Nesting aggregation as a determinant of brood parasitism in mason bees (*Osmia* spp.)

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ABSTRACT

Identifying forces that affect population dynamics can allow us to better understand the distribution and abundance of animals. Both top-down and bottom-up factors can significantly influence animal populations. Mason bees (members of the genus *Osmia*; Hymenoptera: Megachilidae) are important pollinators for agricultural systems and are vulnerable to exploitation by brood parasites, such as kleptoparasitic wasps. High levels of nesting density have the potential to increase rates of brood parasitism by attracting larger numbers of parasites to areas with aggregations of nests. I conducted a field study in subalpine meadows at the Rocky Mountain Biological Laboratory in Colorado, USA, to assess whether mason bees suffer increased brood parasitism as the size of nesting aggregations increases. Mason bees were allowed to nest in artificial nest boxes and establish natural variations in numbers of nesting individuals within nest boxes. Nest cells constructed by bees were then checked for the presence of kleptoparasite larvae shortly after they were completed. Overall, nest cells constructed in blocks containing multiple active bees were significantly more likely to be oviposited in by brood parasites compared to cells constructed in blocks with fewer active nesting bees. This suggests that gathering in large aggregations for nesting can negatively affect populations of mason bees, given the high levels of brood parasitism observed in areas of high nesting density. In addition, the last nest cell in mason bee nests was significantly more likely to be parasitized than inner cells, suggesting bees may be abandoning nests that are parasitized, representing a potential defensive response of bees to brood parasitism. These results have implications for the management of mason bees as agricultural pollinators, as cultivating them in large groups could reduce their survival.

RÉSUMÉ

L’identification des forces qui influencent la dynamique des populations peut nous permettre de mieux comprendre la distribution et l’abondance des animaux. Les facteurs de types ascendants (disponibilité des ressources) et descendants (ennemis naturels) peuvent tous deux grandement influencer les populations d’animaux. Les abeilles maçonnes (des membres du genre d’insectes *Osmia*; Hymenoptera : Megachilidae) sont des pollinisateurs importants pour les systèmes agricoles et sont vulnérables au parasitisme de couvée, tel que par les guêpes cleptoparasites. Des densités de nids élevées risquent d’entraîner une augmentation de parasitisme de couvée en attirant un plus grand nombre de parasites vers ces zones. J’ai réalisé une étude de terrain dans les prés subalpins au Colorado (USA) pour déterminer si les abeilles maçonnes subissent une augmentation de parasitisme de couvée quand l’ampleur des agrégations de nids augmente. Les abeilles maçonnes pouvaient occuper des nichoirs artificiels et établir une variation naturelle du nombre d’individus reproducteurs dans les nichoirs. Les alvéoles de nid construites par les abeilles étaient examinées pour la présence de larves cleptoparasites peu après leur achèvement. En général, les alvéoles de nid construites dans les blocs contenant plusieurs abeilles étaient considérablement plus susceptibles à l’oviposition par les parasites de nid en comparaison aux alvéoles construites dans des blocs contenant moins d’abeilles en cours de reproduction. Cela suggère que le regroupement en grands agrégats pour nidifier peut avoir des effets négatifs sur les populations d’abeilles maçonnes, compte tenu du niveau élevé de parasitisme de couvée observé dans les zones avec une haute densité de nids. De plus, la probabilité que les dernières alvéoles dans les nids d’abeilles maçonnes soient abandonnées était considérablement plus élevée que pour celles construites à l’intérieur. Ceci suggère que les abeilles peuvent abandonner les nids qui sont touchés par les parasites, ce qui représente une méthode de défense potentielle de la part des abeilles. Ces résultats ont des conséquences pour la gestion des abeilles maçonnes comme pollinisateurs agricoles, étant donné que leur élevage en grande concentration pourrait réduire leur survie.

