 2006 contained no brood or foundress. n=number of nests used for that calculation. Bold letters in parentheses refer to the equivalent measure in Figure 2.1.

	2003				**2006**			
Characteristic	**Mean (SD)**	**Minimum**	**Maximum**	**n**	**Mean (SD)**	**Minimum**	**Maximum**	**n**
No. termini	2.6 (0.95)	1[a]	6	30	3.3 (1.89)	2	6	5
Total length (cm) **(A) or sum of (A's)**	39.8 (19.7)	5.5	93.5	30	29.6 (23.2)	13.0	63.0	5
Linear nest length (cm) **(A)**	29.8 (12.2)	3.5	28.7	10	14.0 (1.41)	13.0	15.0	3
Branched nest length (cm) **(sum of all A's)**	57.2 (18.1)	31.9	93.5	11	42.5 (25.1)	28.0	63.0	2
Entrance length (cm) (gallery), all nests **(D)**	17.9 (18.7)	1.0	93.5	30	6.6 (4.5)	1.5	10.0	5
Entrance length (cm) (gallery), branched nests **(D)**	29.0 (26.0)	5.0	93.5	11	5.8 (6.0)	2.0	10.0	2
Entrance length (cm) (gallery), linear nests **(D)**	11.0 (8.4)	1.0	38.0	11	8.2	8.0	8.0	1
Brood cells per nest, all nests **(frequency of C's)**	9.3 (3.4)	0.0[b]	15.0	30	7.8 (9.1)	0.0	21.0	5
No. brood cells branched nests **(frequency of C's)**	10.0 (4.12)	3.0	15.0	11	17.0 (5.6)	13	21.0	2
No. brood cells linear nests **(frequency of C's)**	8.9 (2.9)	0.0	14.0	11	1.7 (2.9)	0.0	5.0	3
Brood cell length (cm) **(G)**	2.2 (1.0)	0.7	6.48	28	2.1 (0.5)	1.7	2.69	5
Brood cell width (cm) **(F)**	1.8 (0.19)	1.2	2.05	25	2.1 (0.14)	1.9	2.25	5
Empty space (cm) all nests **(sum of E's)**	21.2 (20.0)	0.0	93.5	30	14.3 (11.1)	7.5	27.1	5
Empty space (cm), branched nests **(sum of E's)**	35.0 (25.3)	0.7	93.5	11	17.3 (13.9)	8.0	27.0	2
Empty space (cm), linear nests **(sum of E's)**	13.3 (10.3)	0.0	37.8	19	8.2	8.2	8.2	1
No. cells that could occupy empty space	10.1 (10.8)	0.4	51.9	27	7.6 (7.2)	2.8	15.9	5
Entrance diameter	1.03 (0.3)	0.5	2.3	28	1.02 (0.3)	0.5	1.3	5
Number of foundresses, branched nest	3.3 (2.1)	0[c]	6	11	4.0 (2.8)	2	6	2
Number of foundresses, linear nest	1.9 (1.0)	0	4	15	0.3 (0.6)	0	1	3

---

[a] A single "L" shaped nest consisting of one terminus only large enough to accommodate a foundress. The nest contained no brood

[b] nest contained a foundress but no brood (n=1 nest)

[c] nest contained brood but no foundress (n=1 nest)

