Alloparental care in a solitary bee

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Abstract

Research into the evolutionary origins of sociality in insect colonies is changing emphasis from understanding how eusociality is maintained to how insects transition from solitary to social lifestyles. The pygmy carpenter bees (Ceratina spp.) offer an excellent model for investigating such factors as they have been historically thought of as solitary but have recently been shown to be socially polymorphic, which may indicate that they are currently in a transitive phase. By utilizing behavioural observation and experimental removal protocols, I show that extended parental care, as well as sibling care in *Ceratina calcarata* plays an important role in offspring development. I found, upon removal of the mother, that specifically produced 'dwarf' female offspring take over parental care roles in the nest. The existence of alloparental care and generational overlap suggests that although they are classified as solitary bees, *C. calcarata* possess the prerequisite behavioural repertoire for sociality.

Acknowledgments

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Introduction

Parental care in insects

Parental care is broadly defined as any investment in offspring following fertilization of the female gamete (Trivers, 1972; Royle et al., 2012). Trivers (1972) first defined parental care in terms of the reproductive investment of the parent. That is, parental investment is any energy spent to increase the quality or future fitness of an offspring, at the cost of future reproductive success of the breeder (Trivers, 1972). Thus, the amount of investment a parent gives an offspring is modified by the cost to future reproductive success, be it in the form of reduced condition of the parent, or loss of mating opportunity (Trivers, 1972). This inequality between the resource demands of the offspring and the fitness demands of the parent results in conflict (Trivers, 1974). Given the inherent tradeoffs between parental care and future reproductive success, many forms of parental care exist which act to maximize, or at least balance, the needs of the parent and the offspring.

There are many forms of parental care behaviours in insects, and even though patterns of parental care behaviours are generally species specific, it is helpful to loosely categorize them chronologically by offspring development. Parental care behaviours can be divided broadly into three stages: pre-oviposition care, offspring development care, and adult brood care. Prior to oviposition the two main parental care forms are oviposition site selection and nest creation (Royle et al, 2012). During offspring development, from egg until the emergence of the adult, the main parental care behaviours are guarding, maintenance, and food provisioning of the developing offspring.

Once the offspring emerge as adults, parental care behaviours are still generally brood guarding and food provisioning.

Oviposition site selection simply refers to non-randomly depositing a clutch of eggs (Royle et al., 2012). Such site selection reduces the probability of predation and allows for the control of the microclimate in which the offspring develop. In some species of insect, oviposition site selection is accompanied by nest creation behaviour. Nest creation can range from simple burrows dug into soil by lone gravid females like some Gryllidae species (West and Alexander, 1963), to complex structures built by many individuals of a social group like paper wasp hives. The creation of a nest acts to limit predation risk further and better manage the microclimate in which the offspring develop (Royle et al., 2012).

Once oviposition occurs, the next group of parental care behaviours include offspring attendance and mass or progressive provisioning (Royle et al., 2012). Attendance refers to any guarding or maintenance of offspring by the parent. For example male Giant Water Bugs of the family Belostomatidae, rear offspring by attaching a clutch of eggs to their backs (Lauck, and Menke, 1961). The male protects the clutch and aerates the eggs until they hatch. Provisioning refers to the act of providing food for the developing offspring, and is separated into two categories: mass provisioning and progressive provisioning (Royle et al., 2012). Mass provisioning insects provide a one-time store of food to the newly laid eggs, which will be all the food the offspring will have access to until emerging as adults. Progressive provisioning is providing food continually during the offspring’s development. Sand wasps, especially in the tribe Alyssontini, lay their eggs on live, incapacitated, prey items, and then seal the egg chamber (Evans, 2007). These prey items act as the only source of food until the offspring emerge as adults. Alternatively, the burying beetles, genus *Nicrophorus*, continually feed their developing larvae by regurgitation (Smiseth and Parker, 2008). Parental care of adult brood generally refers to offspring attendance and provisioning. Parental care in the primitive cockroach, *Cryptocercus punctulatus*, lasts from oviposition until well into offspring adulthood (Nalepa, 1988). Aside from offspring attendance and progressive provisioning during development, parents of this species provide sustenance for adult offspring through specialized anal secretions and produce only one brood in their life time. Generally, guarding and feeding mature brood rarely occurs in insects (Royle et al., 2012).

A parental care strategy employed by a few insect species is alloparental care. Alloparental care is simply defined as the parental investment given by an individual conspecific that is not the genetic parent (Wilson, 1975). The alloparent can assist in all of the parental care behaviours discussed above (except oviposition site selection), and generally, the alloparent tends to be a relative, a sibling or a past offspring of the breeder (West-Eberhard, 1975). For example, in the African Allodapine bee, *Allodapula dichroa*, communally nesting sisters arrange themselves by size and form a reproductive queue (Tierney, and Schwarz, 2009). While the larger sister breeds, the smaller ones help care for the brood.

The level of investment of an alloparent can vary greatly, from ephemeral, low-investment maintenance behaviours to life-long morphological and behavioural specialization. For example, in the Passalid beetles, adult offspring help care for developing siblings by repairing pupal cases (Schuster and Schuster, 1997). However, this behaviour only lasts until the adult offspring have dispersed. Alternatively, in all the highly eusocial termites, rigid social castes exist in which some individuals become specialized for long-term brood care (Wilson, 1971).

Alloparental care behaviour is interesting because it is considered an altruistic behaviour and represents a costly investment on the part of the alloparent (Alonzo-Alvarez and Velando, 2012). While alloparental behaviour can be very beneficial by increasing brood fitness and the reproductive output of the breeder, the potential costs to the fecundity and fitness of the alloparent can be substantial. Although the alloparental fitness costs may be minimal in a species like the aforementioned Passalid beetle, individuals in a colony of eusocial wasps may lose all future reproductive potential by taking on the role of alloparent (Queller and Strassmann, 1998).

Hamilton (1964) initially proposed a solution to the problem of such altruistic behaviour. He asserted that altruism can appear when the indirect reproductive benefits to the altruist outweigh the direct fitness costs of such behaviour, and that the ratio of costs to benefits is modified by the relatedness between the altruist and the recipient. For example, in a eusocial wasp colony where reproduction is dominated by a single female, the alloparent sisters of the queen may benefit more by helping their highly related sister to rear offspring rather than producing their own (Queller and Strassmann, 1998).

The idea that altruistic behaviour arises due to the reproductive advantage of a species genotype, and the relatedness of the individuals was termed *inclusive fitness* by Hamilton (1964). Inclusive fitness refers to the net fitness garnered from both direct and indirect reproductive routes. As mentioned above, indirect fitness benefits are based on the relatedness of the altruist to the recipient, whereas the direct benefits of altruism can come in the form of extended parental learning, inheritance of territory, or becoming the dominant breeder (Alonzo-Alvarez and Velando, 2012). An excellent example of both indirect and direct fitness benefits of alloparenting comes from the European paper wasp, *Polistes exclamans*. *P. exclamans* sisters nest together and create a reproductive hierarchy where reproduction is left solely to the dominant female (Strassmann, 1998). Cooperative brood care is thought to exist in this group because of the high benefits of inheriting nest substrate and reproductive dominance. Once the dominant queen is lost, the next sister in line has a chance to be the dominant breeder. Hence, not only do females gain indirect fitness benefits from increasing the fitness of their sister's brood, but they also have a chance to gain direct fitness benefits through inheritance of social position.

Social categories in insects

Solitary

A solitary life history generally refers to the behaviour displayed by a species whose females do not share breeding sites and show no parental care toward offspring (Tallamy, 1984; Wilson, 1971). For example, females in the order Ephemeroptera, all of which are solitary, deposit eggs randomly into a source of water, such as a lake or stream, and then die (Allen, 1965). Subsociality is a subcategory of solitary behaviour, and denotes the presence of prolonged parental care (Tallamy, 1984). According to Michener (1964), the mother in a subsocial species guards and progressively feeds her offspring until they reach maturity and leaves thereafter. This definition was further refined by Wilson (1971) who divided subsociality into two intermediate stages which differed in the presence of cooperative brood care behaviour. Currently, a subsocial species is generally accepted as one where a single female deposits her eggs all together in a nonrandom area, guards and maintains the nest site and either mass provisions or progressively feeds the developing offspring (Tallamy, 1984; Plateaux-Quenu, 2008; Crespi and Yanega, 1995).

Parasociality

Parasociality is a broad category which is defined based on the communal nesting of two or more same-generation females, and is further divided into three subgroups based on the presence of cooperative brood care and division of reproductive labor (Wilson, 1971; Crespi and Yanega, 1995). Communal nesting is the first parasocial category and is defined simply by the sharing, amongst conspecifics, of a common nesting substrate. If cohabitating females are found to cooperate in caring for each other's brood, than the group is termed quasisocial. Finally, if reproductive division of labor is present in a cohabitation of same-generation females, then it is considered to be a semisocial group. Generally, a semisocial species consists of a group of nesting females where only one is reproductively active and the rest cooperate in nest construction and brood care.

Eusociality

Eusocial insects are defined as those that show cohabitation by multiple generations of individuals, cooperative brood care, and reproductive caste specialization (Andersson, 1984; Wilson, 1971; Crespi and Yanega, 1995). A typical eusocial colony like that of the honey bee, Apis mellifera, consists of a reproductive queen, specialized simply to reproduce, while the rest of the colony consists of workers that may be a mix of many generations of offspring, all of them specialized for specific tasks based on colony maintenance or defense (Wilson, 1971). Through either behavioural or physiological means, queens control the reproductive potential of the colony, preventing reproduction by workers. The two classes of eusociality, primitively and complex, differ in that castes are morphologically distinct in complex eusociality, but similar in primitive eusociality.

Presocial

Presociality was originally a category of social behaviour which represented subsociality and parasociality combined (Wilson 1971; Crespi and Yanega, 1995). Essentially, presocial is a catch-all term for any species that exhibits social behaviour that falls short of the eusocial prerequisites. Choe and Crespi (1997) note, however, that the term presocial is an inappropriate classification as it implies that presocial species are not yet social. They argue that defining species as presocial underappreciates the complexity of behaviour in non-social species by defining them based on their relation to sociality. Given the difficulties with the term "presocial", and the lack of utility of such a broad category, I propose using "presocial" to refer to any species that exhibits social behaviour but shows no parental care.

Routes to eusociality

The evolution of eusociality is generally thought to progress by two alternate routes: the subsocial route and the parasocial route (Wilson, 1975; Costa, 1997; Fig. 1). The essential defining characteristic which separates them is the order in which eusocial characteristics are developed. The subsocial route is thought to progress through three stages of development. Wilson (1971) summarized the subsocial route to eusociality by separating subsociality into three subcategories. Primitive subsociality is the first step, where only simple parental care exists (see *Solitary* above). From here overlap of generations develops (intermediate subsocial I) and then cooperative brood care occurs (intermediate subsocial II). Finally the route ends at the eusocial stage with the development of reproductive castes. Each step in the subsocial route has been hypothesized to have a specific driver. First, group living and parental care become advantageous if predation or parasitism is high or if food quality is low (Gadagkar, 1990). Second, manipulation of offspring to stay in the nest as helpers leads to cooperative behaviour. Finally, eusociality is reached when genetic asymmetries are exploited by kin recognition, resulting in sterility of helper castes (Gadagkar, 1990).

The parasocial route focuses on increasing degrees of cooperative brood care by same-generation females. The parasocial route begins with communal nesting by same-generation females, and then progresses to quasisociality with the advent of cooperative brood care (Ross, 1983). Next, semisociality develops when a single female starts to dominate reproduction. Finally, if the colony lasts, overlap between generations develops, and eusociality is reached.

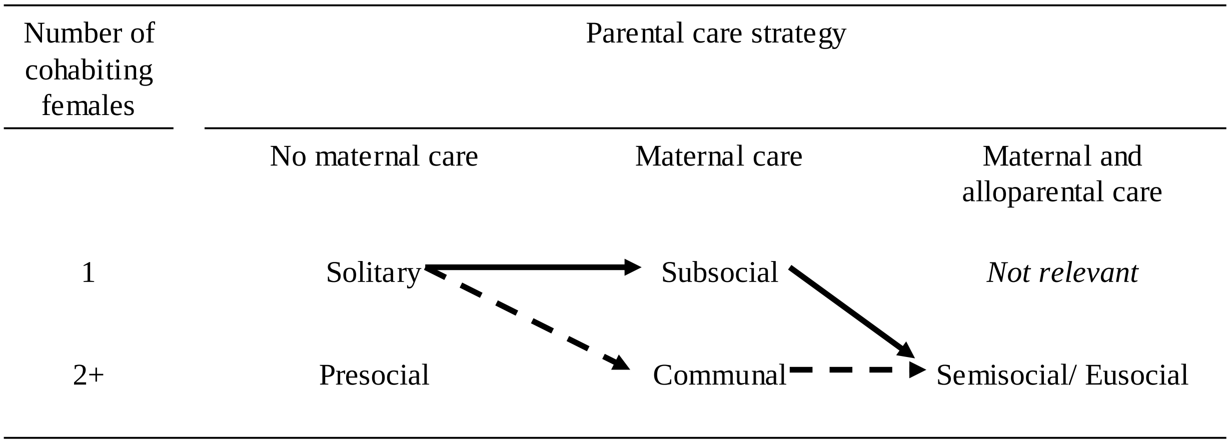


Figure 1: Behavioural categories pertaining to sociality and maternal care in bees. Solid arrows denote the hypothesized subsocial route to eusociality. Dashed arrows denote the parasocial route to eusociality.

General description of nesting biology in the genus Ceratina

The genus *Ceratina* are twig nesting carpenter bees that can be found throughout the world, barring only the polar regions (Sakagami and Maeta, 1984; Michener, 1990; Rehan, Richards, and Schwarz, 2009). Most temperate *Ceratina* species are univoltine, producing a single brood during the warmer months of the year, but bivoltinism is known in some tropical species. Nesting phenology in *Ceratina* begins with the emergence of overwintering adults in the early spring. Newly emerged females forage, mate, and then begin looking for suitable nest sites. The preferred nesting substrate varies by species and region; however, all nest types share the quality of having a soft pithy centre.

Nest creation in *Ceratina* consists of a single female excavating a linear burrow in the pithy center of a twig, within which individual brood cells are constructed (Sakagami and Maeta, 1984; Michener, 1990; Fig. 2). Eggs are laid serially within brood cells, and once an egg is laid the brood cell is closed with a partition molded from the removed pith. *Ceratina* are mass provisioners, which means that each egg laid is provided with a loaf of pollen and nectar that comprises the larva’s entire food supply (Fig. 2). Foraging for the pollen loaf is performed by the mother concurrently with brood cell construction. Offspring development generally last for about six weeks, and following eclosion, adults either disperse or remain and overwinter in the natal nest until the next spring.

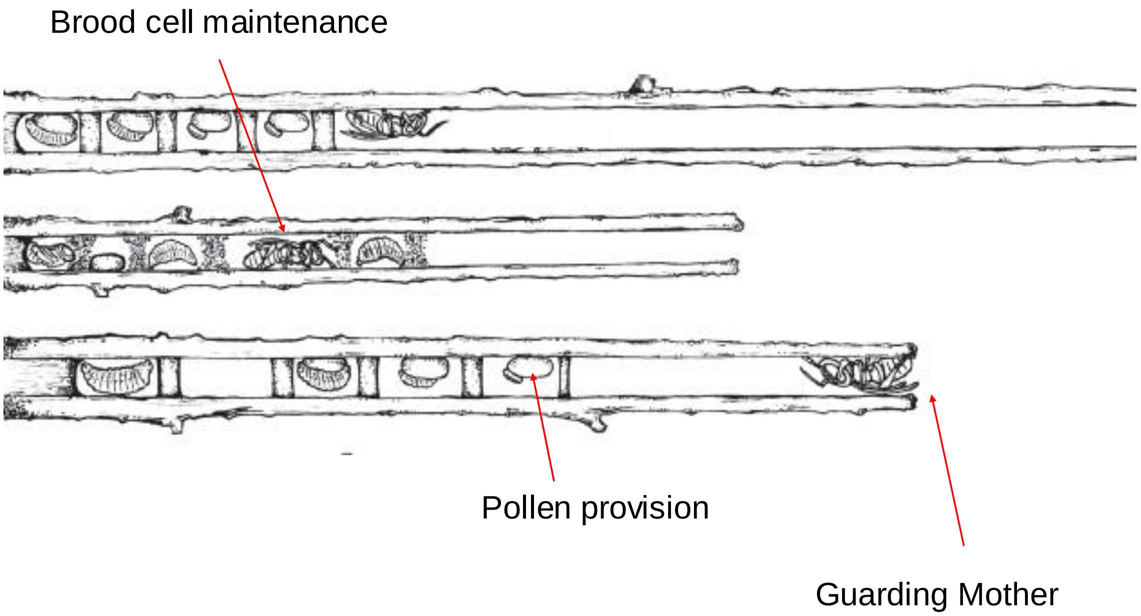


Figure 2: Schematic of generalized *C. calcarata* nests, with maternal care behaviours highlighted. Modified from Rehan, Richards & Schwarz 2009.

Parental care in Ceratina

Parental care in the genus *Ceratina* includes mainly nest creation, food provisioning, and offspring attendance (Sakagami and Maeta, 1984; Michener, 1990). As stated above, all known *Ceratina* lay their eggs in excavated twigs, and mothers remain with their brood at least until they emerge as adults. Mass provisioning is the only form of juvenile food provisioning observed in *Ceratina*. Offspring attendance is separated into two behaviours, brood maintenance and nest defense. Once a female has finished nest construction (including all oviposition and provisioning behaviour), she remains in the nest during offspring development. During the period from oviposition to emergence of the adult offspring, mothers periodically inspect brood cells to remove fecal debris and parasites. They also guard the nest entrance from intruders during this time (Fig 2).

Although the above parental care behaviours are common throughout the genus *Ceratina*, the relative expression of each varies. For example, in *C. megastigmata*, the mother tears down cell walls once and progressively removes debris (Sakagami and Maeta, 1977). Cell walls are never reconstructed, so after a certain larval stage all offspring are raised in a common chamber. In *C. iwatai* brood maintenance is altogether absent, since mothers do not inspect brood cells at all. Instead, brood cell debris is removed en masse once offspring have eclosed (Sakagami and Maeta, 1977).