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CHAPTER 1: INTRODUCTION

Parasitism

Identifying forces that affect population dynamics can allow us to better understand the distribution and abundance of animal species (Kareiva 1990). Both top-down factors (natural enemies) and bottom-up factors (availability of resources) can significantly affect animal populations (Berryman 2001, Eber 2004) and understanding the impact of these processes is an important topic of study (Hunter 2001, Freckleton *et al*. 2006, Moreau *et al.* 2006). This is especially true of managed organisms, such as pollinators used for agriculture, in which large populations are required and the conditions in which organisms are kept may differ significantly from natural conditions.

Parasitism can be a significant top-down factor in insect populations (Steffan-Dewenter and Schiele 2008, Klemola *et al.* 2014) and can be a considerable source of mortality (although parasitism by definition is not lethal, many organisms that are considered parasites, such as parasitoids and brood parasites, are lethal to their host) (Steffan-Dewenter and Schiele 2008, Klemola *et al.* 2014). Parasitism can act as a density-dependent regulating factor, increasing in intensity with increases in population and thereby decreasing population growth rate (Klemola *et al*. 2014).

Numerous factors affect rates of parasitism in insect populations (Rosenheim 1990, Antonini *et al.* 2003, Bischoff 2003, Goodell 2003, Klemola *et al.* 2014, Macivor and Salehi 2014). Increases in population density and size can increase the vulnerability of insects to parasitism or enable protection from these natural enemies (Rosenheim 1990, Antonini *et al.* 2003, Klemola *et al.* 2014). In addition, environmental factors such as resource availability (Goodell 2003) and nesting locations (Macivor and Salehi 2014) can also influence rates of parasitism. Finally, indirect factors can also influence parasitism rate. For example, the release of natural volatiles by plants in response to damage by herbivores can attract parasitoids, increasing the vulnerability of insect herbivores to attack (Charleston *et al.* 2006).

Native Bee Populations

Information on factors affecting population dynamics of native bee species is limited (Potts and Willmer 1997, Wuellner 1999, Steffan-Dewenter and Tscharntke 2000, Minckley *et al.* 2003, Potts *et al.* 2003). However, understanding these factors is crucial given the importance of native bees as pollinators. Availability of pollen resources is thought to be a significant limiting factor for bee populations (Steffan-Dewenter and Tscharntke 2000, Minckley *et al.* 2003, Potts *et al*. 2003, Grundel *et al*. 2010, Palladini and Maron 2014). Bee species depend on pollen as a primary resource; however, pollen can be limited in both temporal and spatial availability (Roulston and Cane 2000). Short flowering periods for plants can leave small windows for bees to collect pollen, and flowers may only be present in specific areas. In addition, availability of suitable nesting sites (Wuellner 1999, Potts *et al*. 2003) or of resources needed to construct nests (Grundel *et al*. 2010) may limit abundance of native bee populations. Frequency of disturbances to the environment (i.e. forest fires) can influence the distribution and abundance of native bee populations by modifying the structure of habitats (Grundel *et al*. 2010).

Despite the fact that natural enemies are thought to be regulators of bee populations (Wcislo and Cane 1996), factors affecting parasitism rates in bee species have only been studied sporadically (Antonini 2003, Goodell 2003, Rosenheim 1990, Rosenheim 1989). Rates of parasitism in bee species are highly variable among years and between regions (Petanidou *et al*. 1995, Wcislo and Cane 1996), but the reasons for this variation are not well understood.

Mason Bees and Kleptoparasitism

Mason bees (*Osmia* spp.; Hymenoptera: Megachilidae) are important pollinators in numerous systems, including agriculture (Ward *et al*. 2010, Schindler and Peters 2011). Mason bees nest in pre-existing burrows in which they deposit their eggs in individual cells along with a cache of pollen for each larva to feed on (Michener 2000). Mason bee females construct these nests as individuals. Nests of these bees are invaded by a variety of brood parasites, which can include parasitoids and kleptoparasites (Wcislo and Cane 1996).