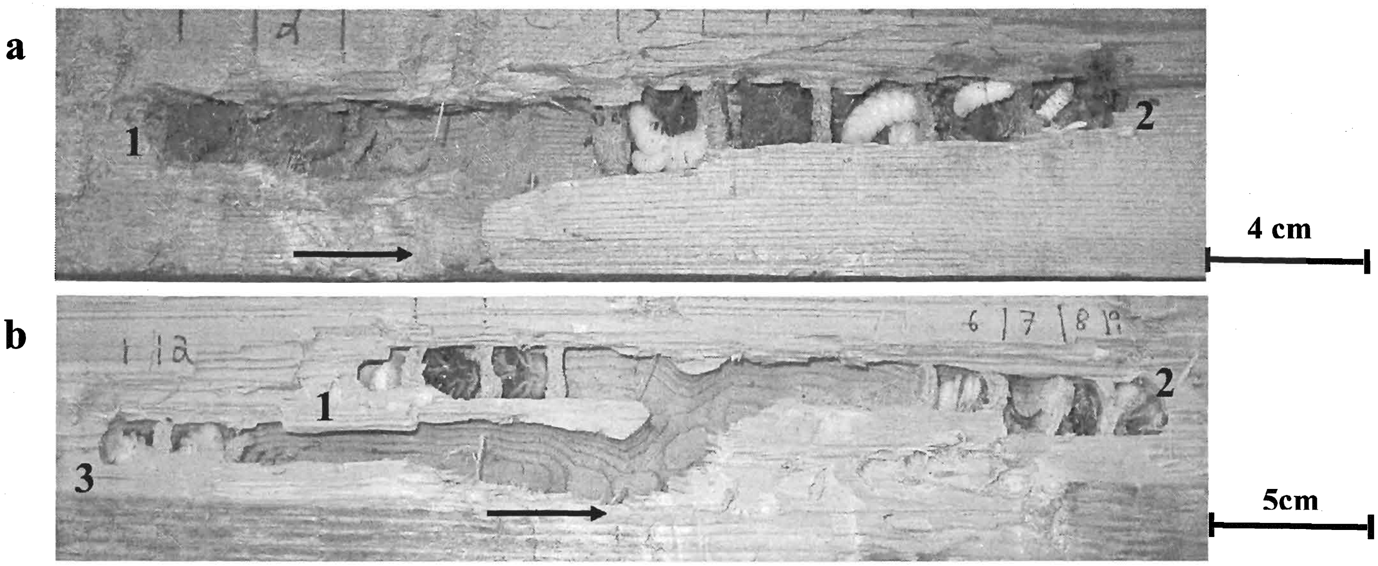


Figure A.10.3: Typical linear (unbranched) nest **(a)**. Typical branched nest **(b).** Arrows indicate nest entrances. Numbers indicate termini. In b, numbers 1 and 2 constitute a single terminus, while 3 indicates the terminus of an additional branch.

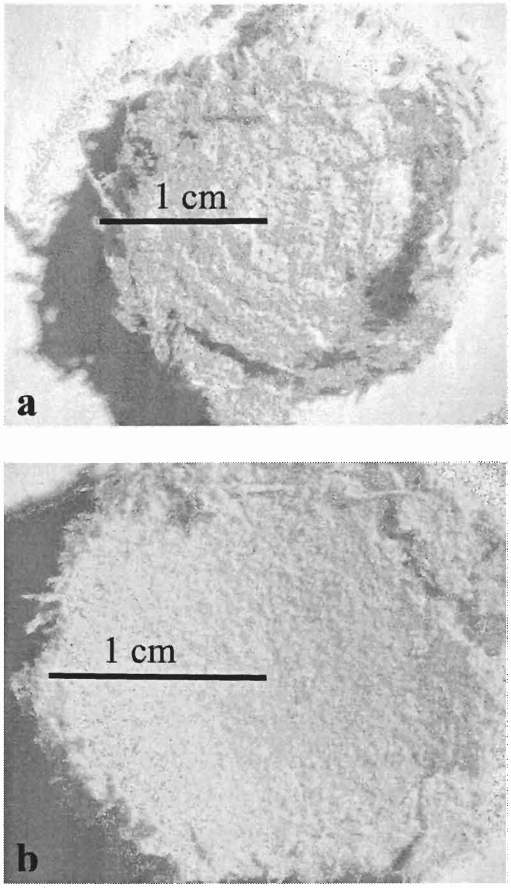


Figure A.10.4: Concave **(a)** and convex **(b)** side of a typical cell partition. The convex side is placed facing a pollen mass, while the concave side faces away. Note the spiral pattern in a.

shaped and placed at a 90° angle to the tunnel floor. This resulted in a concave side facing the tunnel gallery entrance, and the convex side facing the brood pollen mass. Partitions had a spiral pattern, which likely resulted from the female spinning during construction in a manner resembling the action of a drill bit.

The average brood cell was 2.2 ± 1 cm long (n=28) and 1.8 ± 0.19 cm wide (n=25) (Table A.10.2). This is consistent with the length of the tunnel and just larger than a pollen mass, or a pupa (about 2 cm long). Cell partitions fully obstructed the tunnel containing them.