Brood care in the genus *Ceratina* generally concludes with the eclosion of the brood. Even in species which exhibit bivoltinism, adult parental care is very rare, and adult attendance has only been observed in two *Ceratina* species. In *C. japonica* and *C. flavipes* mothers have been observed to forage for adult offspring once they have eclosed as adults (Sakagami and Maeta, 1977). Although the adult offspring were never observed consuming pollen brought by the mother, this was inferred from the fact that pollen was found in the crops of individuals that were never observed to forage themselves.

Ceratina japonica and *C. flavipes* also represent the only *Ceratina* species that may show alloparental care (Sakagami and Fukuda, 1973; Sakagami and Maeta, 1977). In a few *C. japonica* and *C. flavipes* nests, the first female offspring to emerge were found to forage at the same time as their mother. Sakagami and Maeta (1977) were able to infer that these daughters were feeding siblings in *C. japonica* and *C. flavipes* nests. The authors observed female offspring bringing back pollen loads to their nests, and when the nest occupants were later dissected, pollen was found in their crops. However, specific information regarding exact methodology and sample sizes was not included in the report. Sakagami and Laroca (1971) also observed foraging daughters in the Brazilian *Ceratina*, *C. asuncionis* and *C. oxalidis*; however, sample sizes were very low (<5) and feeding of siblings was never recorded.

Social variation in the genus Ceratina

The genus *Ceratina,* as a whole, is considered to be solitary. Given the widespread incidence of parental care behaviours like nest loyalty, offspring attendance, and nest guarding, the genus is generally further classified as subsocial (Michener, 1962; Tallamy, 1986). However, many examples of social behaviour exist throughout the genus, indicating that *Ceratina* are a socially polymorphic group of bees. In most cases social associations are found between females of the same generation, even though some examples of cross generation associations do exist. Ultimately, of the social categories discussed above, examples of quasisociality, semisociality, and even primitive eusociality have all been found in the *Ceratina*.

Semisocial interactions have been observed in the temperate Asian species, *Ceratina okinawana, C. japonica,* and *C. flavipes,* and also in the tropical species *Ceratina megastigmata, C. smaragdula, C. dentipes, C. australensis,* and *C. nigrolateralis (*Rehan, et al., 2009; Sakagami and Maeta, 1977). Sakagami and Maeta (1984; 1977) showed that roughly 20% of *C. japonica* nests were occupied by same-generation females. Cohabitating *C. japonica* tend to form a reproductive hierarchy where size appears to dictate dominance. Generally, the larger of the two cohabitating females acts as the primary egg-layer and nest guard, while the smaller female acts as the forager. Even though the small female is reproductive, the larger female controls reproductive output by eating the small female's eggs. Similarly, in *C. okinawana* semisocial nests could be created by artificially introducing multiple breeding females into a single nest (Sakagami and Maeta, 1984). In *C. flavipes* very rare instances of cohabitation by same generation females were found to result in semisocial associations with reproductive hierarchies similar to those of *C. japonica*; however, the division of labor was very unstable, with task allocation tending to break down. Ultimately, it was problematic to define a *C. flavipes* nests as quasisocial or semisocial since division of labor appeared to fluctuate (Sakagami and Maeta, 1987).

In a few cases, quasisociality has been seen in *Ceratina iwatai*, *C. megastigmata, (*Sakagami and Maeta, 1977) and *C. australensis* (Rehan et. al., 2009), but the rarest social structure found in *Ceratina* is primitive eusociality. To date only *C. megastigmata* (Katayama and Maeta, 1979), *C. japonica,* and *C. okinawana (*Sakagami and Maeta, 1977; Sakagami and Maeta, 1984) have been suggested to nest eusocially in very rare circumstances. One instance of cohabitation was found between two *C. japonica* females of different generations with reproductive dominance by the older larger female, indicating a primitively eusocial association. However, Sakagami and Maeta (1977) were unable to conclude that this was mother-daughter association, as opposed to two unrelated individuals. Similarly, eusocial nests were found to be inducible in *C. okinawana,* and interestingly, relatedness appeared to dictate reproductive role (Sakagami and Maeta, 1984). When kin cohabitation nests were induced, that is, when a previous daughter was added to her mother's second brood, the mother was found to take on the role of 'queen'. Conversely, in non-kin nests where an unrelated newly eclosed female was introduced to a breeding female's nest, the young female tended to take on the 'queen' role.

Current state of sociobiological understanding in Ceratina calcarata

Ceratina calcarata is a univoltine, mass-provisioning species of small carpenter bees (Vickruck and Richards, 2012; Rehan and Richards, 2010b). In early spring, generally around the end of April, *C. calcarata* emerge from overwintering nests, termed hibernacula (Fig. 3). Generally, the hibernacula are the natal nests of offspring that emerged the previous summer. Newly overwintered females forage and mate and begin to found new nests by the beginning of May (Fig. 3). *Ceratina calcarata*'s preferred nesting substrate is raspberry canes; however, they will also nest in teasel and sumac (Vickruck and Richards, 2012). New nest creation consists of excavating a linear tunnel through the pithy centre of a twig. Once a suitable length of tunnel is completed, reproductive behaviour begins and consists of oviposition, brood cell construction, and brood provisioning. Reproductive behaviour generally lasts from late May until the middle of June (Vickruck and Richards, 2012; Rehan and Richards, 2010b). During this time the foundress forages for pollen and nectar which she provides to her eggs as she lays them. Brood cells are arranged serially in the nests, with the first cell positioned at the bottom of the burrow. The arrangement of the brood cells dictates sequential construction as well as oviposition; that is, one brood cell is completed before construction of the next begins. Once a brood cell is finished, it is capped with a partition molded from pith scraped from the nest's walls. This process is repeated until 7 to 14 brood cells are finished.

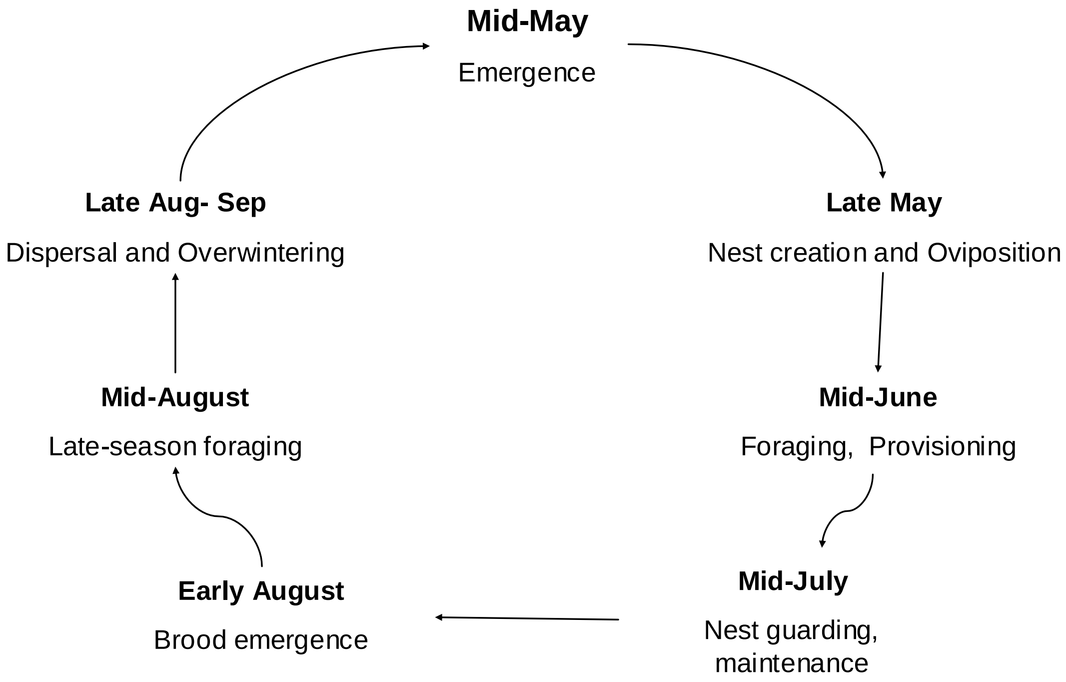


Figure 3: *C. calcarata* nest cycle in southern Ontario.

Once oviposition behaviour is complete, mother bees cease foraging activity and remain at the nest while their offspring develop (Vickruck and Richards, 2012; Rehan and Richards, 2010b). During offspring development, a mother periodically inspects her brood while guarding the nest from intruders, and this behaviour lasts until her brood emerges in early August. Offspring emergence coincides with a second bout of foraging activity. The precise purpose of this phase of foraging activity is unknown, but both mothers and newly emerged daughters are active (Rehan and Richards, 2010b). Activity tapers off during August until early September, at which point one last small peak is seen in activity, considered to be a bout of overwintering dispersal, and then all activity ceases as overwintering behaviour begins (Rehan and Richards, 2010b).

Like all its *Ceratina* congeners, *C. calcarata* is considered to be subsocial (Rehan and Richards, 2010b). Females are long-lived, nest-loyal mass provisioners who periodically inspect brood cells and guard the nest from intruders. In terms of parental care strategies, *C. calcarata* appears unremarkable. To date no examples of cooperation among adults have been observed in *C. calcarata.* However, Rehan and Richards (2010a) noted that in many *C. calcarata* nests, the first two brood cells usually produced female offspring, and these first two daughters tended to be smaller than their siblings.

Like many hymenopterans, breeding females have precise control not only over the sex of their offspring, but also their size (Michener, 1974). Haplodiploid sex determination allows females to choose offspring sex by controlling fertilization. Mass provisioning allows a mother to manipulate how much pollen and nectar is provided to her eggs. With respect to *C. calcarata*, Rehan and Richards (2010a) found that brood sex ratios and body sizes were a function of female size. That is, large, robust, females tended to produce larger offspring with a heavily female-skewed sex ratio, whereas small females tended toward male-biased broods. Ultimately, they found that *C. calcarata* females are able to adjust reproductive investment in response to ecological and biological factors such as somatic quality and resource availability.

In *C. calcarata*, female offspring are larger than males and consequently represent a greater reproductive investment (Rehan and Richards, 2010b). Moreover, overwintering survival, at least of females, is dictated in part by body weight (Rehan and Richards, 2010b). Thus, in the event that either the mother is of reduced quality or resource availability is low, a *C. calcarata* mother is expected to produce proportionally more males. The production of extremely small daughters represents a puzzle, since it is counterintuitive that a mother would produce an offspring which has less of a chance to survive overwintering and reproduce. Instead, a mother would be expected to produce a large robust daughter early when she is still unworn and in her best condition.

Rehan and Richards (2010a) suggested that mothers produce one small daughter in *C. calcarata* to help with brood care. This hypothesis is supported by observations that *C. japonica* and *C. okinawana* both produce early small daughters (Sakagami & Maeta, 1984; Sakagami & Maeta, 1995), and that small daughters feeding siblings is common in Allodapine bees, which are a sister tribe to Ceratinini (Aenmey, et. al., 2006; Tierney, et. al., 2002). Furthermore, Michener (1990) notes that some species of *Xylocopa* and *Ceratina* require a bout of feeding prior to overwintering. If this is true for *C. calcarata*, then the late summer foraging activity noted by Rehan and Richards (2010a) after brood emergence, suggests that the mothers feed adult offspring. Furthermore, the fact that approximately 75% of *C. calcarata* mothers either disperse or die off once their brood has emerged (Rehan and Richards, 2010b), suggests that a survival strategy may exist to overcome the cost of nest orphaning. Ultimately, the production of a small daughter may be a strategy to allow for progressive provisioning of adult offspring in the event of nest orphaning. Testing this hypothesis is the main objective of this thesis.

Hypotheses

The overarching goal of this thesis is to investigate whether *C. calcarata* daughters can be induced to care for their siblings. As alloparental care is the hallmark of caste-based sociality in bees, demonstrating alloparental care would suggest that *C. calcarata* is facultatively social. In order to determine if such social plasticity exists, I have separated this thesis into two parts, each dealing with a different fundamental question. In the first section I ask what the normal reproductive behaviour of an average *C. calcarata* mother is, and then describe such behaviour across a full reproductive season. This step is inherently important as it serves as a baseline for normal *C. calcarata* behaviour, with which to compare alternate social strategies. **My first hypothesis is that *C. calcarata* require a second bout of provisioning in order to survive overwintering**. In the second section I ask how offspring respond to being orphaned and then artificially create such a scenario. In this way, I would be able to induce behaviour which may be rarely expressed in the population; that is, alloparental care behaviour. **My second hypothesis is that a dwarf female is the first offspring to be produced in order to take over brood care in the event of orphaning**.

Objectives and predictions

Section 1: Baseline foraging behaviour and overwintering survival

The first objective is to characterize the foraging effort of Ceratina calcarata mothers across both spring and summer activity phases. I predict that summer phase activity is a second bout of foraging behaviour. The second objective is to show that newly eclosed brood need feeding during the summer phase in order to survive overwintering by food depriving a group and measuring overwintering mortality. I predict that significantly higher mortality rates will be seen in brood which are food-deprived compared to brood which are fed before overwintering. If *C. calcarata* offspring depend on a second bout of provisioning to survive overwintering, then it follows that an alternate parental care behaviour may exist to overcome orphaning.

Section 2: Offspring response to orphaning

The **third objective** is to investigate the response of *C. calcarata* offspring to orphaning by artificially removing mothers prior to summer phase activity. I predict that in the event of orphaning, a single female daughter will take over brood care behaviours. Furthermore, the foraging daughter will predominantly be a dwarf. The **fourth objective** is to show that mothers and alloparents are feeding brood. I predict that all brood in nests with a forager will show signs of both pollen ingestion and fat body growth. The **fifth objective** will be to quantify and compare foraging effort between *C. calcarata* mothers and alloparent daughters foraging in the summer phase. I predict that foraging effort will be comparable between the two groups. If foraging rates are similar between mothers and alloparent daughters, than alloparental care is a sufficient strategy for overcoming orphaning. Furthermore, the presence of alloparental care in *C. calcarata* suggests that this species possesses the social plasticity to be consider facultatively social.

Methods

Site location

Collection and observation sites during both the 2012 and 2013 field seasons were localized to various forest edges on the periphery of the Brock University campus in St. Catharines, Ontario, Canada (latitude 43.12, longitude -79.25; Fig. 4**)**. Sunny, edge environments bordering wooded areas with large patches of raspberry growth were chosen based on known *Ceratina calcarata* nesting site preferences (Vickruck and Richards, 2011). Field sites used in 2012 were destroyed due to maintenance activity by the University; hence, 2013 field sites differ from 2012 sites in location.

Field season timeline

The breeding season of *Ceratina calcarata* comprises three distinct phases: spring foraging, mid-summer guarding, and summer foraging. In 2012, the spring foraging phase began on 30 May and ran to 14 July, the mid-summer guarding phase ran from 15 July to 1 August, and the summer foraging phase ran from 1 August to 5 September (Table 1). In 2013, spring phase foraging observations did not commence until mid-June due to inclement weather. The spring foraging phase was shorter in 2013, lasting from 17 June until 6 July (Table 1). Mid-summer guarding began on 7 July and lasted until approximately 7 August, at which point summer phase foraging began. Summer foraging in 2013 observation nests was stopped before foraging behaviour ceased so that I could collect nests prior to pre-overwintering dispersal. Thus, the timeline for the 2013 summer foraging phase observations lasted from 7 August until 28 August (Table 1).

2013

2012

Figure 4: Aerial map of the University of Brock campus. Red boxes denote collection and observation sites for both field seasons.

Table 1: Date range of *C. calcarata* nest cycle phases with associated number of data collection days. Total number of days in the phase represents all days from first day foraging was observed until last day foraging was observed. Observation days represent actual data collection , while non-observation days represent days skipped or days missed due to poor weather. Total possible foraging days were defined as the number of days foraging behaviour was observed plus the number of missed observation days that foraging was likely to have occurred given mild weather.

	Activity phase					
Field work	2012			2013		
	Spring foraging 30 May- 6 July	Guarding 7 July – 4 August	Summer 6 August – 10 September	Spring 17 June – 6 July	Guarding 7 July – 3 August	Summer 5 August – 29 August
Number of days in phase	38	29	36	20	28	25
Possible foraging days	24	NA	29	12	NA	21
Observation days	18	NA	20	12	NA	20
Non-observation days	20	NA	16	8	NA	5

Preparation of nesting substrates

During the 2012 and 2013 field seasons, 105 and 100 raspberry twigs, respectively, averaging 40 cm in length, were collected from the forest edges surrounding Brock campus (Fig 5a; Fig 5b). Twigs were selected if they were dead but still in a natural position on the shrub (i.e. not trampled and broken on the ground). Upon collection of twigs, the ends were clipped perpendicular to the long axis of the twig so as to expose the pith (Fig. 6). Twigs were zip-tied to a 1m long bamboo stick, so that approximately half of the twig length extended out from one end (Fig. 7). Twig/bamboo combinations were placed within raspberry bushes growing along the forest edge near the observation sites, then left to attract nesting females. Nest founding progress was checked daily, and a twig was marked as founded if a burrowed hole was found in the pithy center of the exposed, clipped surface of the twig (Fig. 6). By mid-June, founding of new nests in the prepared raspberry twigs had finished. All founded nests not used for spring phase observations were left undisturbed at the field site until I initiated summer phase methodology.