Kleptoparasitic behaviour is defined by the stealing of resources another animal has already procured (Morand-Ferron 2007), most typically food items. This behaviour is seen in a multitude of animal systems, including kill stealing by scavengers (Hayward *et al*. 2006), the stealing of food from spider webs by ants (Agnarsson 2003, Leborgne *et al*. 2011), and even the raiding of wasp nests by avian scavengers (Benttinen and Preisser 2009). Kleptoparasites of bees, such as sapygid wasps (Hymenoptera: Sapygidae), will typically invade nests to lay eggs, whose larvae, when hatched, will kill the host egg and feed on the collected pollen. Parasitism rates can be high in mason bees, with up to 15-56% of brood cells in a population being parasitized by kleptoparasites and 100% fatality rates in parasitized cells (Steffan-Dewenter and Schiele 2008, J. Forrest unpublished data). Although the behaviour of these brood kleptoparasites is more akin to that of typical brood parasites such as cowbirds (Rothstein 1990, Clodfelter 1999), which typically oviposit within the nests of host species and kill the host offspring, they do still consume the pollen caches within mason bee nests, and are therefore considered kleptoparasites.

Economic Significance

The economic importance of mason bees has become more apparent in recent years. A large proportion of agricultural crops depend on animals to provide pollination (Klein *et al.* 2007, Aizen *et al.* 2009), and many agricultural systems depend on large numbers of managed pollinators such as European honey bees (*Apis mellifera*) to provide these services (Klein *et al*., 2007, James & Pitts-Singer 2008). Recent reductions in populations of such economically important pollinators have been a cause for concern (Klein *et al.* 2007, Winfree *et al*. 2009, Ward *et al.* 2010, vanEngelsdorp and Meixner 2010). Demand for honey bee colonies outstrips the growth of available colonies for agricultural pollination, resulting in increased costs for these services (vanEngelsdorp and Meixner 2011). Overall, crops benefit from having larger number of pollinators available to deliver sufficient pollen (Winfree *et al*., 2007, Burgett *et al*. 2010), making supplementary pollination by alternative pollinators an attractive prospect for agricultural systems.

The usage of mason bees as alternative pollinators for agricultural systems has been suggested as a possible supplement or replacement for traditionally used pollinators (Fliszkiewicz *et al.* 2011, Gruber *et al.* 2011, Jauker *et al.* 2012, Schindler and Peters 2011, Ward *et al*. 2010). Mason bees will readily nest in artificial housing (Gruber *et al.* 2011, Bosch and Kemp 2001), including cardboard tubes, reeds and paper straws, that enable them to be transported and cultivated in agricultural settings. (This behaviour also makes mason bees an excellent group in which to study brood parasitism, as their nests are accessible for observation.). In addition, Jauker (2012) determined that although mason bees do suffer significant reductions in brood production when the flowering period of an agricultural crop ends, early increases brood production from high pollen availability offset these reductions. Given the typical monocultures of contemporary agriculture, this is an important consideration for the maintenance of these pollinators. Mason bees are effective pollinators for multiple crop systems including almond, pome and stone fruit (Schindler and Peters 2011, Ward *et al*. 2010). The introduction of mason bees has also been found to reduce the economic costs of pollination by providing a supplement to honey bee pollination (Ward *et al.* 2010). Despite the growing agricultural importance of this pollinator group and the prevalence of brood parasitism in natural and managed populations of megachilid bees, parasitism rates and factors affecting parasitism have only been sporadically assessed (Torchio 1979, Munster-Swendsen and Calabuig 2000, Goodell 2003, Seidelmann 2006).

Nesting Aggregation

Nesting density is a potentially significant factor for rates of brood parasitism in mason bees. For the purposes of this study, "nesting density" is defined as the number of bees nesting in a single location simultaneously. While female mason bees forage and construct nests individually, they have been found to nest gregariously in some areas (Torchio 1984), each bee constructing nests individually in the same location. However, mason bees in agricultural settings are more likely to abscond from nests when forced to nest in larger aggregations (Artz *et al*. 2013, Bosch 1994) suggesting these sorts of aggregations may not always be preferred or beneficial to bees.