Many disassembled nests featured "empty" space not occupied by brood cells (Table A.10.2). Some of this space was comprised of the entrance gallery and thus was not suitable for brood cells, but nests also contained empty space or branches. On average, disassembled nests contained 18.5 ± 15.0 cm (n=35) of space not filled with brood cells (Table A.10.2). When this value was adjusted for the entrance gallery, the average nest contained enough space to accommodate an average of 3.9 ± 8.8 (n=27) additional brood cells (Table A.10.2). Branched nests (n=11) contained, on average, more empty space (34.9 ± 25.3 cm) than linear nests (n=19) (13.4 ± 10.2 cm), and also proportionately more empty space (53% versus 47% in linear nests). However, this difference is not statistically significant (t-test for unequal variances $t_{14.4}$= -1.63, p=0.123) (Table A.10.2).

Geographic variation

Since climate, and presumably season length, vary with geography, it is plausible that nest architecture might vary with geography. The length of a season may be related to the amount of time that females can spend constructing and renovating nests, since relative to shorter seasons, longer seasons allow more for nest construction and renovation. I compared nest architecture among latitudes using nests collected in southern Ontario and Maryland, and using published data from Missouri (Rau, 1933) and Georgia (Gerling and Hermann, 1976) (Table A.10.3). I statistically compared patterns among the Ontario, Maryland and Missouri nests (raw data were not available for Georgia). The number of branches in nests differed significantly among locations (ANOVA on ranks: $F_{2,59}=4.61$, $p<0.01$); pairwise comparisons with Tukey's post-hoc test demonstrated that Missouri nests differed from Ontario and Maryland, but Ontario and Maryland did not differ from each other. The total length of nests also varied significantly among locations (ANOVA on ranks: $F_{2,58}=6.71$, $p<0.01$); this effect was also the result of a difference in Missouri nests. I compared brood cells between Georgia (southernmost latitude) and Ontario (northernmost latitude), and found that southern Ontario nests contained more brood cells (8.43 ± 4.2 cells per nest) than nests in Georgia (6.6 ± 3.5 nests; t-test: $t_{50}=9.3$, $p<0.05$) and also had greater variation in the space occupied by brood cells (3.5-39 cm) relative to Georgia (14.5-20 cm), although this could not be examined statistically.

Table A.10.3: Comparison of nest elements for nests collected in southern Ontario, Maryland, Missouri, and Georgia. Maryland nests were collected in winter and no data are available for foundresses or brood cells. NA indicates data are not available, n indicates the number of nests. Data are arranged from north (Ontario) to south (Georgia).

Location	**Total length (cm) Mean ± SD (range)**	**No. of termini Mean ± SD (range)**	**Cells per nest Mean ± SD (range)**	**Females per nest Mean ± SD (range)**	**Brood cell length (cm) Mean ± SD (range)**	**Source**
Ontario	38.0 ± 19.9 (5.5-93.5) n=35	2.6 ± 1.1 (1-6) n=35	8.4 ± 4.2 (0-21) n=37	2.2 ± 1.73 (0-6) n=36	18.1 ± 11.03 (0-39) n=33	This study
Maryland	45.5 ± 26.0 (11.5-100.0) n=20	2.8 ± 0.9 (2-5) n=20	NA	NA	NA	This study
Missouri	102.0 ± 57.0 (50.8-193.0) n=6	4.5 ± 2.3 (3-9) n=6	NA	NA	NA	Rau, 1933
Georgia	17.5 ± 9.3 (3.9-47) n=39	2.4 ± 0.7 (1-4) n=29	6.6 ± 3.5 (2-14) n=23	2.1 ± 1.48 (1-3) n=19	17.5 ± 1.48 (15-20) n=39	Gerling, 1976