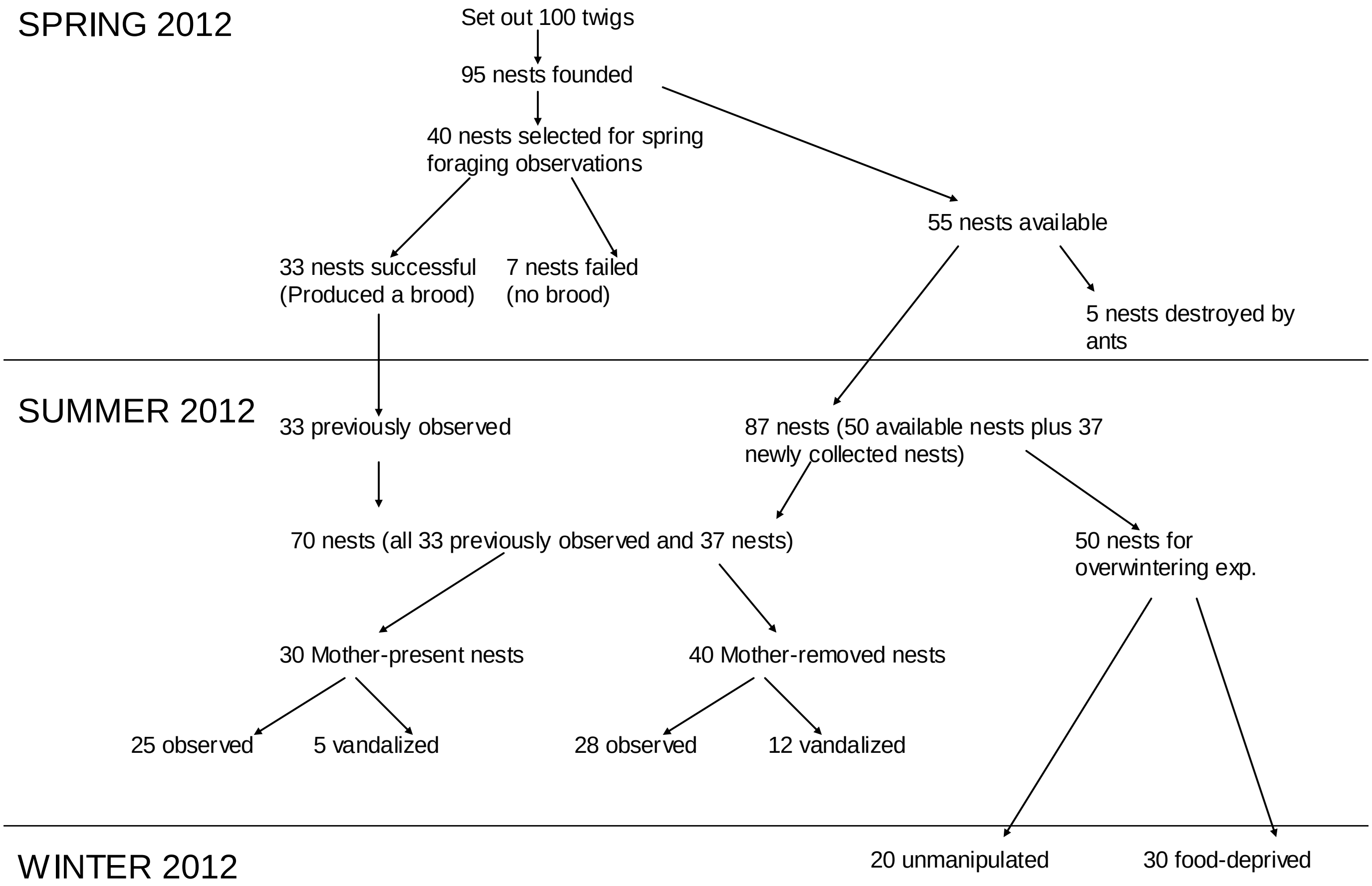


Fig 5a: Flowchart of nest use during 2012. A nest was considered successful if it produced a brood. Mother-present nests refer to nests with a mother present and Mother-removed refers to nests without a mother.

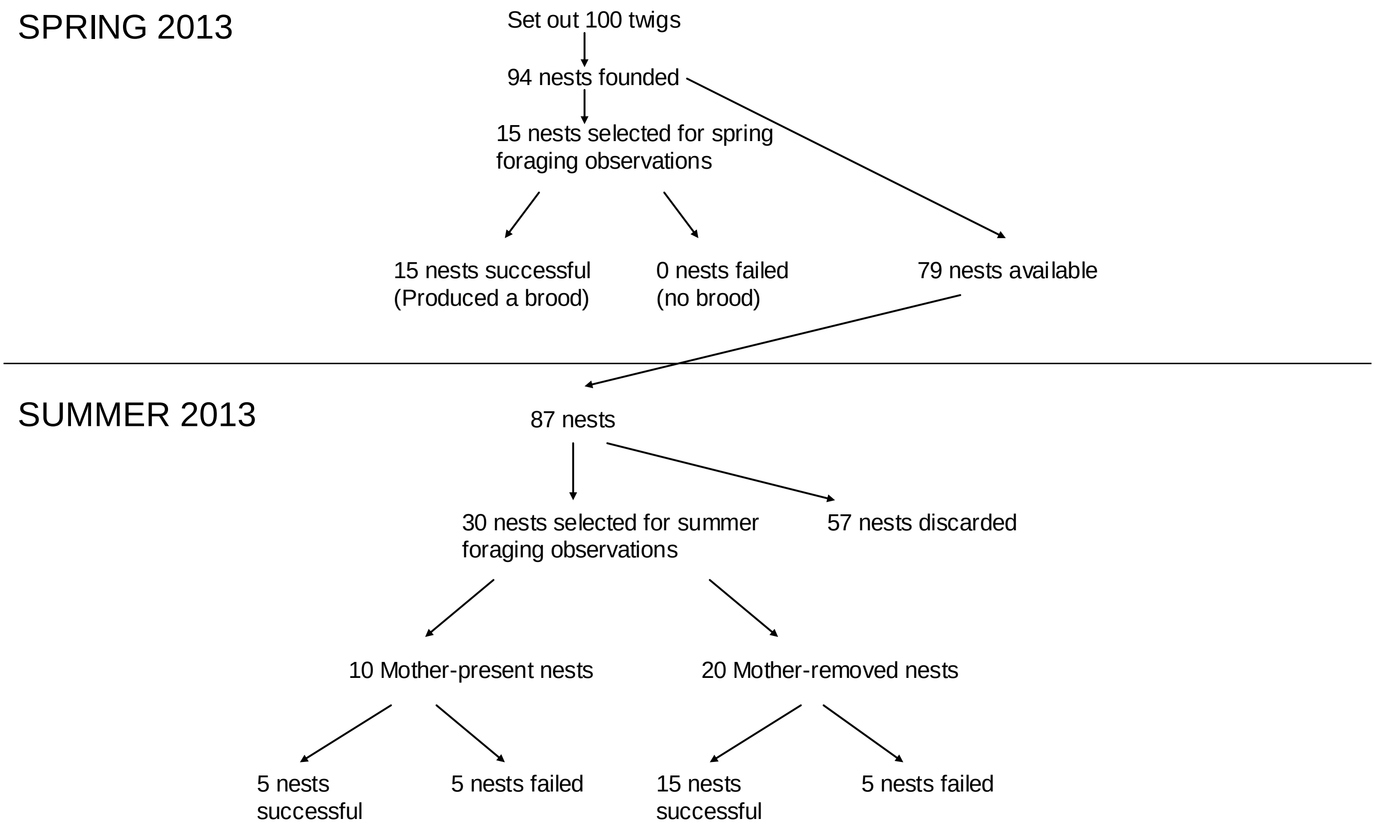


Fig 5b: Flowchart of nest use during 2013. A nest was considered successful if it produced a brood. Mother-present nests refer to nests with a mother present and Mother-removed refers to nests without a mother.

Figure 6: Field pictures of observation nests. A: active nest entrance with attached lid apparatus. B: Ejection of pith in a newly founded nest, with full cup/lid apparatus. C: Fine gauge mesh-covering over a nest entrance. D: Fresh cut raspberry twig, with exposed pithy center.

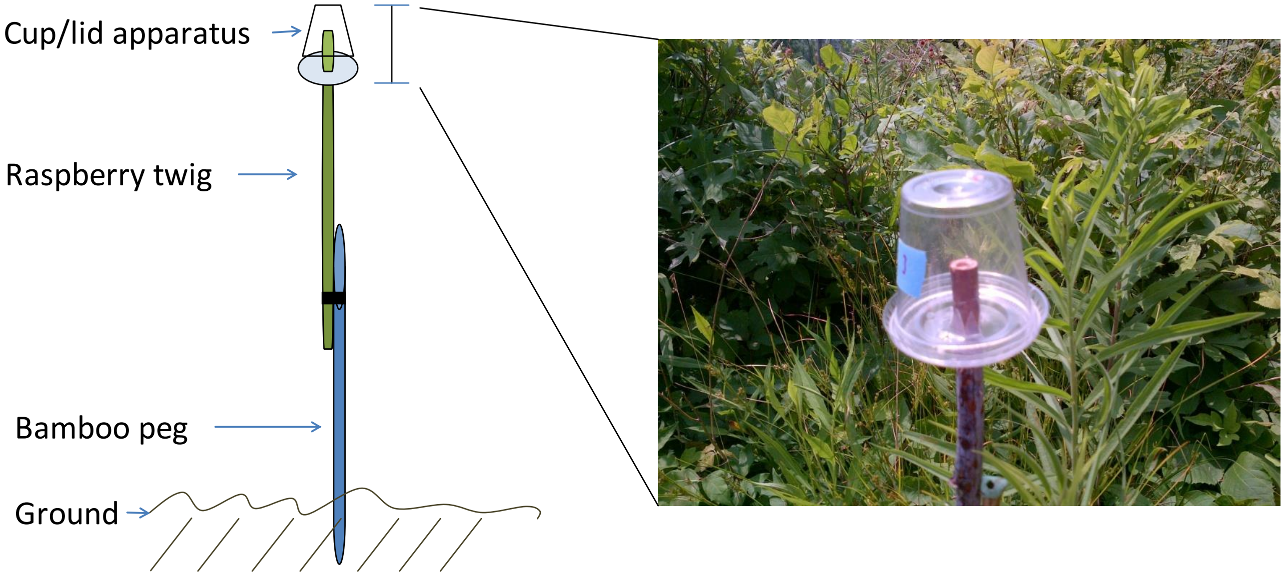


Figure 7: Schematic diagram of generalized observation nest .

Preparation of observation nests

Spring phase

One week prior to the estimated time of spring phase foraging initiation in each field year, a random subset of the founded raspberry twigs was moved to the observation site (Fig. 5a; Fig. 5b). The nests were moved between 07·00 h and 08·00 h to ensure that the foundress was inside. Nests were set up in blocks of 10 in order to facilitate efficient observation, because with more than ten nests there is a greater chance of overlooking an attempted bee departure or arrival (Fig. 8). During the 2012 field season, 40 nests were selected and placed into 4 blocks of 10 each, whereas in 2013, 15 nests were selected and placed into a single block (see Behavioural Observations; Fig. 8)

The nest blocks were set up with each bamboo/twig combination placed into the ground at approximately a 65 degree angle so that the nest entrance was directed towards the spot where I would be seated (Fig. 8). In 2012, nests were placed in a 5 x 2 rectangular grid pattern, with a 25 cm separation between twigs, whereas 2013 nests were placed in a 2 x 8 grid pattern (Fig. 8). I sat approximately 1 m from the centre of the block of nests, perpendicular to its long axis (Fig. 8), which allowed a full view of all nest entrances without the need to rotate my head. Bee departures and arrivals from the nest entrance, as well as other behaviours, could be observed as the individual events happened.

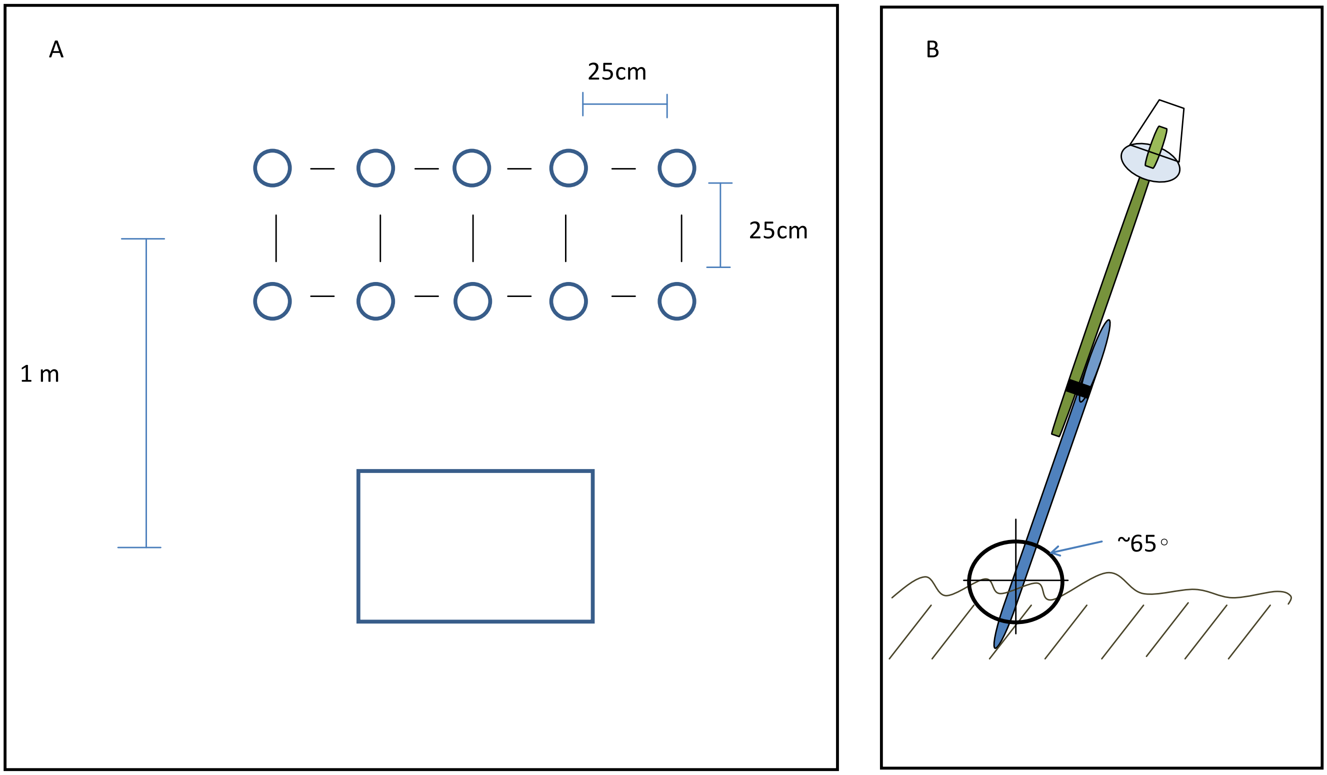


Figure 8: Observation nest block schematic. A: Diagram of typical nest observation block. Circles represent nests, and rectangle represents observation point. B: Diagram of individual nest’s orientation. Nest entrances angled toward observation point.

In order to accurately time the departures and arrivals of foraging bees, nest entrances were enclosed using a plastic cup with a detachable lid to prevent bees from exiting or entering unobserved (Fig. 6; Fig. 7). Preparation of the detachable lid consisted of cutting a hole in its centre, placing it in an inverted position over the raspberry twig, and sliding it down the twig so that it would sit just below the level of the nest entrance. This placement of the lid allowed the companion plastic cup, also inverted, to be placed over the nest entrance, resulting in the creation of a small volume of confined space above the nest entrance. Thus, bees could exit the nest but were prevented from leaving until I removed the cup. After the bee departed, the cup was replaced, blocking re-entry. Once the bee returned to the nest, the cup was lifted again, allowing re-entry into the nest. Lids were set as a permanent fixture on observation nests, but the accompanying cup was only in place during observation periods. This setup allowed for quick and easy removal of the cup, which thus facilitated accurate scoring of departure and arrival times.

Summer phase

Nests selected for 2012 summer phase observations were taken from the pool of spring observation nests and from the unused founding nests group (Fig. 5a). In 2013, summer phase observation nests were taken only from the unused founding nest pool because all 2013 spring phase observation nests were collected following spring phase observations. (see Nest Survival; Fig. 5b).

Nests selected for summer phase observation were collected between 07·30 h and 08·00 h to ensure that mothers were present. Prior to collection, but on the collection day, each nest was covered with a fine gauge mesh to prevent unwanted departures (Fig. 6), then brought to the lab.

To ensure that nests used for the summer phase observations were not already abandoned or occupied by transient males, each nest was checked for the presence of a female. *C. calcarata* females, when disturbed, emit a buzzing sound and/or a lemon-like odour. To check whether females were present in nests, a long stem of grass was inserted into a nest entrance and slowly pushed inside until the female reacted or the stem could go no further. Nests were only selected for summer phase observations if I was able to elicit the olfactory cue. The timing of mother removal was selected to allow for mothers to maintain offspring until they had developed at least into late stage pupae.

Once in the lab all nests were randomly separated into a mother-present group (control) and a mother-removed group (treatment). Females in the control group were left un-manipulated while treatment females were removed. Since the predominant activity during the nest guarding phase of the colony cycle is sitting at the nest entrance (Vickruck, 2010), clipping off the top inch of the nests allowed for the efficient removal of the entrance guarding mother. Nests in both groups were placed back in the observation site after the mother was removed from the treatment group.

Once the presence of a female had been established and mothers were removed from treatment nests, all nests were separated into blocks of ten each. Mother-present and mother-removed groups were placed in their respective summer phase observation sites on the same date. In mother-present nests the mesh was removed when the nest was placed back in the field since the mother was present and able to guard the nest.

However, in order to prevent intruders or parasites from entering the nests, treatment group nests were left in the field with the fine gauge mesh intact. Once summer foraging had ceased, all nests were capped with masking tape, brought into the lab, and stored in a -20^{o}C freezer until nest dissections began (see below).

Marking of foragers

In order to reliably identify individuals within a nest and to differentiate nest residents from usurpers or transients, each resident foundress was marked with a spot of non-toxic, enamel-based model paint on the dorsal surface of the abdomen. Bees were marked over a two day period, at the beginning of foraging observations of both the spring and summer phases.

Females were marked on their first observed departure from the nest by removing the entire cup/lid enclosure as a unit, and placing it on ice for 30 min. This functionally paralyzes the bee but does no damage, and within minutes the individual is fully motile again. Bees were never in direct contact with the ice. After 30 minutes a cooled bee was removed from the ice and held gently by the thorax, with the wings pushed down, away from the abdomen. Testors© enamel-based model paint was applied to the abdomen with a size 0/4 acrylic tipped paint brush. Once the paint was applied, the bee was held near the ice for approximately 15 minutes in order to let the paint dry and to prevent the wings from becoming adhered to the drying paint, a scenario which usually results in severe damage or loss of the wings. Once the paint had dried, the bee was returned to the plastic cup, and the cup was placed back over the nest entrance to allow the bee to retreat back into the nest.