Nesting aggregations are thought to arise in Hymenoptera for a number of reasons outlined by Rosenheim (1990). Selection of appropriate nesting locations by individual bees could potentially lead to aggregations (Rosenheim 1990) if bees require specific conditions to construct their nests. Similar to this, spatial availability of floral resources could concentrate bee nests in single locations (Rosenheim 1990). Bees may also use the presence of conspecifics as an indicator of appropriate nest locations, as a form of social information, similar to that used by vertebrates (Fletcher 2007) as well as other invertebrates such as cockroaches for predator evasion (Coolen *et al.* 2005) and social bees for nest selection and pollen foraging (Chitkka and Leadbeater 2005). Finally, philopatry (organisms selecting nesting locations close to the place of their birth) could lead to aggregations of related individuals close to their maternal nests (Rosenheim 1990).

Previous studies have reported conflicting results regarding the effect of aggregation on parasitism rates. There are large numbers of studies observing positive density dependent parasitism, including parasitic flies victimizing moths (Umbanhowar *et al*. 2003), parasitoid wasp oviposition on scale insects (Matsumoto *et al*. 2004) and cowbird brood parasitism of songbirds (Woolfenden *et al.* 2004). However there are also studies suggesting inverse density dependent parasitism rates including parasitism of leafhoppers by dryinid wasps (Gonatopus sp.) (Wiesenborn 2001) and sugarcane borer wasps by parasitoid flies (Rossi and Fowler 2003). Instances of both positive and negative density-dependent parasitism have been reported in solitary nesting Hymenoptera (Wcislo 1984, Rosenheim 1989, Rosenheim 1990, Antonini *et al.* 2003), although the majority of studies suggest positive density dependence, in which bees nesting in larger aggregations suffer increases rates of parasitism (Rosenheim 1990). Similarly, species of solitary wasps that nest in larger aggregations have been found to suffer increased parasitoid oviposition and larval mortality, compared to species that did not form nesting aggregations as frequently (Freeman 1982).

There are proposed explanations for why positive or negative density dependence could occur. Larger nesting aggregations might deter parasites from attempting to attack simply due to a larger target presence in a single location (Wcislo 1984, Antonini *et al.* 2003) leading to predator confusion, in which the presence of large numbers of prey individuals overwhelm predators, making it difficult for them to locate a single target (Jeschke and Tollrian 2007).

Dilution of risk of victimization may result in many individuals being spared parasitism when large groups of animals gather together as there are a large number of targets for natural enemies to exploit (Hamilton 1971). This effect has been observed in a large number of animal systems, including fish (Frommen *et al*. 2009), seals (De Vos and O'Riain 2010) and birds (Beauchamp and Ruxton 2008) as well as in solitary nesting Hymenoptera including wasps as a defense against parasitoids (Coster *et al*. 2002). Finally, larger aggregations of nesting individuals may provide increased defense against natural enemies by having multiple individuals guarding nesting locations from potential oviposition (Thorp 1969), which has been observed in other groups, such as blackbirds forming colonies for protection against nest predators (Robinson 1985), and spiders building webs in groups to avoid predation by birds (Henschel 1998).

Conversely, aggregations might attract larger numbers of parasites to areas with many individuals. Parasites have been demonstrated to aggregate in areas in response to increases in host densities, suggesting increased risk of parasitism with higher aggregation, an effect that has been noted with parasitoid wasps (Matsumoto *et al.* 2004). This effect is also noted with parasites of solitary nesting bee species (Rosenheim 1989). Higher nesting densities may also result in the persistence of parasites within these areas, i.e., brown-headed cowbirds gather and persist in locations with larger songbird populations to exploit (Woolfenden *et al.* 2004), as they represent significant resources for exploitation (Rosenheim 1989). In addition, it has been suggested that persistent high nesting density may lead to increases in parasite populations through multiple seasons from continued availability of nests to exploit at these locations (Rosenheim 1989).

The tendency of some solitary species (Torchio 1984, Freeman 1982, Rosenheim 1989) to form nesting aggregations has led to the speculation that this behaviour is the precursor to the sociality found in other hymenopteran groups (Rosenheim 1990). High nesting density may lead to interactions between nesting females such as communal brood care, nest sharing, and progressive feeding, all of which have been observed in other primitively social Hymenoptera, and which are thought to be precursors to more advanced forms of sociality (West-Eberhard 2005, Flores-Prado 2012). The effects of aggregation on parasitism rates could influence selection toward or against sociality depending on whether parasitism is positively or negatively density-dependent. Positive density-dependent parasitism would tend to discourage social behaviour, while negative density dependence would encourage it.