Discussion

Nest site and substrate selection

In southern Ontario, *X. virginica* appears to nest almost exclusively in wooden man-made structures. This behaviour is a common trait in *X. virginica*, as both Rau (1933) and Gerling and Hermann (1976) also reported that nesting was rare in natural materials, although, Rau (1933) did observe bees in a dead branch. Unlike in *X. sulcatipes* (Gerling et al., 1983), there is an association between compass direction and nest location. As with other species, *X. virginica* may locate nests based on shade and sun; nests appear oriented to maximize the surface area that receives light. In the populations studied, *X. virginica* nests were frequently in aggregations within the same boards or structures. It was rare to find a single nest in either one piece of wood or one structure. In addition, some seemingly suitable substrates were unused while new nests appeared in densely occupied boards. When I added "artificial nests" to the courtyard site in summer 2003, none were occupied, nor boards with "pre-drilled" 2 cm diameter nest entrances. These patterns may be because some substrates in the same location are more suitable than others in factors such as orientation or water content. It is also possible that there is a nesting cue that has yet to be detected. Finally, there may be a benefit to nesting in aggregations either for protection or because of proximity to relatives.

The seeming specificity of *X. virginica* nesting substrate may lead to limited nest sites. While a given location may have plenty of wood, it may not have wood that meets the requirements for establishing a nest. Further, conditions may change so that wood that was suitable becomes unsuitable in subsequent years. In chapter 3, I demonstrate that the proportion of nests changes annually but that some nests are permanently abandoned. This may reflect a change in nest suitability, variation in nest quality, or it may indicate that some nests reach a point at which they are permanently unsuitable for re-use. Females may also prefer to re-use the nests in which they overwintered. If this is true, then nests unused one year would be available to females the next year. The apparent tendency to increase the number of branches within a nest may result from females re-using a nest but replacing branches that are no longer suitable for use. This corresponds with the videoscope in which a single nest appears to have both used and unused termini. Specifically, a nest may be re-used as long as new tunnels can be constructed, and then abandoned when the available space for tunnels is consumed. Taken as a whole, these factors indicate that nesting substrate is more limited than would otherwise appear, at least on a local level.

Abandoned *X. virginica* nests and termini are often occupied by other insects. In video observation, I occasionally found nests containing insects other than *X. virginica*. It is not clear if these insects had infected an otherwise suitable nest or if they were using nests that were abandoned, but it is more likely that when nests or termini are unused other insects adopt them. With the videoscope, I have observed a leaf-cutting bee (Megachile) in an abandoned nest. In the summer of 2006 I observed what appeared to be a female mason bee (*Osmia*) using an abandoned nest, and in one nest I found earwigs (Dermaptera) in one branch and *X. virginica* in another. Finally, the wasp species *Ancistrocerus adiabatus* was observed near and within nests at Courtyard bench 1; this bench is known to have few active *Xylocopa virginica* nests (Chapter 3).

Factors influencing nest architecture

Xylocopa nests can have two basic shapes (Table A.10.1, Figure A.10.3). The more simple shape is linear and resembles a letter "T". The "T" shape appears to be the default shape, and the first form of a nest. As nests are re-used, females may add additional branches to replace unsuitable branches or to provide additional room for brood cells. For instance, two new nests were examined at Courtyard with the videoscope in 2005; one nest had a single terminus (and one foundress), the second was linear (2 termini) and also contained one foundress.

In all of the nests I observed, nests were oriented so that entrances were vertical, although they could be on the side or face down. Some authors have suggested that vertical entrance orientation prevents rain from entering nests (Dhaliwal and Kapil, 1968). However, since nests are constructed along the grain of the wood, a vertical entrance is inevitable as long as branches are perpendicular to the entrance. It is also possible that downward facing entrances offer protection from parasitism because they are more difficult to locate, or in the case of many bombyliid flies, to drop eggs in. While some authors have observed species of *Xylocopa* (Rau, 1933; Stark, 1992a) in nests that contain multiple entrances, none of the nests that I collected had more than one entrance, and nests never intersected.

Many nests that were collected at Farmhouse contained extra space not occupied by brood cells. Since provisioning appears to occur one branch at a time, and since nests are provisioned starting at the terminus of the branch (Gerling and Hermann, 1976; Rau, 1933), extra space in nests may reflect a situation in which a nest has more room for brood than a female can produce. For instance, if climate or weather factors, such as the number of suitable foraging days, limit the provisions a female can acquire, than she may not be capable of using all the space within her nest. Duchateau and Velthuis (1988) suggested that in *Xylocopa sulcatipes*, which is multivoltine, extra space is associated with a need to control development time between individuals at differing positions within the nest (Duchateau and Velthuis, 1988). In chapter 1, I note that an adult must feed juveniles after they eclose; this may establish a time limit for provisioning. If a female lays eggs too late in the season, the larvae may not develop to eclosion, or may not be an adult in time to be fed. This would have the effect that a female must stop provisioning cells before she filled all the available space, resulting in unused space.