The location of the paint mark on the anterior-dorsal surface of the abdomen was selected to increase the time needed to remove the paint by the individual bee's grooming, as the anterior-dorsal section of the abdomen is least accessible to the middle appendages. Most individuals were eventually able to remove most, if not all, of the paint. Hence, paint was applied repeatedly throughout the season. During initial marking and re-application of the paint, newly painted bees tended to cease all activity until the following day, irrespective of how early in the day painting was done. Hence, no foraging observations were made during marking days.

In the event that a paint mark was lost from a bee, or marking never took place (as in the overwintering survival experiment), wing wear scores were also recorded. The presence of damage on the margins of the wings is an indication that the bee has been active outside the nest, and hence, foraging. Wing wear scoring was based on a scale of 0 to 5, with 0 representing no damage and 5 representing complete obliteration of wing margins (Vickruck, 2010).

Behavioural observations

Daily activity schedule

During the 2012 spring and summer foraging phases, one block of nests was observed each day; since there were four blocks of nests, this resulted in a 4 day observation cycle. In 2013, one block of 15 nests was observed continually throughout the spring phase.

In both 2012 and 2013, an observation day consisted of 5 to 6 hours of behavioural observations, beginning at 09·00 h - 10·00 h and ending 14·00 h - 17·00 h. The 09·00 h start time was determined by observing 3 nest blocks over the course of three days, from 07·30 h until one hour after all activity ceased. Foraging activity was found to start between 09·00 h and 10·00 h, vary rarely was an individual observed foraging before 09·00 h and foraging was never observed before 08·30 h. In the event of bad weather (rain, low temperatures), when *C. calcarata* generally do not forage (personal observations), the observation day was skipped, but the next nest block in line for observation was not changed.

Each full day of behavioural observations on a single bee was recorded as a single bee day. A bee day is defined as the observed behavioural activity of a single individual over the course of a full day. During each observation day, from the beginning of the observation period to the cessation of activity, the nest blocks were observed constantly. Therefore, all departures and arrivals were recorded. In the 2012 spring foraging phase, each of the 33 nests was observed for 2 to 5 non-consecutive days, resulting in a total of 140 bee-days of observations (Table 3). In the 2012 summer foraging phase, 4 blocks of nests were observed (2 mother-present blocks and 2 mother-removed blocks), resulting in a total of 31 nests observed over 163 bee days (Table 3). During the spring phase of 2013, 15 nests were observed continually for 10 days, resulting in 154 bee-days of observation (Table 3). In the summer phase of 2013 only 2 blocks of nests were observed, one mother-present block and one mother-removed block. This resulted in a total of 15 nests being observed over 115 bee days (Table 3).

Quantifying foraging effort

In order to quantify foraging effort, a watch was used to record the departure and arrival times of each flight. Accurate scoring of these times was facilitated by the use of the cup apparatus. When a female bee attempted to leave her nest, the cup was removed from the entrance by hand, and once she had departed, the cup was returned and the time was recorded. During a return trip, the bee approached the nest and hovered near the cup until it was removed, at which point she entered the nest. The time was recorded once the cup was replaced. This pattern was repeated for all nest departures and arrivals, in both spring and summer foraging phases.

The initial presence of the cup barrier was novel, and as such, many individuals were distracted by the cup itself and by the subsequent manipulation of the cup. This resulted in delayed nest returns as the individual would retreat when the cup removal action was initiated. However, once an individual had experienced these events once or twice, she no longer retreated, but would hover near the nest entrance until the cup was removed. Once an individual had become accustomed to the cup removal protocol, any departure from the normative sequence of the action sometimes resulted in the individual leaving the nest entrance area and attempting to enter a different nest. In order to prevent this, the cup was removed immediately upon arrival of the individual, which meant that sometimes I did not have time to notice the presence of a pollen load.

Foraging effort was quantified using five variables: average number of trips per day per bee, average flight time per day per bee, average flight duration per day per bee, average handling time (time spent in the nest between foraging bouts) per day per bee, and proportion of foraging days on which an individual was active. Proportion of foraging days active represents an estimate of the number of days that an individual was active over the full season (spring and summer phases) based on the proportion of active days actually observed (no bee observed was active on every day). These variables allowed for the calculation of total flight time and total handling time per bee for the whole season, which in turn allowed me to estimate total provisioning time per bee for the season. To calculate total provisioning time, the total flight time and total handling time were summed and then multiplied by the proportion of total days active. All the preceding variables and estimates were compared between the spring and summer phases, as well as across the two field seasons.

Types of foraging trips

The presence or absence of pollen carried on a bee's hind femora allowed me to differentiate between pollen-foraging trips and nectar-foraging trips, since nectar is carried internally. Accurate scoring of small pollen loads was difficult due to C. calcarata's small size, flight velocity, and speed of nest entering. On most occasions, once the cup was removed the individual would immediately enter the nest and would be lost from view. Very rarely did an individual stop on the entrance long enough for me to see a pollen load. As a result, foraging observations are biased towards those with large pollen loads, as these were the most obvious on an individual in flight.

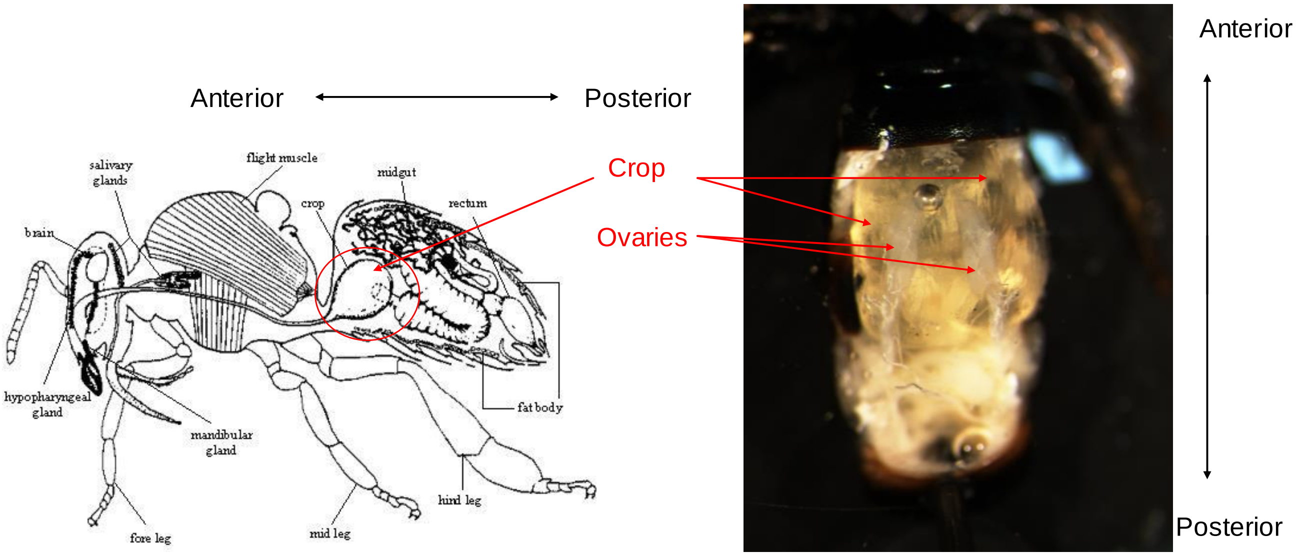
Brood survival

In order to determine brood survival rates, all nests observed during the 2012 and 2013 summer observation phase were collected once summer foraging activity had ceased. Nests were opened using an old, blunt scalpel to split the nest length-wise, removing a section of the twig from one side of the nest while leaving the contents of the nest relatively undisturbed. A general qualitative description of each nest was recorded, as well as the order of individuals in brood cells, the sex of all individuals, the presence of parasites and dead individuals, uneaten pollen loaves, and empty cells. Each individual was stored in a 0.2 ml PCR tube with 70% ethanol.

Assessing gut contents

In order to assess gut contents and ovarian development, all collected bees were dissected. To prevent digestion, and thus loss of gut content data, all collected nests were immediately stored in a -20ºC freezer until the nest occupants could be removed; this acted to quickly euthanize all nest occupants. Once individuals were removed from their nests, they were stored in 70% ethanol until dissected.

Dissections were performed on bees that were immersed in ethanol in a petri dish, using Micro Spring scissors. Fat body presence was noted, and for females, ovaries were removed. The degree of inflation and colour of the crop (stomach) were noted, and then crop, midgut, and hindgut were dissected to ascertain whether they contained pollen (Fig. 9). It is possible to discern whether or not nectar is in the crop because when the crop is punctured, the mixing of the nectar and ethanol is quite apparent due to their differing viscosities; the effect resembles honey being mixed into water. Dissection of the whole alimentary canal was done to increase the chance of seeing evidence of feeding, since the elapsed time between feedings may allow for full digestion.



Dade, 1985

Lewis, 2013

Figure 9: Diagram of generalized apid internal anatomy, and a photograph of the relevant internal abdominal structures in a *C. calcarata* female.

Overwintering survival experiment

In order to assess whether or not summer phase provisioning of newly eclosed brood is necessary for offspring overwintering survival, broods from 20 founding nests not used in previous experiments were food-deprived during the 2012 summer foraging phase. Prevention of foraging was accomplished by attaching the observation cup to the lid with adhesive tape, preventing individuals from foraging, but allowing ejection of nest debris from the removal of brood cell walls. Debris was periodically removed from the cup enclosure. Immediately following the end of summer phase observations on 7 September 2012 (indicating the beginning of overwinter behaviour), the cup apparatus was removed, two layers of fine mesh material were adhered to the nest entrance, and the cup was replaced. This prevented individuals from exiting the nest and intruders from entering during the winter. The cup was left on to prevent moisture accumulation in the mesh.

Overwintering survival nests were placed together within the forest interior near the observation sites to prevent wildlife trampling, and were left until spring temperatures had reached 10 degrees for the first time (April 2013). The nests were brought into the lab on 25 April 2013 and immediately dissected in order to record surviving individuals. Once nest dissection was done, individuals were stored in ethanol.

To control for food deprivation prior to over-wintering, and to assess baseline levels of offspring overwintering survival, a group of wild nests were collected during the same period as the deprivation treatment nests (27 April, 2013). As with the treatment nests, the control nests were brought into the lab and dissected, and the number of living individuals was recorded.

Data analysis

All statistical analysis was accomplished using R, package version 1.5.2. All numerical variables were tested for normality using the Kolmogorov-Smirnov test. Subsequently, normal data were analyzed using parametric tests (Chi-square and ANOVA), or non-parametric tests (Kruskal-wallis and the Fisher’s exact test). All results were assessed with a confidence interval of 95%.

Results

Section 1: Foraging patterns of Ceratina calcarata mothers

Rates of nest founding

During the last week of April in both 2012 and 2013, 100 raspberry twigs were set out to attract nesting *Ceratina calcarata* females. In 2012, nest founding began the first week of May and lasted until the first week of June. Females founded nests in 95 of 100 twigs (Fig. 5a). In 2013, nest founding began in the second week of May and ceased on 30 May. In the initial block of 100 twigs set out to attract females in 2013, 94 nests were founded (Fig. 5b).

Daily patterns of foraging activity by C. calcarata mothers

During the spring phase of 2012, mothers began foraging anywhere from 09·00 until 14·00 h, but peak first departure time generally fell between 10·30 and 11·45 h daily. Foraging activity ranged from 09·00 to 15·00 h, and individual mothers averaged a total 4-5 hours of activity daily, with peak activity (most bees out of the nest) around 12·00 h (Fig. 10). In 2013, spring phase mothers started daily foraging between 09·00 and 13·30 h, with peak activity time ranging from 11·00 to 13·30 h. Spring mothers never foraged before 09·00 h or after 16·30 h during the spring. During an average day, the most active period was between 11·30 - 12·00 h (Fig. 10). Interestingly, 2013 mothers also showed a second activity peak in early afternoon from 13·00 to 14·30 h, as compared to 2012 mothers (Fig. 10).

In the summer phase of 2012, mothers began foraging between 09·15 and 14·30 h with peak first departures falling between 10·00 and 11·30 h. All daily foraging fell between 09·00 and 15·30 h but peak foraging activity occurred between 11·30 h and 13·00 h (Fig. 11) with mothers averaging roughly 4.5-5.5 hours of activity daily. In the summer of 2013, mothers began a foraging day anytime between 09·00 and 15·00 h, with peak first departure time falling between 10·45 - 11·45 h. Similar to the activity ranges in spring, summer mothers never began foraging before 09·00 h; however, summer mothers foraged longer into the afternoon, generally ceasing activity by 15·30 h (Fig. 11). Daily foraging in the summer of 2013 was found to be multi-modally distributed, with mothers showing two distinct peaks of activity throughout the day, whereas 2012 summer foraging was found to be unimodally distributed (Fig. 11).

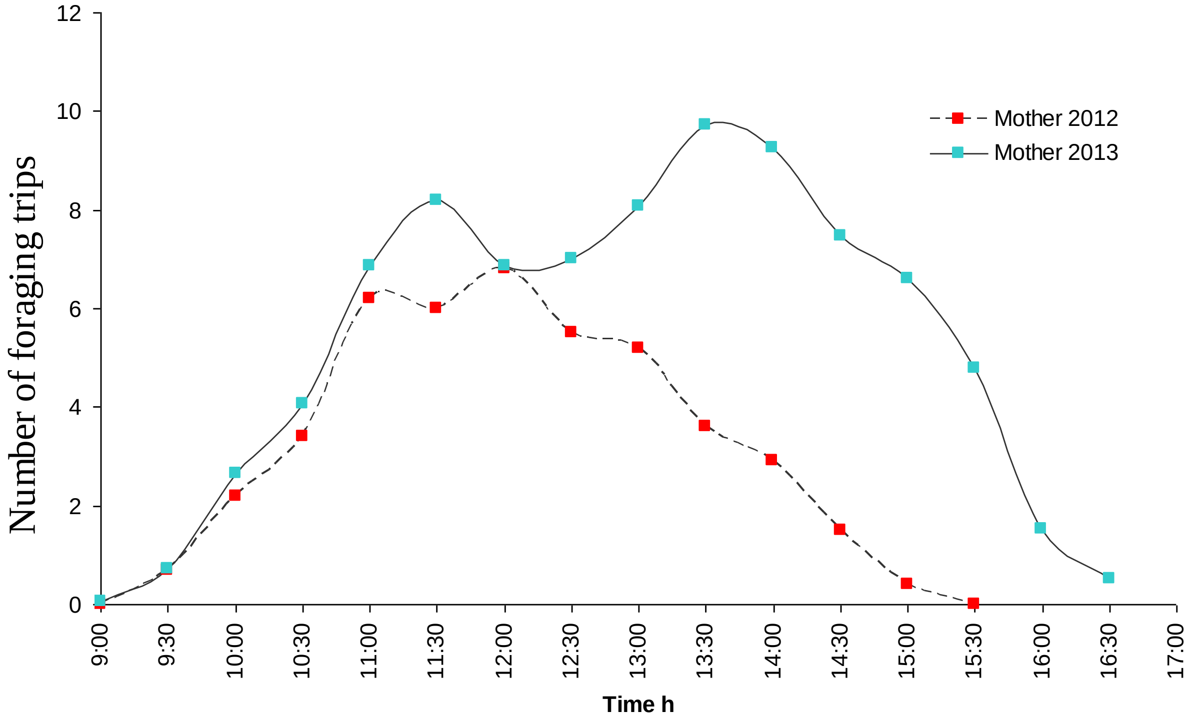


Figure 10: Differences between 2012 and 2013 in the time distribution of foraging trips observed in *C. calcarata* mothers during the spring phase. Each point represents the number of forging trips observed within the indicated period, pooled across all observed bee days, divided by the number of bees observed.

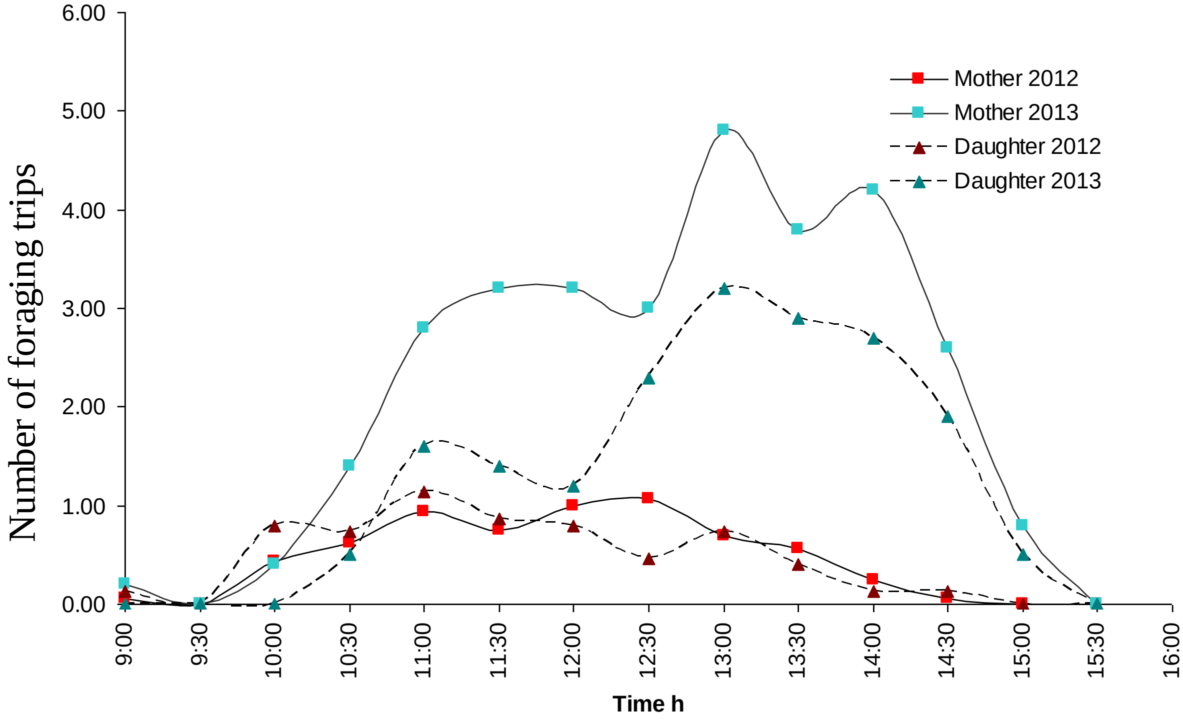


Figure 11: Variation in the time distribution of foraging trips observed in *C. calcarata* mothers and foraging daughters during the summer phase compared across 2012 and 2013. Each point represents the number of foraging trips observed within the indicated period, pooled across all observed bee days, divided by the number of bees observed.