The effect of nesting density on brood parasitism is also likely to be of particular management importance for informing the type of artificial nesting structures utilized for maintenance of mason bee population. If mason bees nesting at particular densities are at higher risk of larval mortality from parasitism, maintaining populations of these pollinators may be more difficult if these factors are not taken into consideration. Reduced populations of these managed pollinators may decrease the ability of these bees to provide effective pollination.

Distance From Pollen Resources

The availability of foraging resources (primarily floral resources) could influence vulnerability of mason bee nests to brood parasitism. Multiple studies have found that solitary hymenopterans suffer significant reductions in brood sizes when their nests are situated far from natural floral resources, presumably because of the increased energy expenditure required to reach distant resources (Williams and Tepedino 2003, Peterson and Roitberg 2006, Zurbuchen *et. al* 2010). Bees may also be at increased risk of parasitism due a lack of nearby floral resources. Female solitary hymenopterans are required to both forage for resources and protect their nests from numerous natural enemies including parasites (Linsley 1958), and must balance these tasks in order to maximize their reproductive output. They may therefore be vulnerable to increased exploitation by natural enemies if they are required to devote more energy or time to foraging when faced with low resource availability.

In a flight cage experiment, Goodell (2003) showed that mason bees suffered higher parasitism rates, and had reduced reproductive output when provided with fewer floral resources. Although it was suggested that lower resource availability might have forced females to forage for longer periods, leaving nests more vulnerable to parasite invasion, Goodell (2003) did not observe longer foraging bouts in bees in low-resource cages, leaving the mechanism of this effect unclear. Goodell (2003) was limited to a cage experiment and was unable to consider the effect of resource availability in a natural setting. The effect of natural floral resource availability on parasitism rates remains unstudied.

Hypotheses and Predictions

For this study, I set out to assess the effects of both nesting aggregation and floral resource availability on brood parasitism of mason bees by kleptoparasites. I hypothesized that larger aggregations of nesting bees would provide brood parasites with a more easily located target for oviposition, leading to increased levels of brood parasitism in areas with larger numbers of bees nesting. This hypothesis generated a number of predictions: 1) Nesting blocks with larger numbers of bees nesting in them simultaneously would have a larger proportion of individual nest cells parasitized by kleptoparasites compared to blocks with fewer bees. 2) The amount of time where at least one bee was present at a nesting block would be longer when larger numbers of bees were nesting. 3) Increased numbers of kleptoparasites would be present and more per capita incursions by parasites into bee nests would be observed in nesting blocks with more active bees.

The second hypothesis to be tested by this study was that in locations with fewer floral resources available to bees close to their nests, bees would be forced to spend more time foraging for pollen. This would lead to increased levels of brood parasitism as nests would be vulnerable to kleptoparasite incursion for longer periods of time. This hypothesis led to the following predictions: 1) Nesting locations far from floral resources would have a larger proportion of individual nest cells oviposited in by kleptoparasites compared to locations closer to floral resources. 2) Foraging bouts of bees in locations with more distant floral resources would be longer than those in areas with close floral resources.

In addition, in order to determine whether the mason bees in this study naturally formed aggregations while nesting, I assessed the first nesting choices of all mother bees. I observed whether bees chose to construct nests in nesting blocks with other nesting bees or in ones in which there were no other bees nesting.

I tested my hypotheses using a field study of natural populations of mason bees conducted at the Rocky Mountain Biological Laboratory in Colorado, USA in summer of 2014. This area has been previously used for studies involving mason bees (Forrest and Thomson 2011). To my knowledge, this study is the first to evaluate predictors of parasitism of mason bees in a field setting and will enhance understanding of how these factors can influence the population dynamics of these organisms. Given the emerging importance of mason bees as alternative pollinators, and the limited extent of literature on parasitism of this group, this study will likely assist in understanding and inform the management of this group for agricultural pollination.