Geographical variation in nest architecture

X. virginica nest architecture varies geographically. Although all the locations examined show similar patterns of architecture with branched and linear nests, a single entrance, and 2 cm wide tunnels, specifics vary. Nests in Missouri contained more branches and were longer than those in other locations; however, this may be a result of the age, and consequently re-use, of nests in the Missouri population, which was reported to be older than any of the others studied (Rau, 1933). Older nests might have been extended more often or contain more abandoned branches, which would result in longer nests. Nests in Ontario have a greater overall length than those from Maryland and Georgia, but this space does not arise from more branches. Finally, while there was no difference in the number of foundresses between Ontario nests and Georgia nests; there was a difference in brood size. On average, Ontario nests contained larger broods, and displayed a larger maximum brood size. We might expect that a univoltine bee in a southern habitat would have a longer breeding season, which would result in longer nests and larger broods. However, my findings contradict this expectation as the southernmost nests (Georgia) were shortest and contained smaller maximum broods; they are also univoltine. The finding that nest architecture varies with geography agrees with findings for members of the subgenus *Lestis* (Steen and Schwarz, 2000) and for *X.* (*Xylocopa*) *violacea* where variation occurs in both in length and number of brood cells (Vicidomini, 1996).

Interspecific variation in nest architecture in the genus *Xylocopa*

While all species of *Xylocopa* except the members of the subgenus *Proxylocopa* nest in wood or stems (Hurd, 1978), the choice of nesting substrates varies widely by species (Table A.10.4). In addition, variation exists in specific aspects of nest architecture, including total length, and shape that may be influenced by nesting substrate (Table A.10.4). For instance, a bamboo nesting species cannot make branched nests, while longer substrates can support longer nests and larger broods.

Authors have noted that branched nests in many species can lead to communal nesting where each foundress has a separate branch within a nest (Iwata, 1964; Sabrosky, 1962; Steen, 2000). While *X. virginica* does not display communal colony social organization, communalism is reported for *Xylocopa augusti, X. brasilianorum, X. frontalis,* and *X. hirsutissima* (Bonelli, 1976; Sakagami and Laroca, 1971). If branched nests facilitate social evolution then one might expect to see a relationship between branched nests and cooperation. I examined whether nest shape (linear or branched) is associated with social organization (only solitary or some multi-foundress) in 30 species of *Xylocopa* (Table A.10.4) and found no significant association (Chi-square: χ^2_1=0.35, p=0.07). While this is by no means a definitive analysis and may be too simplistic, it

Table A.10.4: Comparison of aspects of nest architecture for various species of *Xylocopa*. NA indicates data not available.