Spring foraging patterns of C. calcarata mothers

In 2012, spring foraging began on 31 May, peaked during the first and third weeks of June, and then began to taper off, until all activity had ceased by the first week of July (Fig. 12). In 2013, spring foraging was delayed until 17 June due to poor weather, but activity ceased during the first week of July, similar to 2012 (Fig. 12). Summer phase foraging began the first week of August in both 2012 and 2013 (Fig. 13). Summer activity in 2012 peaked during the third week of August and then ceased fully by 5 September. In 2013 a similar pattern was observed; however, foraging activity was stopped on 28 August, when I collected the nests (Fig. 13; see methods). The substantial increase in foraging activity from 2012 to 2013 is discussed below (see 'Activity rate of C. calcarata mothers').

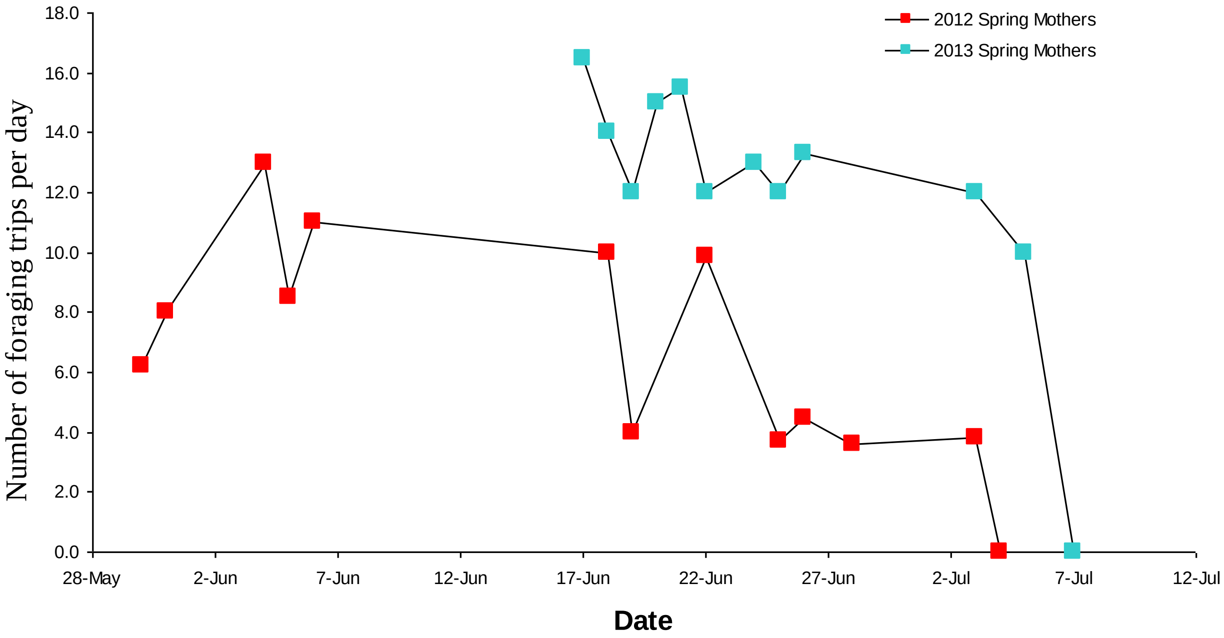


Figure 12: Variation in spring phase foraging activity by date by *C. calcarata* mothers, comparing 2012 and 2013. Mothers in 2013 did not begin to forage until 17 June due to poor weather conditions. Each point represents the number of forging trips observed on the indicated date, divided by the number of bees observed.

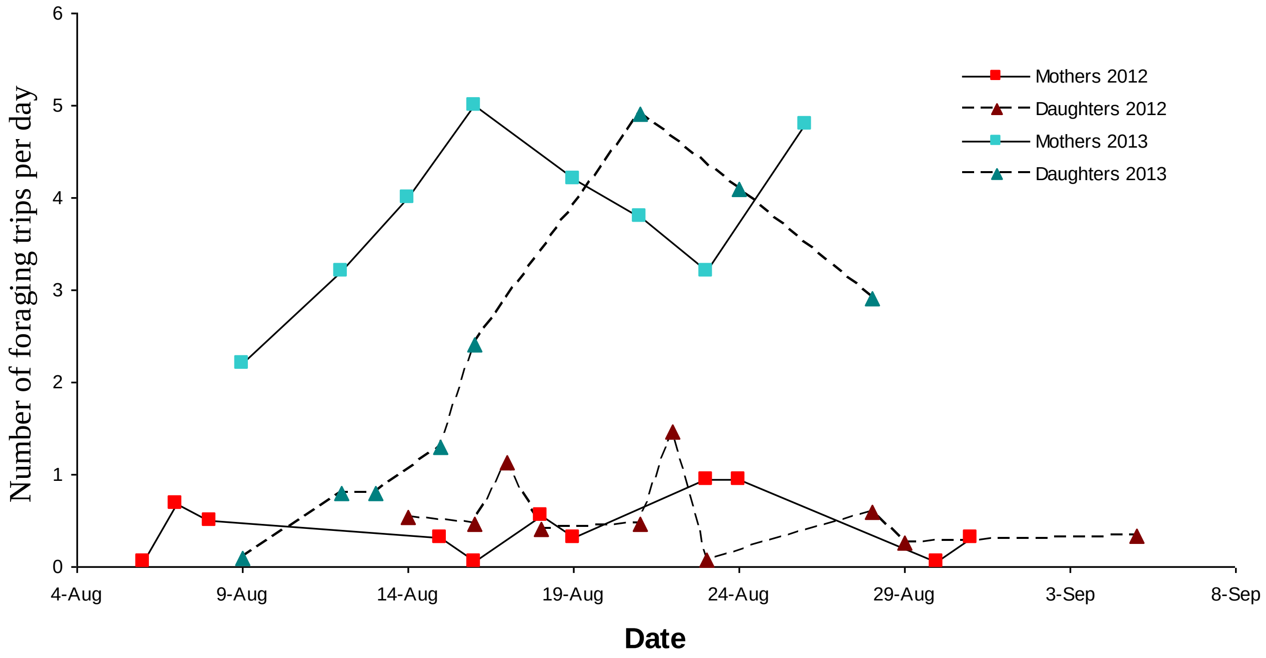


Figure 13: Variation in summer phase foraging activity by date by *C. calcarata* mothers and daughters, comparing 2012 and 2013. In 2013, data collection was ended on 28 August (see methods). Each point represents the number of forging trips observed on the indicated date, divided by the number of bees observed. Note the difference in scale compared to figure 12.

During the 2012 spring phase, 40 newly founded C. calcarata nests were selected for observation from the original pool of 95 founded nests (Fig. 5a). A nest was considered to be successful if a foraging individual was observed on at least one observation day; if not the nest was scored as failed. Of the 40 observation nests, 33 (83%) contained a foraging mother and were considered to be successful, while the rest showed no activity and were scored as failed (Fig. 14; Fig. 5a). Daily observation of the 33 successful nests revealed that spring mothers foraged on about two out of every three days (Table 2). Roughly 60% of flights during the spring phase were pollen trips, while 40% were for nectar (Table 2). In 2013, 15 founding nests were selected from the pool of 100 for spring observations and all nests contained a foraging mother (Fig. 14; Fig. 5b). Bees were active in the spring of 2013 about 80% of the time, and approximately 87% of flights were pollen trips (Table 2). Overall, mothers were more active and were able to collected pollen and nectar faster in 2013 compared to 2012 (Chi-square: x2=6.18, df=2, p=0.001; Table 2). Furthermore, the ratio of pollen to nectar trips was lower in 2012 compared to 2013 (Chi-square: x2= 9.29, df=2, p<0.0001; Table 2).

Table 2: Comparison of spring mother activity rates and pollen vs. nectar foraging trips between phases. An active bee day is defined as an observation day where the focal bee was observed to forage at least once. 2013 mothers spent more time active compared to 2012 mothers. The ratio of pollen to nectar trips was lower in 2012 compared to 2013.

Year	Bee type	No. of bees observed	Total bee days	Active bee days	Non-active bee days	Total foraging trips	Pollen trips	Nectar trips
2012	Spring mothers	33	140	94 (67%)	46 (33%)	428	258 (60%)	170 (40%)
2013	Spring mothers	15	154	127 (82%)	27 (18%)	1202	1041 (87%)	161 (13%)
Comparison				Chi-Square: x^2= 6.18, df=1, p = 0.001			Chi-Square: x^2= 9.29, df=1, p<0.0001	

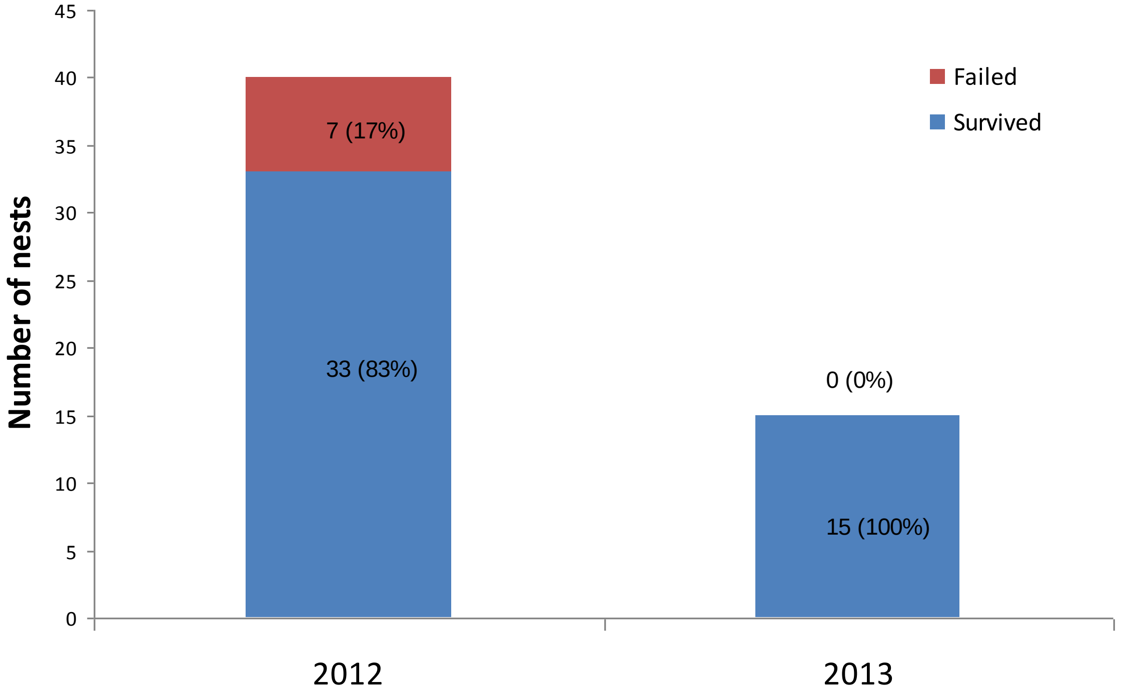


Figure 14: Nest survival rates during spring phase in both 2012 (n=40) and 2013 (n=15) field years. Bars represent the number of nests that survived until the end of the spring phase vs. those that died based on the presence of the foraging mother (Fisher’s exact probability test: Phi = 0.23, df = 2, p =0.2).

Summer foraging patterns of C. calcarata mothers

The blocks of nests used for summer phase observations were those in the control group of nests in which the mothers were present (Fig. 5a). During the summer phase in both 2012 and 2013, only one female was observed to forage per nest. High wing wear and paint marks confirmed that these females were the original nest foundresses (see methods; Fig. 15). Surviving 2012 mothers foraged on about 2 out of every 3 days observed, and roughly 33% of flights were pollen trips (Table 3). Of the 25 control nests selected in 2012, 16 (64%) contained a foraging mother during the summer phase, whereas 36% of nests had failed and contained no occupants (Table 4). In 2013, 10 founding nests were selected for summer phase observations, with 5 (50%) nests containing a foraging mother while the other 5 were empty (Table 4). Surviving 2013 mothers foraged on about 88% of days observed, and 90% of flights were pollen trips (Table 3). Overall, summer mothers were more active in 2013 compared to 2012 (Chi-square: x2= 8.68, df=1, p=0.003; Table 3).

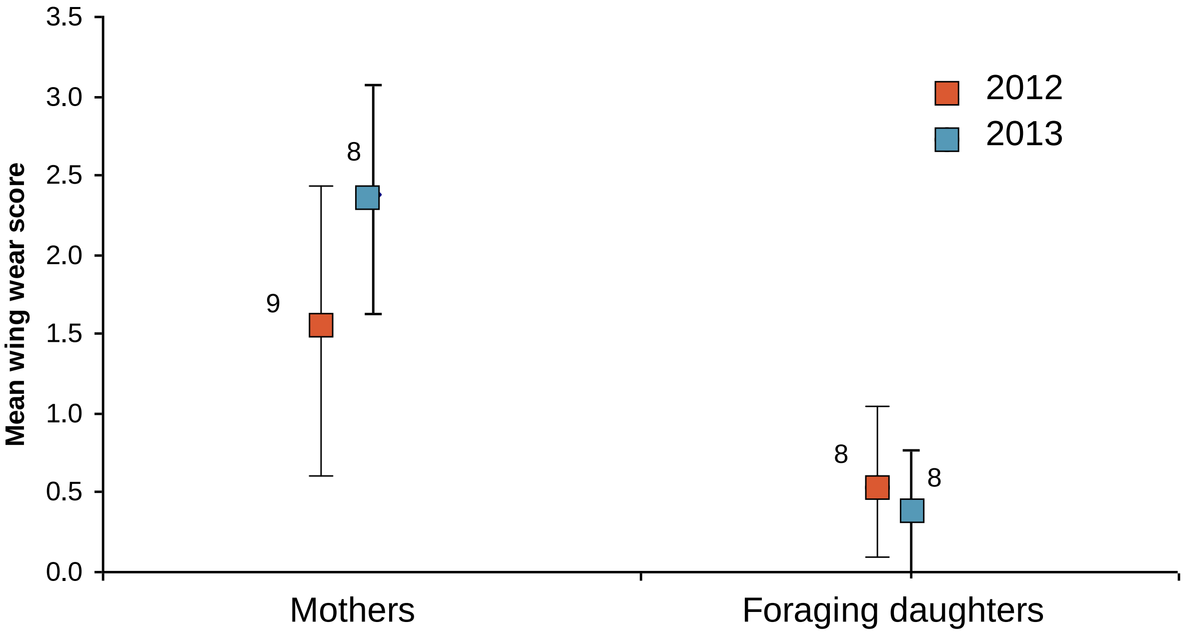


Figure 15: Mean wing wear scores between summer mothers and summer daughters +/- StDev. Number above data point is the sample size. Wing wear scores for foraging daughters are significantly lower than mothers (Kruskal-Wallis: H= 17.31, df = 3, p < 0.001)

Table 3: Comparison of summer mother and summer daughter activity rates and pollen vs. nectar foraging trips between phases. An active bee day is defined as an observation day where the focal bee is observed to forage at least once. Control and treatment refer to nests with a mother present and nests where the mother has been removed, respectively. Mothers were found to be more active in 2013 than 2012, whereas daughters were not (Chi-Square: Mothers: $x^2 = 8.68$, df = 1, p=0.003; Daughters: $x^2 = 1.42$, df = 1, p=0.1)

Year	Bee type	# of bees	Total bee days	Active bee days	Non-active bee days	Total foraging trips	Pollen trips	Nectar trips
2012	Mother (control)	16	88	54 (61%)	34 (39%)	103	34 (33%)	69 (67%)
	Foraging daughter (treatment)	15	75	51 (68%)	24 (32%)	96	37 (39%)	59 (61%)
2013	Mother (control)	5	35	31 (88%)	4 (11%)	154	139 (90%)	16 (10%)
	Foraging daughter (treatment)	10	80	47 (59%)	33 (41%)	182	166 (91%)	16 (9%)

Table 4: The number of nests with a forager and the identification of the forager. When a mother was present she was the only forager observed. Conversely, when the mother was artificially removed a single female offspring was the only individual to be observed foraging. Control refers to mother-present nests and Treatment refers to mother-removed nests. Nest fate refers to nests which produced a brood (Survived nests) vs. nests which did not (Failed nests; see fig 5b). Rate of mother replacement in treatment group between 2012 and 2013 is comparable (Fisher Exact Probability Test: Phi= 0.5, df=2, p>0.7).

Year	Type of nest	Total nests	Nest fate		Surviving nests with brood at collection	Surviving nests without brood at collection	Forager identity in the summer phase
2012	Control	25	Survived 16 (64%)	Failed 9 (36%)	8	8	All mothers
	Treatment	28	Survived 15 (54%)	Failed 13 (46%)	9	6	Single daughters
2013	Control	10	Survived 5 (50%)	Failed 5 (50%)	5	0	All mothers
	Treatment	15	Survived 10 (66%)	Failed 5 (33%)	10	0	Single daughters

Activity rates of C. calcarata mothers

The average number of flights per bee per day was assessed for both the spring and summer foraging phases during 2012 and 2013. In 2012, spring mothers averaged 4.2 ±2.6 foraging trips per day, while summer mothers showed a significantly lower number of foraging flights at 1.8 ±0.5 per day (Man-Whitney U: z= 3.04, p= 0.002; Fig. 16). In 2013, mothers also foraged more in spring (8.3 ±2.1 foraging flights per day) than in the summer phase (4.8 ±1.3; Mann-Whitney U: z= 2.57, p= 0.01; Fig. 16). Ultimately, foraging mothers made fewer flights during the summer in both 2012 and 2013, even though overall number of flights increased from 2012 to 2013 (Kruskal-Wallis: H=36.2, df=3, p < 0.0001; Fig. 16).