Xylocopa species	Nesting substrate	Multiple females	Nest type	Mean nest length	Mean no. brood cells	Reference
aeratus	*Xanthorrhoea*	Yes	Branched & linear	30.5-linear 40.0-branched	8.09 ± 0.4[a] 6.74 ± 1.1[b]	Steen, 2000
aeratus	*Banksia*	Yes	Branched & linear	19.2-linear 39.7-branched	1.00 ± 0.6[a] 5.05 ± 0.7[b]	Steen, 2000
appendiculata	NA	NA	Linear	15.6	8.67 ± 3.2	Sugiura, 1995
arizonensis	Redwood	NA	Branched & linear	NA	NA	Hurd, 1958
artifex	NA	Yes	Linear	NA	8.00	Sakagami, 1971
augusti	Hardwood	Yes	Branched	NA	NA	Hurd, 1958
bombylans	*Xanthorrhoea*	Yes	Branched	149.7 182.9 164.2	3.71 ± 1.7[a] 6.83 ± 0.5[c] 4.87 ± 0.6[d]	Steen 2000
bombylans	*Banksia*	Yes	Branched & linear	301.4	4.85 ± 0.5	Steen 2000
capitata	Decayed, hard twigs & branches	No	Branched	NA	7.83 ± 0.6	Watmough, 1983
carinata	NA	Yes	Branched	NA	10.0	Bonelli, 1976
combusta	NA	Yes	NA	NA	4.50	Bonelli, 1976
erythrina	NA	No	Branched	6.4	1.15	Watmough,1983; Vicidomini ,1996
fenestrata	Bamboo	No	Linear	25.4	4.60	Dhaliwal, 1968
fimbriata	Seasoned wood fencepost, eaves	Yes	NA	NA	NA	Janzen,1966; Bonelli, 1976
flavicolis	NA	Yes	Branched & linear	NA	3.75 ± 1.0	Watmough, 1983
flavorufa	Aloe, dead wood	No	Branched & linear	8.8	3.57 ± 0.2	Watmough, 1983
frontalis	Hardwood fence posts & tree stumps	Yes	Branched	21.0	6.09	Camillo, 1982; Hurd 1958
imitator	Plants (*Annona*)	No	Branched	NA	NA	Gerling, 1989
inconstans	Decayed twigs & branches, agaves and aloe	Yes	Branched	NA	5.88 ± 0.7	Watmough 1983
inconstans	Decayed twigs & branches, agaves & aloe	Yes	Branched	NA	7.75	Bonelli 1974; Bonelli, 1976
Iris	NA	No	Branched & linear	NA	5-7 or 6-11	Sakagami ,1971; Bonelli, 1967

Table A.10.4 continued

---

[a] Woy Woy site

[b] Kangaroo Island site

[c] Yamba site

[d] Fraser Island site

Xylocopa species	Nesting substrate	Multiple females	Nest type	Mean nest length	Mean no. brood cells	Reference
nigrita	Decayed & hard tree branches	No	Branched	11.2	5.50	Watmough,1983, Watmough, 1974; Sakagami, 1971
nogueirai	NA	NA	NA	NA	5.00	Sakagami, 1971
orpifex	Redwood, pine and sequoia	No	Branched	10.2	NA	Nininger, 1916
pubescens	Wood, poplar	Yes	Branched	60.0	2-3 per branch	Van Der Blom,1988; Gerling, 1981
rufitarsis	Decayed twigs & branches	No	Linear	NA	7.0 ± 2.2	Watmough, 1983
sonoria	Hibiscus, redwood & man-made structures	Yes	Branched & linear	12.5	4.4 ± 2.5	Gerling, 1983
sulcatipes	Dead twigs & branches, Arundo	Yes	Linear	NA	4.7 ± 1.7	Stark,1990; Stark, 1992 a,b
sulcatipes	Dead twigs & branches, Arundo	No	Linear	NA	3.0 ± 0.03	Stark,1990; Stark, 1992 a,b
suspecta[a]	Dead dry rotting wood, some man-made structures	Yes	Branched & linear	NA	4.5 ± 1.6	Camillo, 1982
suspecta[b]	Dead, dry, rotting wood, some man-made structures	No	Branched & linear	NA	11.8 ± 4.9	Camillo, 1982
tranquebarorum	Bamboo	Yes	Branched	22.4 ± 14.0	6 .0 ± 1.0	Sakagami and Laroca, 1971
varipuncta	Oak, pepper, eucalyptus	Yes	Branched	12.7-30.5	NA	Nininger, 1916; Maeta 1996
violacea	Poles, branches, cane	No	Branched & linear	15.3	7.5 ± 2.7	Vicidomini,1998; Vicidomini, 1996; Friese, 1923
virginica (Georgia)	Structural timbers (soft wood)	Yes	Branched & linear	6.6	3.5	Gerling, 1978
virginica (Ontario)	Structural timbers (soft wood)	Yes	Branched & linear	9.1	3.9	This study

---

[a] New nests

[b] Re-used nests

does suggest that the role of nest architecture on social evolution in *Xylocopa* may vary among species, or that the relationship may be weak.

Conclusions

In this study, I have added to the general knowledge of *Xylocopa* nesting habits. I have presented the first comparative information on *X. virginica* nesting with respect to geography. Specifically, I have demonstrated that *X. virginica* nests do not vary in overall shape with geography, but do vary in size and ability to accommodate brood. Finally, I have presented data that suggest little association between nest shape and sociality across species of *Xylocopa*.