The average duration of foraging flights per day per bee was assessed for both the spring and summer foraging phases during 2012 and 2013. 2012 spring mothers were found to fly an average of 13.4 ±5.3 minutes per foraging trip, whereas summer mothers showed significantly higher trip times at an average of 32.2 ±10.4 minutes per trip (Mann-Whitney U: z= -5.24, p<0.001; Fig. 17). In 2013, the relative increase in average flight duration from spring to summer was found to be similar to 2012 levels, with spring mothers spending 10.5 ±1.6 minutes per flight and summer mothers spending significantly more time foraging at 22.9 ±3.1 minutes per flight (Mann-Whitney U: z= -3.32, p<0.001; Fig. 17). Overall flight duration increased in both spring and summer from 2012 to 2013 (Kruskal-Wallis: H=42.94, df=3, p < 0.0001; Fig. 17)

The time spent in the nest by foragers, between foraging trips, was compared between spring and summer. In 2012, summer mothers invested an average of 19.7 ±15.8 minutes of handling time between foraging trips, while summer mothers were found to have significantly more handling time at 37.5 ±22.3 minutes between trips (Mann-Whitney U: z= -2.87, p<0.005; Fig. 18) . In 2013, spring and summer mothers were shown not to have a statistically different average handling time, at 18.3 ±8.0 minutes and 19.5 ±9.1 minutes, respectively (Man-Whitney U: z= -0.04, p=0.96; Fig. 18). Overall, handling time decreased from 2012 to 2013 (Kruskal-Wallis: H=12.1, df=3, p = 0.007; Fig. 18).

In general, spring mothers completed many short foraging trips with comparatively short handling times in between. In contrast, summer mothers completed only half as many trips with trip durations and handling times being twice as long.

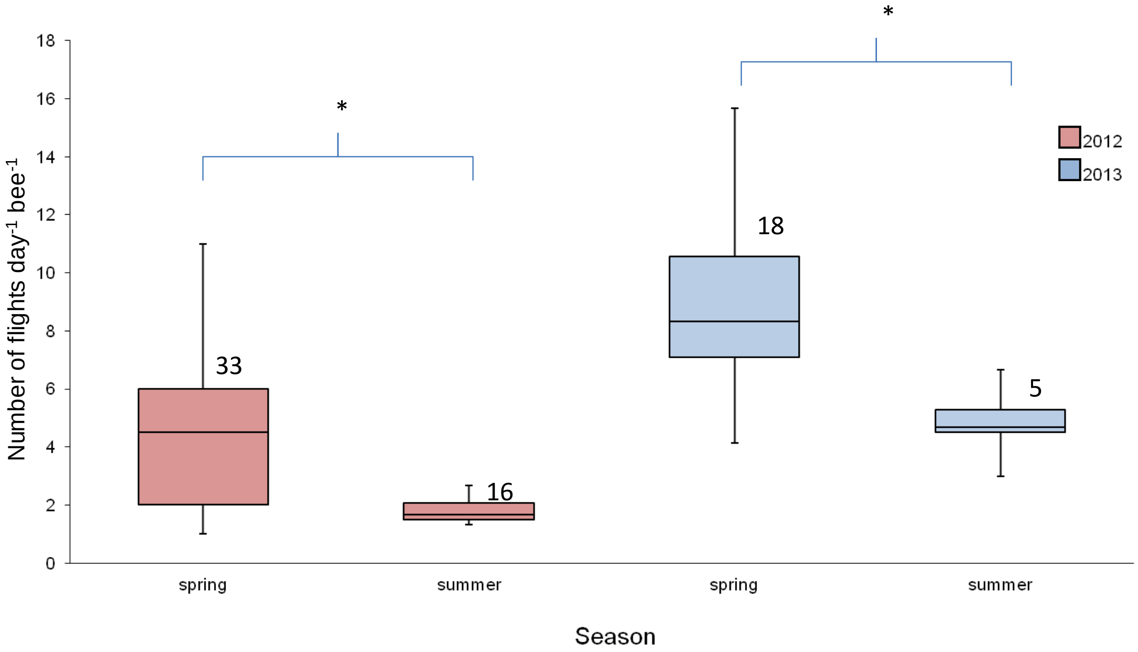


Figure 16: Comparison of spring and summer foraging rates in *C. calcarata* mothers, across 2012 and 2013. Boxes represent the mean, 1st and 3rd quartile, and maximum and minimum values of the average flight number distribution. Numbers above boxes represent nest sample sizes. Asterisk indicates significant difference (Mann-Whitney U: 2012, z= 3.04, p= 0.002; 2013: z= 2.57, p=0.01). Overall number of flights increased from 2012 to 2013 (Kruskal-Wallis: H=36.2, df=3, p < 0.0001)

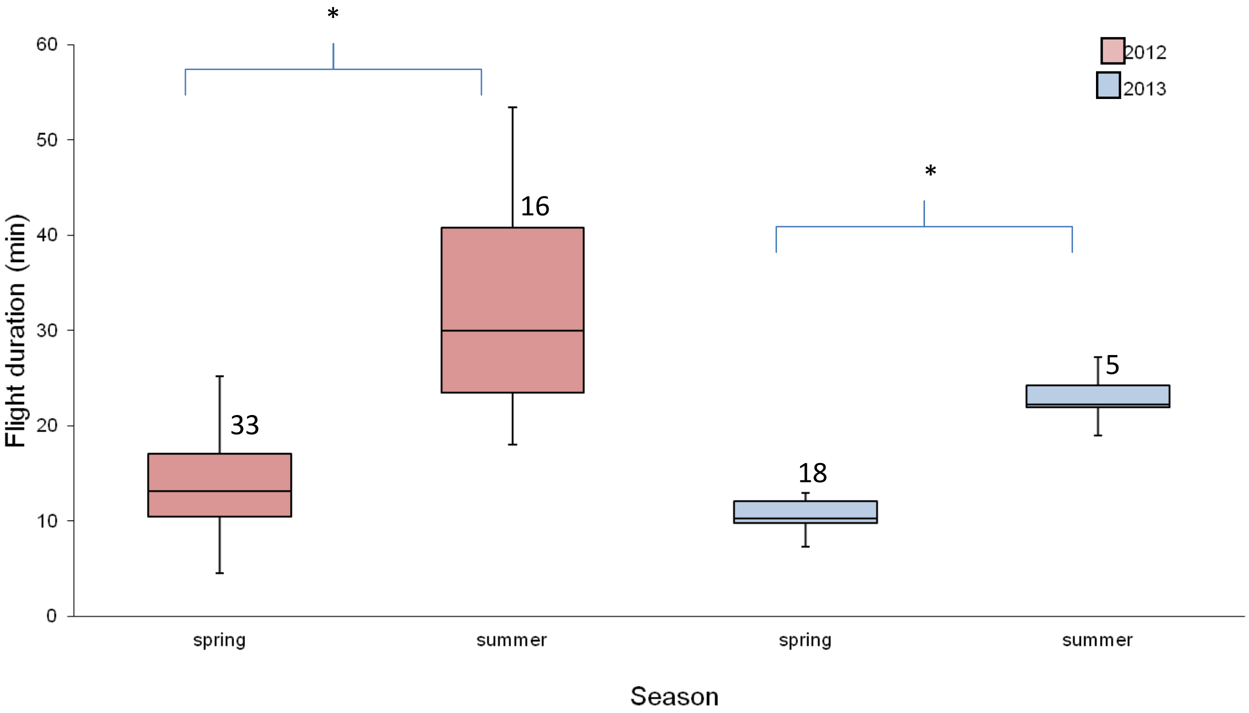


Figure 17: Comparison of spring and summer average flight duration in *C. calcarata* mothers, across 2012 and 2013. Boxes represent the mean, 1st and 3rd quartile, and maximum and minimum values of the average flight duration distribution. Numbers above boxes represent nest sample sizes and asterisk signifies significant difference (Mann-Whitney U: 2012:, z= -5.24, p=<0.001; 2013, z= -3.32, p= <0.001). Overall flight duration decreased from 2012 to 2013 (Kruskal-Wallis: H=42.94, df=3, p < 0.0001).

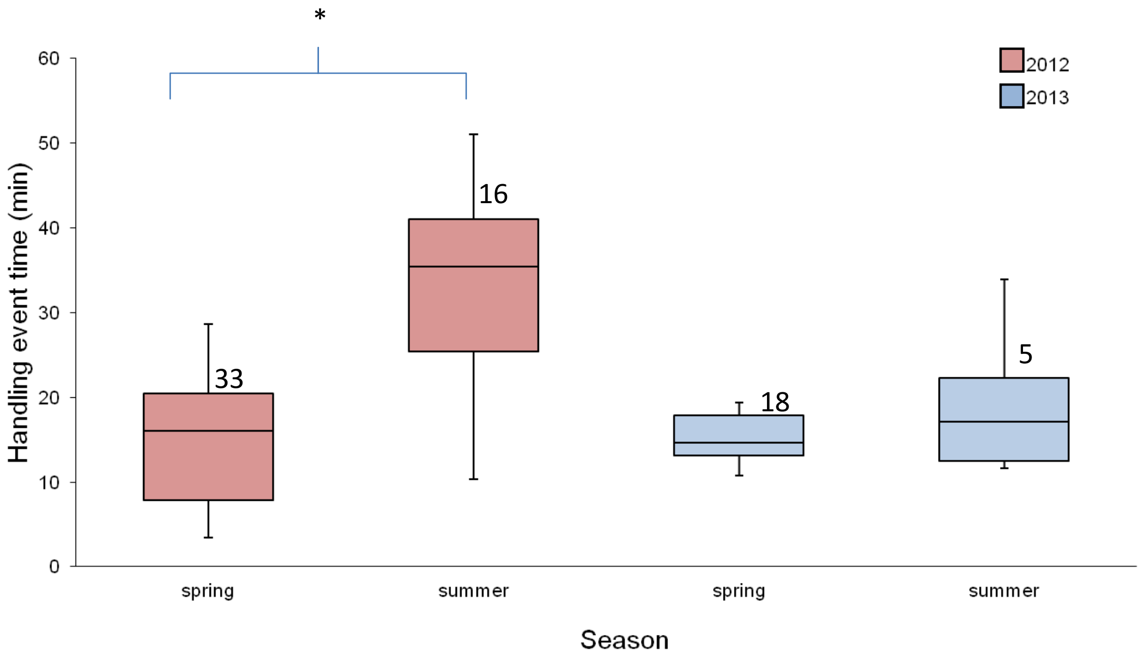


Figure 18: Comparison of spring and summer average handling time in *C. calcarata* mothers, across 2012 and 2013. Boxes represent the mean, 1st and 3rd quartile, and maximum and minimum values of the average handling duration distribution. Numbers above boxes represent nest sample sizes and asterisk signifies significant difference .(Mann-Whitney U: 2012: z= -2.87, p= <0.05; 2013: z= -0.04, p>0.05). Overall handling time decreased from 2012 to 2013 (Kruskal-Wallis: H=12.1, df=3, p = 0.007)

Section 2: Offspring response to brood orphaning

In order to assess the response of offspring to orphaning, a block of nests was selected from the original group of founded nests and then separated into two groups. In the mother-present group (control), the nests were un-manipulated (Fig. 5a). In the mother-removed nests (treatment), the mother was removed prior to brood eclosion (see methods; Fig. 5a). Combining data from both years, pooled across 2012 and 2013, a total of 78 nests were monitored during the summer phase (Fig. 5a). Of these 78 nests, 32 (59%) nests did not produce a foraging individual or contained no occupants when the nest was opened. Of 35 mother-present nests, 21 (60%) were observed with the mother as the sole forager during the summer phase (Table 4). Of 43 mother-removed nests, 25 (58%) had a single female offspring completing foraging trips (Table 4). The rates of mother replacement in 2012 and 2013 were similar (Fisher's exact probability test: Phi=0.5, df=2, p>0.7; Table 4)

Brood survival

In both mother-present and mother-removed groups, a nest was considered to be successful if an individual was observed to forage on at least one day during the summer foraging phase and if live bees were collected from the nests at the end of the phase. Successful nests generally yielded live individuals upon nest collection at the end of the summer phase; however, half of nests from 2012 were empty when opened. As these nests were unparasitized and cleaned of debris, I assumed the brood had dispersed and deemed the nests to be successful. If no foraging activity was observed in a nest and it was found to be parasitized, it was scored as failed. In 2012, only 8 of 16 mother-present nests contained live bees, and only 9 of 15 orphaned nests contained live bees (Table 4).

In 2013, all nests scored as successful in both mother-present and mother-removed groups contained bees when collected (Table 4).

Identity of foraging daughter

Head width was measured in all foraging daughters in order to investigate the hypothesis that the smallest daughter would become the forager. Pooled across 2012 and 2013, of 25 foraging daughters observed, 16 were collected. In 6 nests the foraging daughter was the smallest female in the nest, and in 4 nests she was the second smallest female (Fig. 19). In 2 nests a single female offspring was found, and they were both considered the smallest female in their respective nests (Fig. 19). Lastly, in 4 nests the foraging daughter was the third smallest female (Fig. 19). Overall, foraging daughters in orphaned nests tended to be the smallest female offspring (Kruskal-Wallis: H= 27.01, df = 3, p = 0.0001; Fig. 19).

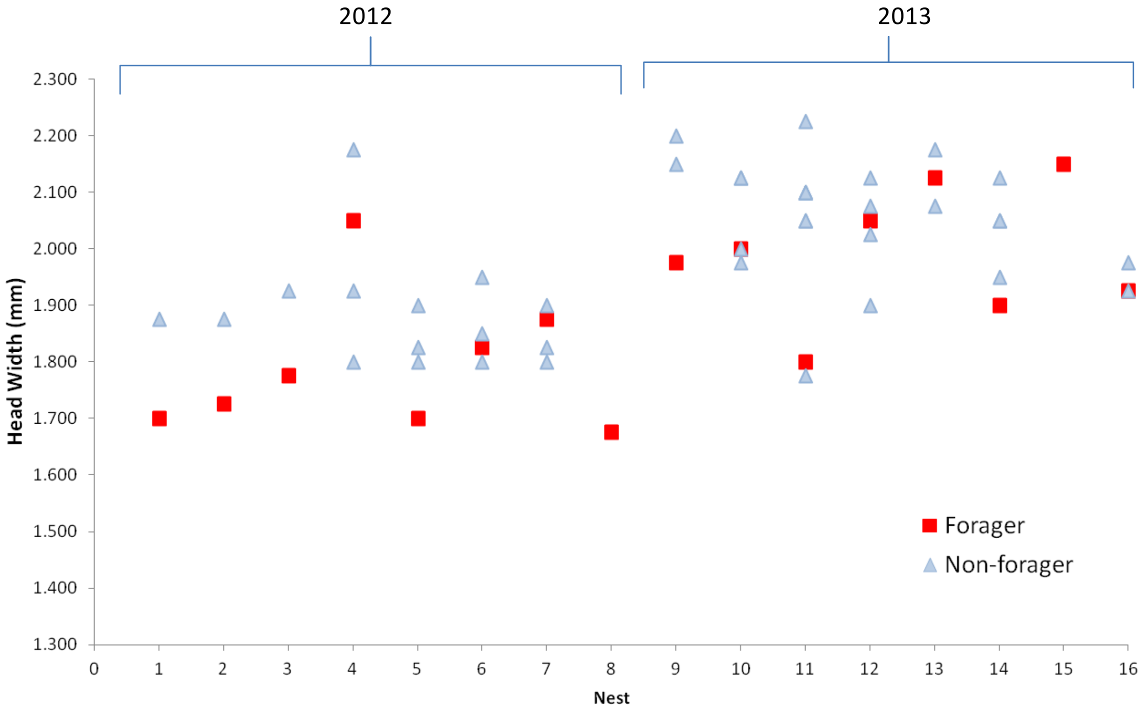


Figure 19: Comparison of body size between foraging daughter and female siblings across 16 treatment nests. On average, foraging daughters had significantly smaller head widths than female siblings (Kruskal-Wallis: H= 27.01, df = 3, p = 0.0001.)

Comparison of foraging mothers vs. foraging daughters

Seasonal foraging patterns of foraging daughters

Foraging daughters began collecting pollen and nectar during the first week of August in both 2012 and 2013. In 2012, foraging activity reached a peak during the second week of August and then tapered off until all activity ceased by the first week of September (Fig. 13). The same pattern was observed in 2013; however, observations were stopped on 28 August due to early collection of nests (Fig. 13).

In 2012, 28 orphaned nests were created from the original pool of 100 founded nests and used for summer phase observations (Fig. 5a). Out of these 28 nests, a daughter was observed to forage in each of 15 (54%) of them (Table 4). Foraging daughters were subsequently observed to forage on 2 out of every 3 days in 2012, with the majority of foraging flights (61%) being nectar collection trips (Table 3). In 2013, 15 mother-removed nests were created for summer phase observations, and a foraging daughter was observed in 10 (~66%) of them (Table 4; Fig. 5b). Foraging daughters in 2013 foraged during approximetly 60% of days observed, and most of their foraging flights (90%) were pollen trips (Table 3). Overall, foraging daughters were equally as active in 2013 compared to 2012 (Chi-square: $x^2 = 1.42$, df = 1, $p=0.1$; Table 3)

Daily foraging patterns of foraging daughters

In 2012, foraging daughters (mother-removed nests) averaged 1.9 ±0.6 flights per day, which was not significantly different from the summer mother (mother present nests) flight number average of 1.8 ±0.4 flights per day (Mann-Whitney u: $z= -0.02$, $p=0.98$; Fig. 20). The same pattern was found in 2013; foraging daughters averaged 3.8 ±0.9 flights per day, whereas summer mothers averaged a similar 4.8 ±1.3 flights per day

(Mann-Whitney U: z= -1.35, p=0.09; Fig. 20). The overall number of flights per day increased from 2012 to 2013 (Kruskal-Wallis: H= 27.41, df = 3, p < 0.0001; Fig. 20); however, this increase was similar between mothers and foraging daughters.

The average duration of foraging flights was compared between mothers and foraging daughters in both 2012 and 2013. In 2012, foraging daughters were found to average 30.8 ±8.1 minutes per flight, and summer mothers had a similar average of 32.1 ±10.4 minutes per flight (Mann-Whitney U: z= 0.26, p=0.8; Fig. 21). Again, the same pattern was seen in 2013, with summer mothers and foraging daughters averaging 22.9 ±3.1 minutes per trip and 23.1 ±4.5 minutes per trip, respectively (Mann-Whitney U: z= 0.18, p=0.9; Fig. 21). Average flight duration was lower overall in 2013 (Kruskal-Wallis: H= 9.7, df = 3, p = 0.02; Fig. 21); however, both mothers and foraging daughters showed the same decrease.

Lastly, average handling time durations were compared between summer mothers and foraging daughters in both 2012 and 2013. In 2012, foraging daughters averaged 26.8 ±20.8 minutes per handling event, and mothers were found to average a similar 37.5 ±22.3 minutes per event (Mann-Whitney U: z= 1.56, p=0.11; Fig. 22). In 2013, average handling time durations were also similar between mothers and foraging daughters, with 16.7 ±8.0 minute per event and 19.5 ±9.1 minutes per event, respectively (Mann-Whitney U: z= -0.18, p=0.85; Fig. 22). Average handling time was found to decrease from 2012 to 2013 (Kruskal-Wallis: H= 10.12, df = 3, p = 0.01; Fig. 22).

In general, foraging daughters completed relatively few, but quite long, foraging trips with comparatively long handling times in between. Furthermore, foraging activity rates between summer mothers and foraging daughters was not significantly different.

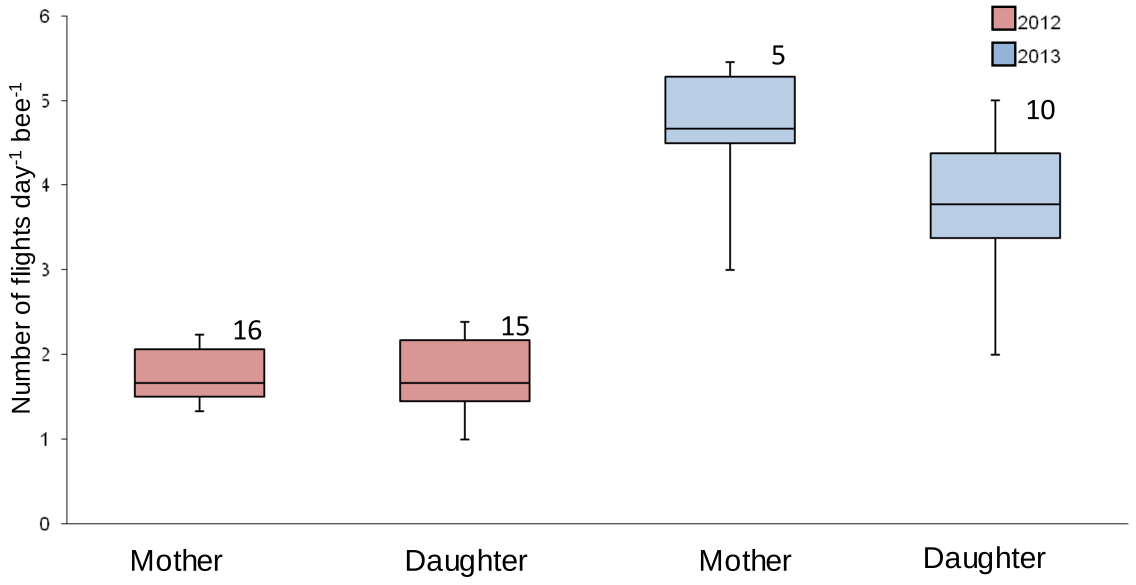


Figure 20: Similarity in foraging rates between mothers (in mother-present nests) and orphaned daughters (in mother-removed nests) during the summer phase in 2012 and 2013. Boxes represent the mean, 1st and 3rd quartile, and maximum and minimum values of the average foraging rate distribution. Numbers above boxes represent nest sample sizes. Foraging rates between mothers and daughters are not significant within years (Mann-Whitney U: 2012: z= -0.02, p= 1.0; 2013: z= -1.27, p=0.2). The overall number of flights per day were found to increase from 2012 to 2013 (Kruskal-Wallis: H= 27.41, df = 3, p < 0.0001).

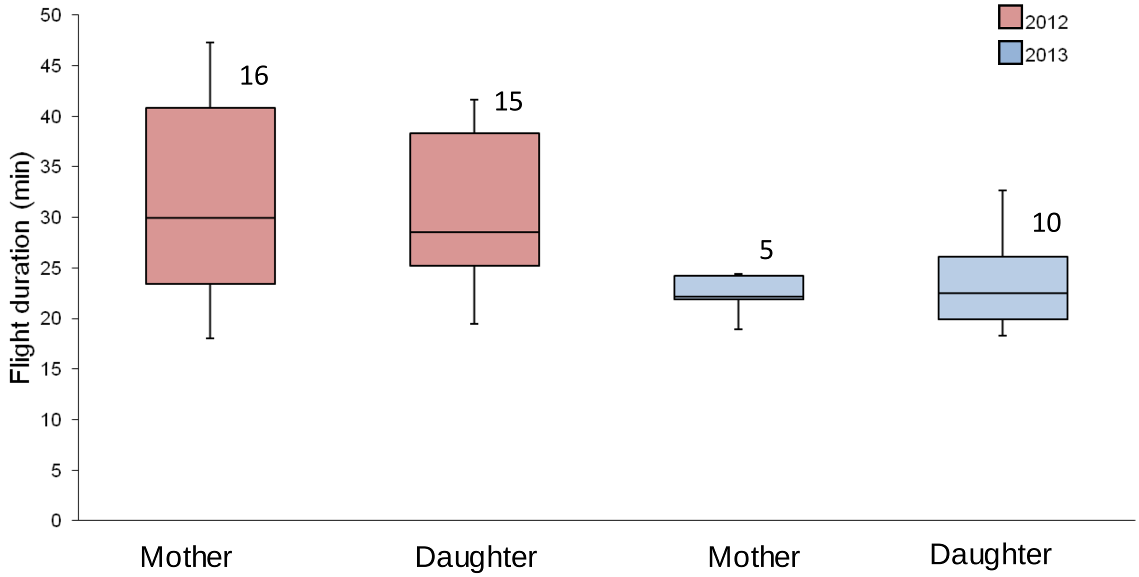


Figure 21: Similarity in average flight duration between mothers (in mother-present nests) and orphaned daughters (in mother-removed nests) during the summer phase in 2012 and 2013. Boxes represent the mean, 1st and 3rd quartile, and maximum and minimum values of the average foraging duration distribution. Numbers above boxes represent nest sample sizes. Average foraging duration between mothers and daughters are not significant within years (Mann-Whitney U: 2012: z= 0.26, p= 0.8; 2013: z= 0.18, p=0.8). Overall flight duration decreased from 2012 to 2013 (Kruskal-Wallis: H= 9.7, df = 3, p = 0.02)

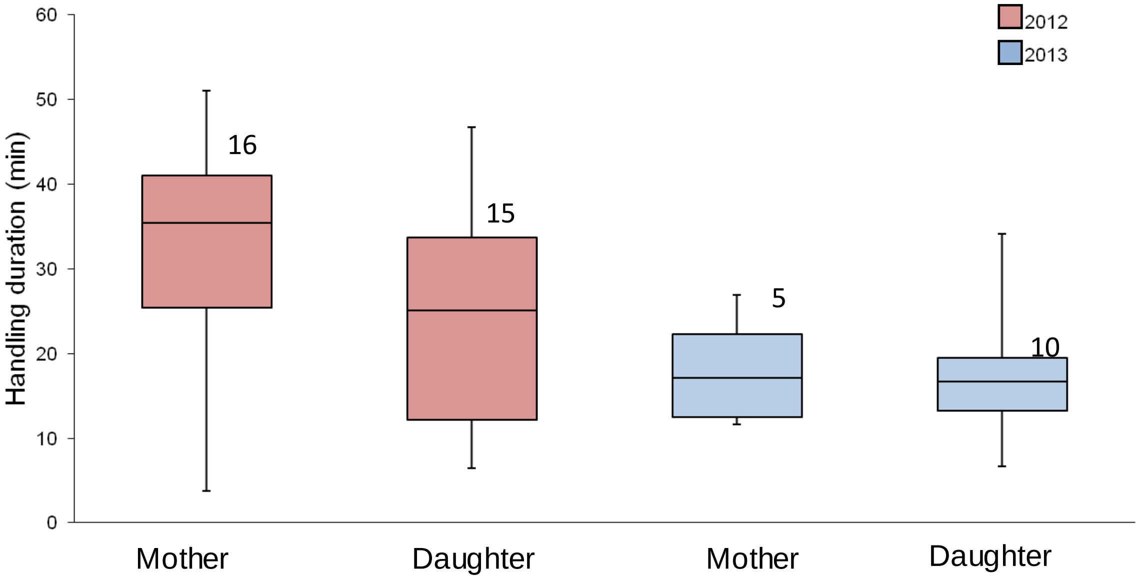


Figure 22: Similarity in average handling time between mothers (in mother-present nests) and orphaned daughters (in mother-removed nests) during the summer phase in 2012 and 2013. Boxes represent the mean, 1st and 3rd quartile, and maximum and minimum values of the average handling duration distribution. Numbers above boxes represent nest sample sizes. Average handling duration between mothers and daughters are significantly similar within years (Mann-Whitney U: 2012: z= -2.87, p= <0.05; 2013: z= -0.04, p>0.05. Average handling time was found to be slightly decrease from 2012 to 2013 (Kruskal-Wallis: H= 10.12, df = 3, p = 0.01)

Brood feeding by the forager

Feeding of *C. calcarata* offspring was not observed directly due to the behaviour happening inside the nest. Instead, feeding was inferred by dissecting offspring and noting the presence of pollen in the offspring's digestive system and fat-body accumulation in the abdomen at the end of summer. No nests that lacked a foraging individual contained adult bees (dead or alive) when opened. These nests were found to be either unfinished or to contain parasitized larvae and pupae. Thus, the reported dissections necessarily came from successful nests only.

In 2012, 22 of 23 (96%) brood in the mother-present (control) group had pollen in their crops, and all of them showed stored fat-bodies (Table 5). Similarly, all mothers dissected from the same group showed pollen in their crops and fat-body storage (Table 5). In the 2013 mother-present group, pollen was found in 20 of 24 (84%) dissected offspring, and all of them had fat-bodies (Table 5). Similarly, pollen was found in 6 of 8 (75%) dissected mothers from this group as well as fat-bodies in all of them (Table 5).

In 2012, 20 of 26 (77%) offspring that were dissected in the mother-removed group (treatment) had pollen in their crops, and all of them showed stored fat-bodies (Table 5). Similarly, 6 of 8 (75%) foraging daughters dissected from the mother-removed group showed pollen in theirs crops and fat-body storage was found in all of them (Table 5). In the 2013 mother removed group, pollen was found in 50 of 58 (86%) dissected offspring, and all of them had fat-bodies (Table 5). Similarly, pollen was found in 6 of 8 (75%) dissected foraging daughters from this group as well as fat-bodies in all of them (Table 5). Ultimately, even though only one individual per nest was ever observed to forage, all offspring were fed. Furthermore, brood feeding rates were similar between control and treatment groups in both years (Chi-square: 2012 Phi = -0.27, df=2, p = 0.1; 2013 Phi = 0.04, df = 2, p = 0.99; Table 5).

Table 5: Presence of crop pollen and fat-bodies in summer phase nest occupants across 2012 and 2013. All bees, nest forager and brood included, collected at the end of the summer phase showed the presence of fat-bodies, with 75%-100% of those individuals found with crop pollen. Control refers to mother-present nests and Treatment refers to mother-removed nests. Brood feeding rates were not significantly different between control and treatment groups (Chi-square: 2012 Phi = -0.27, df=2, p = 0.1; 2013 Phi = 0.04, df = 2, p = 0.99)

		Control			Treatment		
Type of bee	Year	Total individuals	Individuals with pollen in crop	Individuals with fat-bodies	Total individuals	Individuals with pollen in crop	Individuals with fat-bodies
Foragers	2012	9	9 (100%)	9 (100%)	8	6 (75%)	8 (100%)
	2013	8	6 (75%)	8 (100%)	8	6 (75%)	8 (100%)
Brood	2012	23	22 (96%)	23 (100%)	26	20 (77%)	26 (100%)
	2013	24	20 (83%)	24 (100%)	58	50 (86%)	58 (100%)

Necessity of summer phase brood provisioning

In order to assess the importance of brood provisioning prior to overwintering, the overwintering survival of a group of food deprived *C. calcarata* was compared against an un-manipulated control group. At the beginning of August 2012, 20 un-manipulated nests (control) and 25 nests which were capped with mesh to deprive them of food (treatment) were placed outside at the study site (see methods) and were left until early spring. A total of the 76 individuals from un-manipulated nests were collected at the beginning of spring, of which 53 offspring and 9 mothers survived (Table 6; Fig. 23). Dissection of all 76 individuals revealed fat-bodies in all 62 surviving bees as well as the 4 dead offspring and 2 of the dead mothers. In the food-deprived group, a total of 103 individuals were collected (25 mothers and 78 offspring), but none survived and no fat-bodies were found in any individual upon dissection (Table 6; Fig. 23).

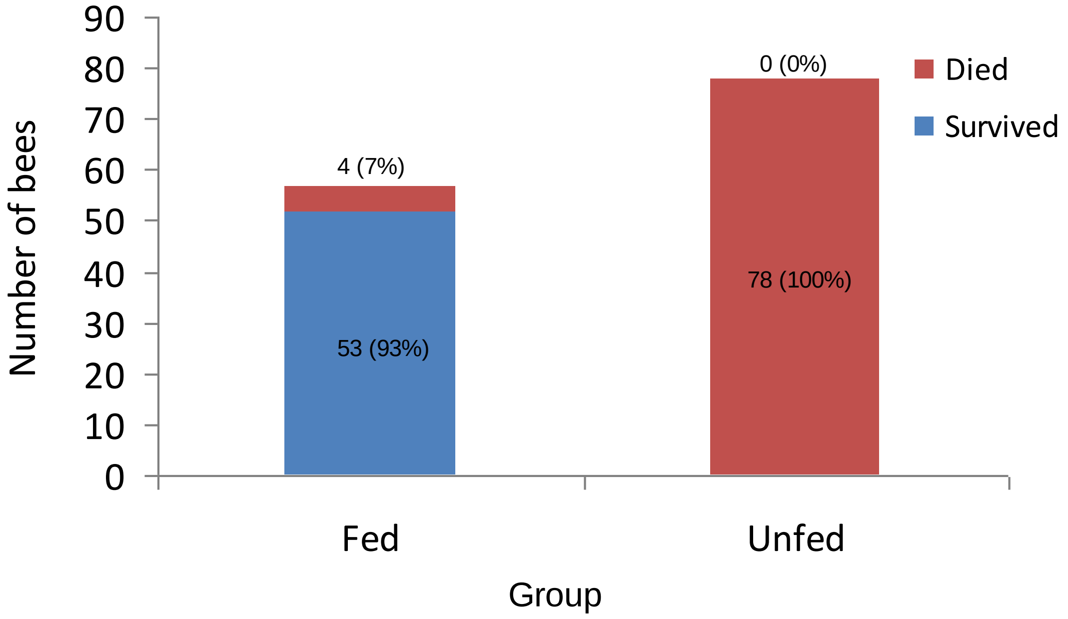


Figure 23: Overwintering survival of fed (n=57) and unfed (n=78) *Ceratina calcarata* brood. Each stacked bar represent the number of individuals surviving or not surviving the winter. Unfed treatment represent the block of nests prevented from foraging prior to overwintering. Only offspring numbers are included in graph, brood mother survival has been omitted since age may confound the results. The Fed bar represents the un-manipulated control. (Fisher's exact probability test: Phi = -0.93, $p < 0.001$).

Table 6: Survival rates and fat-body presence following winter diapause in *Ceratina calcarata*. Only bees which were fed prior to overwintering diapause show fat-body accumulation and were able to survive until spring. The forager in each nest was identified by wing wear (see Methods). Significantly more individuals survived in the fed treatment.

	Fed (n = 20 nests)		Food deprived (n = 25 nests)	
	Forager	Brood	Forager	Brood
Number of nests with live occupants	15 (75%)		0 (0%)	
Number of nests with all dead occupants	5 (25%)		25 (83%)	
Number of individuals	19	57	25	78
Individuals surviving	9 (47%)	53 (93%)	0 (0%)	0 (0%)
Individuals with fat-bodies	11 (58%)	47 (82%)	0 (0%)	0 (0%)

Discussion

Section 1: Baseline reproductive behaviour in Ceratina calcarata females

The first objective of this thesis was to characterize foraging effort behaviourally across a full reproductive season in *C. calcarata*. Quantifying the foraging effort would provide a description of parental care behaviours and, thus, act as a comparison when characterizing alloparental care strategies. Recent work looking at the phenology of Niagara region *Ceratina* described three peak activity periods during the reproductive season (Rehan and Richards, 2010). The first two were identified as emergence of overwintering individuals and ovipositional food provisioning behaviour, but the third peak was uncharacterized. Based on the previous findings I hypothesized that the third peak of activity, observed during late summer, was a second round of brood provisioning. Furthermore, I hypothesized that this second bout of provisioning is required for overwintering survival.

Seasonality in foraging activity

Foraging *C. calcarata* mothers showed two distinct peaks of foraging activity during their reproductive phase. The first peak was originally characterized by Rehan and Richards (2010), and was determined to be brood provisioning behaviour. The second peak of foraging activity occurring after offspring emerged as adults in late summer, was determined in the present study to be a second bout of brood provisioning. *Ceratina calcarata* mothers were found to provision food for adult brood in the summer phase. Not only does this result support my original hypothesis, but it is interesting because *C. calcarata* offspring become fully independent once they emerge and some actually disperse from the natal nest (Rehan and Richards, 2010). However, many offspring remain within the natal nest until the following spring, and the mother feeds them in late summer. Such behaviour constitutes extended parental care, given that the offspring are independent by this time. Extended parental care is relatively rare, especially in solitary bees. Generally, parental care behaviour ends once the offspring are nutritionally independent, and are able to fend for themselves (Choe and Crespi, 1997).

Contrary to my original hypothesis, foraging behaviour by *C. calcarata* mothers was found to be very different in spring and summer (Fig. 16-18). Spring foraging consisted of a high number of short duration flights with correspondingly short handling times. Conversely, summer foraging consisted of a reduced number, but longer flights, with longer handling durations in between. However, the number of days individual bees were active out of the season was found to be comparable between spring mothers and summer mothers in both 2012 and 2013 (Table 2). Seasonal differences can be attributed to the dominant parental care behaviours being employed by the mother and to environmental variation between the spring and summer foraging phases.

Parental care and foraging activity

The difference in foraging activity from spring to summer can be explained partly by the parental care behaviours being employed. Spring phase foraging is dominated by brood cell construction and mass provisioning behaviours. That is, a *C. calcarata* female during the spring phase is foraging for pollen and nectar in order to create the provisions for each brood cell. Concurrently, during foraging the female is also ovipositing and constructing cell partitions. This contrasts with summer phase foraging activity, which consists solely of progressive provisioning of adult brood.

Mass provisioning during the spring phase means that foraging is dependent on the number of eggs being laid. Even though a mother has the ability to control birth weight by manipulating the size of the pollen provisions (Richards and Rehan, 2010), there is still a minimum amount of pollen that must be provided. This means that for every egg there is a compulsory minimum investment, which is independent of the survival of the egg. Thus, the minimum amount of maternal investment is dependent upon the quantity of eggs, and independent of how many actually survive. Conversely, the summer phase foraging peak is focused on providing for adult brood that have survived. What this suggests is that not only will summer provisioning rates be reduced simply as a function of offspring mortality, but we may also assume that since summer phase foraging is for adult offspring provisioning, the mother will only forage when the offspring need feeding. This agrees with findings in other bees where the parent will only forage when the brood signals her to do so (Blom, and Velthuis, 1988; Sommeijer et. al., 1982). Ultimately, foraging effort is reduced during the summer phase because mothers are progressively provisioning for surviving adult offspring as opposed to mass provisioning for a full clutch of eggs.

Resource abundance and foraging activity

Aside from the differences in activity patterns between the spring and summer foraging phases of the *C. calcarata* reproductive cycle, environmental variation appears to have a direct effect on activity. During the spring foraging phase, the weather is generally wetter and cooler than during the following summer foraging phase (Environment Canada, 2014). Such a marked difference must affect resource availability, and consequently, foraging activity rates (Aldridge et al., 2011). We can expect floral resources during spring to be high, which leads to a high number of short foraging trips, as more resources can be gathered in a shorter amount of time. Conversely, in the summer we can expect fewer floral resources than in spring, due to a rise in temperature (Aldridge et al., 2011). The heat and dryness of summer acts to lower floral resources, and increase the time a flower needs to refill its complement of pollen and nectar. This results in floral resources being limited, and leads to bees needing to forage longer, and thus fewer trips can be accomplished per day. Thus, over the same amount of foraging time during a day**,** a bee will collect fewer resources.

Seasonal effects on *C. calcarata* mother foraging rates are further evidenced by the changes in activity found between 2012 and 2013 (Fig. 16-18; Fig. 20-22). The temperature and precipitation recorded from 2012 indicate that it was a drought year (Environment Canada, 2012). As such, we can expect seasonal differences in activity patterns to be greater than in a year with relatively normal rainfall and temperatures, such as 2013. Mothers were found to make fewer foraging trips in both activity phases in 2012 compared to 2013, indicating limited floral resources. Furthermore, foraging for nectar in 2012 was much lower than in 2013, indicating that nectar was limited (Table 2 & 3). Since nectar production is highly dependent upon moisture availability to flowers (Zimmerman, 1998), drought conditions must lead to a decrease in nectar collection.

Taken together, the above information suggests that yearly and seasonal variability in floral resource likely contributed greatly to variation in foraging rates of *C. calcarata* mothers. Furthermore, foraging activity was further modified between seasonal phase due to the change in brood provisioning behaviour (mass provisioning vs. progressive provisioning). Cool and wet conditions in spring leads to many foraging trips of short duration, given the abundance of resources**,** and mass provisioning dictates a minimum investment per egg laid. Dry and hot conditions in summer limit resources and result in foraging trips taking longer to accomplish, leading to fewer trips per unit time. Progressive provisioning of adult brood means that foraging rates are dictated by both the number of offspring that survive to adulthood**,** and individual offspring resource needs. Hence, the reduction in foraging rates between spring and summer foraging phases in both 2012 and 2013 makes sense within the context of seasonal resource availability and the changing resource needs of the offspring.

Foraging rates in *Ceratina calcarata* are highly variable both by individual and by season. Seasonal and yearly variation in foraging activity is ultimately a function of resource availability, in that resource availability drives reproductive decisions made by *C. calcarata* mothers. Also, extended brood care is prevalent in *C. calcarata*. Independent, adult brood are progressively provisioned by the nest mother, which suggests that not only is such provisioning necessary for survival, but also that such foraging behaviour by the brood must be costly. In the next section I will explore why extended brood care is necessary and also what follows when the parental care system breaks down as a result of orphaning.

Necessity for summer phase brood provisioning

The second objective of this thesis was to show that Ceratina calcarata offspring require a second bout of provisioning to survive overwintering. The observation that mothers forage for the brood once it has emerged suggests that newly emerged offspring require a store of energy in order to survive the winter. Summer phase foraging was hypothesized to be necessary for offspring to store enough energy to sustain the metabolic costs of overwintering diapause. Food deprivation of a group of C. calcarata nests prior to overwintering behaviour resulted in 100% mortality of the nest occupants (Fig. 23). Hence, summer phase foraging is a necessary behaviour.

Generally, in insects that must survive a prolonged period of inclement weather, such as a winter season, a resource reserve must be accumulated before any diapause or quiescent behaviour is initiated. This reserve is essential not only for repair functions relating to damage from the cold, but also to power post-overwintering behaviours like dispersal (Hahn and Denlinger, 2007). The negative effect of low winter temperatures on diapausing bees has only been studied in a few bee species, but the majority of studies agree that diapause during low temperature is associated with weight loss (Hahn and Denlinger, 2007, and references therein). This result agrees with the findings of Rehan and Richards' (2010) that overwintered populations of C. calcarata are on average lighter than pre-overwintered populations. Furthermore, many temperate bee species which must endure a low temperature season show a feeding period prior to overwintering diapause (Michener, 1974). It is therefore safe to conclude that summer food provisioning is necessary for overwintering survival.

Section 2: Ceratina calcarata's response to brood orphaning

The purpose of the second section of this thesis was to investigate how *C. calcarata* offspring would respond to orphaning. Brood mothers were removed prior to summer phase foraging behaviour, and any subsequent activity by the offspring was recorded. Substitution of the removed mother by a dwarf female was predicted. Summer phase foraging observations of orphaned nests showed that not only will a single female take over brood care behaviour in the event of nest orphaning, but also that this female is predominantly the smallest female in the nest (Fig. 19). The significance of such alloparental care behaviour is discussed below.

Foraging rates in mothers vs. alloparents

Across 2012 and 2013 a total of 43 orphaned nests produced a brood, with each being accompanied by a foraging daughter (Table 4). The foraging rates of these alloparents were quantified and compared against foraging rates in summer mothers in order to determine if the parental care behaviour was comparable. Alloparents were found to forage at the same average rates as mothers from non-orphaned nests (Fig. 20-22). This suggests that newly emerged daughters are able to provide the necessary amount of brood care in the event of orphaning, and thus represent a stable, adaptive response to maternal mortality. Furthermore, this also indicates that not only is alloparental care an adaptive behaviour in the event of nest orphaning, but that it represents a great enough benefit to brood to prevent dispersal following nest orphaning.

The existence of alloparental care in *C. calcarata* represents a puzzle. Such behaviour is not displayed until the brood begin to emerge as adults and this means that brood are being cared for that are able to feed themselves. Generally, in species that show alloparental care**,** the alloparent is much older than her siblings. In *Exoneurella eremophia*, females lay eggs constantly throughout the summer and end up with adult brood alongside larvae and pupae (Hogendoorn et. al., 2001). Even though the alloparent and her siblings are roughly of the same generation, only immature siblings are cared for. Similarly in the allodapine bee, *Exoneura bicolor*, alloparents take care of immature siblings; however, the siblings are produced as a second clutch later in the summer (Bull and Schwarz, 1997). The point is that *C. calcarata* alloparents care for independent adult brood at the same rate as a mother would. This suggests that newly emerged adult brood feeding themselves somehow represents a cost.

Benefits of extended parental care

If orphaned *C. calcarata* are able to forage for themselves, why is it adaptive to forego feeding themselves and rely on a parent during the summer foraging phase? It seems much simpler for the offspring to feed themselves than to rely on the development of a complex behaviour such as alloparental care. In order to answer this question, we must look at the inherent costs and benefits associated with summer phase foraging.

The main costs inherent to brood provisioning are predation and loss of maternal quality (decreasing maternal condition). Foraging is physically demanding for bees, in that the longer an individual flies, the more accumulated damage will be suffered by the wings (Higginson and Barnard, 2004; Higginson and Gilber, 2004). Such damage has the effect of reducing flight efficiency, and thus, foraging efficiency. Also, foraging increases predation risk as the forager must traverse the environment for food. As such, mortality is necessarily higher in foraging individuals than those that stay in the safety of the nest.

Predation and flight damage during foraging could be very costly for a newly emerged adult *C. calcarata.* Females that are orphaned and forage for themselves likely incur increased total flight times compared to females which were fed by a mother or sister. Hence, these females will already experience a higher mortality rate, as well as accumulated flight damage, before they begin their breeding season.

Costs such as accumulated damage and increased mortality represent an adaptive pressure favoring extended parental care. A mother in the summer phase is nearing the end of her life. Thus, there is no reproductive cost for her to risk further foraging behaviour; there is only a benefit for her offspring. However, in the event that the mother is lost, what drives the expression of parental care behaviour in an alloparent which will incur such reproductive risks?

Ultimate mechanisms driving alloparental care behaviour

Queller (1989) asserted that worker behaviour (i.e. alloparental care) can be an advantage when offspring require extended care and when adult mortality is high. In *C. calcarata*, extended brood care is needed because the mortality of mothers during summer ranges from 36% to 50% (Table 4). Thus, worker behaviour may represent an adaptive benefit in this species. The most interesting question is what underlying mechanisms drive alloparental care behaviour in *C. calcarata* daughters? The most obvious answer is haplodiploid sex determination, as this mechanism is well established in hymenopteran literature (for a review see Heimpel and de Boer, 2008). Haplodiploid sex determination in *C. calcarata* means that caring for a sibling is equivalent to raising one's own offspring, due to the relatedness between siblings (Hamilton, 1964). In the event of brood orphaning, alloparental behaviour can thus be beneficial because the alloparent (a female sibling) receives indirect fitness benefits from caring for her siblings. This mechanism, however, does not explain why *C. calcarata* alloparents tend to be dwarf daughters; if it was sufficient to produce altruistic behaviour, then there would be no need for the production of a specialized alloparent. Thus, I would argue that other mechanisms are helping drive the expression of alloparental behaviour.

Nest mate manipulation

One mechanism that can drive the development of alloparental care is nest mate manipulation (Bull and Schwarz, 1997). Dominance interactions, such as manipulation by the brood mother, have been suggested as a mechanism to drive altruistic behaviour. That is, nest mate coercion may be an avenue for the expression of worker-like behaviour. In this study *C. calcarata* females were removed before the emergence of offspring. This timing prevented interaction between the mother and her brood, thus preventing direct coercive behaviour. Since alloparental care behaviour was observed only in orphaned nests, we can conclude that direct coercion is not necessary. However, this does not preclude pre-emergence manipulation. Two methods of coercion that do not depend on the interaction between the adult offspring and the parent are protogyny and the production of subfertile females. Taken together, these two methods of coercion can drive worker behaviour by reducing the costs of altruism to the altruist.

Insurance of protogyny

Bull and Schwarz (2001) defined protogyny as the production of a female-biased early brood to serve as workers in the event of orphaning**,** and asserted that in any species in which alloparental care is present, protogyny should also be found. Protogyny is the result of what Bull and Schwarz (2001) call the ‘insurance model’ of sex allocation. The model states that a reproductive mother that experiences high adult mortality and produces offspring with a prolonged period of dependence will ensure the survival of her brood by producing the alloparent sex first. Thus, such a species will show a time-dependent female sex bias. As stated above, Richards and Rehan (2010) were the first to show a female bias for early brood in *C. calcarata* (the first two offspring produced) and furthermore, found that after the first two offspring**,** the rest of the brood was unbiased. As Bull and Schwarz (2001) suggested, protogyny has been seen in a number of solitary species which show alloparental behaviour (Aenmey, et al., 2006; Tierney, et al., 2002).

Dwarf daughter production

In both 2012 and 2013, a proportion of orphaned nests were observed with alloparents that were the smallest female in the nest (Fig. 19). The production of the small daughter could represent a bet-hedging strategy that minimizes the cost of alloparental care. If we assume that foraging is inherently damaging, and thus acts to lower reproductive output, then a small daughter represents less of a reproductive cost than a large female. That is to say, the cost to future reproductive success due to accumulated damage from foraging activity is much greater for a large daughter than a small daughter (Rehan and Richards, 2010). Furthermore, large females preferentially produce large daughters, whereas small females produce more male offspring, and since females represent a greater reproductive investment than males, the loss of a large female offspring represents a greater cost to fecundity than the loss of a small female to a brooding mother.

Rehan and Richards (2010) suggested that only large robust mothers were able to produce a small daughter. The authors argue that this is interesting, but also counterintuitive since an offspring produced specifically to be a worker would benefit a small mother much more than a large one, given that small mothers have lower reproductive potential. I would argue that the solution is simply that large mothers have enough of a reproductive advantage (due to their size) to be able to afford to produce a subfertile daughter, whereas a small mother cannot afford the possible reproductive waste. Furthermore, a large mother may have a higher rate of motility due to wearing herself out attempting to produce a high quality brood. This, thus, increases the utility of producing a dwarf daughter. Simply put, a large mother has the resources to bet-hedge against her own mortality, whereas a small mother cannot take the chance.

The Head Start and Assured Fitness Returns models

Two more related mechanisms which can drive the expression of alloparental care are the Reproductive Head Start' and 'Assured Fitness Returns' models. These models differ from nest mate manipulation in that they describe how alloparental care can benefit the alloparent instead of how the mother can force worker behaviour. Queller's (1989) 'Reproductive Head Start' model describes how a worker caste can be selected for in a solitary species. This model states that alloparents can gain fitness by helping to raise siblings that have already been invested in by the genetic parent, whereas a future mother must wait until her reproductive period to gain any fitness. This means that the alloparent is able to increase its indirect reproductive output by inheriting partially developed brood. Queller (1989) argues that the alloparents gain reproductive output equal to the average gain from raising their own offspring, but at half the reproductive investment. Furthermore, whereas pre-reproductive mortality results in zero fitness for a non-alloparent, a successful alloparent has positive fitness irrespective of future mortality.

Gadagkar (1990) argued that Queller (1989) overestimated the benefit of the head-start model, but ultimately agreed that such a model does produce a fitness advantage comparable to haplodiploidy, but without the relatedness requirements. Gadagkar (1990) asserted that the alloparent does not gain the same fitness benefits as the genetic parent for raising its siblings, but the alloparent does receive an ‘assured fitness return’ for its work. This ‘assured fitness returns’ model relies on the salvaging of energy that would have been lost due to orphaning, instead of giving full credit of rearing to the alloparent. In the context of orphaned *C. calcarata*, the alloparent daughter receives fitness gains months before she will be ready to reproduce. Thus, increased mortality and foraging damage will be less costly to her life-time reproductive fitness if she helps to raise siblings.

Normal alloparent vs. subfertile alloparent

I have shown that in the event of nest orphaning, a female *C. calcarata* offspring takes over brood care behaviour. Furthermore, I have shown that in a proportion of nests, a dwarf daughter carries out this role; however, normal sized daughters are also able to fulfill brood care needs. It is thus puzzling why both alloparental types are conserved in the population.

In the event of nest orphaning the potential alloparent can either forage for herself, and let her siblings do the same, or she can feed her siblings. Either choice will result in incurring the costs of foraging; however, alloparental care also includes indirect fitness benefits. Queller (1994) suggested that a normal sized daughter may become an alloparent because she will receive a guaranteed fitness advantage irrespective of the probability of adult mortality, whereas her sisters have zero fitness if they die anytime before nest creation. As discussed, there is a certain probability of mortality associated with both overwintering and post-overwintering flight activity. However, if the female is an alloparent, she will already have generated a contribution to the next generation by the time she begins overwintering behaviour; thus, future mortality will have less of an impact on total reproductive output (Queller 1994).

One final issue is why some *C. calcarata* mothers produce dwarf daughters at all if alloparental care is found in normal sized daughters. Queller (1994) noted that there is still the problem of how it can be adaptive for an individual (worker caste/alloparent) to give more to the genetic parent than to herself. One possible explanation is subfertility; if the helper is physiologically unable or unlikely to become reproductive (West-Eberhard, 1978), then altruistic behaviour is favored because the opportunities for direct fitness gains are limited (Bull and Schwarz, 1997). Furthermore, given the fact that foraging is inherently damaging, producing a normal sized daughter alloparent may represent a waste of reproductive energy. Thus, the dwarf daughter not only has more incentive to care for siblings, but she is also less of a reproductive cost to the mother if she does not survive to reproduce herself.

General conclusions

The major objective of this thesis was to argue that *C. calcarata* are facultatively social, and indeed the results support this. Even though *C. calcarata* exhibits all the characteristics of subsociality, given the right context, eusocial characteristics can be expressed. In order to be considered primitively eusocial, a species needs to have overlapping generations, cooperative brood care and reproductive division of labor (Andersson, 1984; Wilson, 1971; Crespi and Yanega, 1995). Previous work on *C. calcarata* phenology has shown that mothers live long enough to interact with their offspring (Rehan and Richards, 2010), and my work adds the finding that this species is also able to produce a worker class. Specifically, the production of a dwarf female represents a primitive version of a worker class. Furthermore, the dwarf female, given her reduced size, is at a reproductive disadvantage, and is thus manipulated by the brood mother to be a nest helper. This indicates simple reproductive division of labor. Lastly, in the event of nest orphaning the dwarf daughter usually takes over brood care. Thus, given the above findings, we must conclude that *C. calcarata* is not only social polymorphic, but facultatively, primitively eusocial.

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