CHAPTER 2: METHODS

Study Species

Mason bees (cavity-nesting members of the genus *Osmia*; Hymenoptera: Megachilidae) are solitary hymenopterans. Most mason bees in subalpine Colorado (location for this study) emerge in early June and live for three to four weeks; males usually emerge before females and are shorter lived. Female bees construct nests individually, typically in existing holes in decaying plant material. Female bees construct multiple cells within single nests, each containing a single egg (Michener 2000).

Nest cells are constructed first by provisioning food for developing larvae: the female bee deposits pollen collected from flowers and it is mixed with saliva and nectar to form the food mass. Once the pollen mass is complete, the female lays a single egg on it. She then partitions off the completed nest cell by constructing a wall made from collected mud or chewed-up leaf material and saliva in order to protect the egg. Multiple cells are constructed sequentially from the rear to the entrance of the nest. Finally, the female constructs a thicker wall at the entrance of the nest to further protect the developing eggs inside (Torchio 1989, Michener 2000). Mason bees only construct one nest at a time, but an individual can construct multiple nests in her lifetime. Her offspring develop over the course of the summer and then overwinter for one or two years before emerging as adults. Some species of mason bee will readily nest in artificial wooden nesting blocks (Taki *et al*. 2004). Species of *Osmia* that have previously been observed nesting at the field location include *O. iridis*, *O.lignaria* and *O. coloradensis* (Forrest and Thomson 2011).

Sapygid wasps (Hymenoptera: Sapygidae) are brood parasites of mason bees, ovipositing within the cells of their nests. Sapygid females enter mason bee nests after female bees have finished provisioning cells with pollen and oviposited. They will enter either while the cell wall is incomplete, or when the wall is freshly constructed and still malleable, in which case they will oviposit through the wall (J. Forrest pers. comm.). Sapygids will lay a single egg within a nest cell which is then sealed in with the bee egg. The sapygid egg hatches before the mason bee egg and the larva consumes the unhatched bee egg and the pollen provision within the cell.

Study Site

This study was conducted at the Rocky Mountain Biological Laboratory in Colorado, USA from 30 May to 21 July 2014, from the beginning of the nesting period of mason bees until past the peak nesting period. Sub-alpine meadows adjacent to stands of trembling aspen (*Populus tremuloides*) were used as study sites for this experiment, representing typical locations where mason bees nest and providing herbaceous flowering plants for bees to gather pollen for nest provisioning.

Experimental Set-Up

Artificial nesting blocks were constructed from untreated pine wood. Each block measured 15.24 cm x 15.24 cm x 10.15 cm and had 16 6.35 mm diameter holes drilled through lengthwise. The sides of the nesting blocks that constituted the entrances to the bee nests were scorched with a blowtorch. The exterior of the blocks and the nesting holes were treated with non-toxic water-based polyurethane to allow blocks to survive exterior conditions and the side opposite the nest entrances was covered in foil tape. The sides adjacent to the nest entrances were covered in burlap, with approximately three centimeters of burlap protruding forward on the entrance side. Each nesting hole had a single 4.76 mm diameter paper straw inserted into it to allow for removal and observation of bee nests. These construction methods correspond to those used by Taki *et al.* (2004).

Three individual sites (D1:38.953320, -106.981910, D2: 38.950525, -106.978197, D3: 38.949632, -106.975805) were selected for use in the nesting density experiment; each one was placed at least 200 m from the others. At each site, nesting blocks were placed at the margins of meadows. Each block was placed approximately 0.5m above the ground, attached to the side of a single aspen trunk with metal strapping. Nesting blocks were positioned close to or on dead or dying aspens if possible, to be close to areas where mason bees would naturally nest. Sixteen nesting blocks were used at each site (for a total of 48 blocks). Eight blocks were placed on trees individually approximately 10 m apart ("low density" treatment); the other 8 blocks were placed in groups of 4 blocks on single trees at least 15 m from the other blocks within the site ("high density" treatment).

Assessing Parasitism Rates

Mason bee females were allowed to construct nests within the nesting blocks at all sites. All nesting blocks were checked for the presence and progress of bee nests by removing the paper straws from the nests and observing them at the maximum every two days for each of the three nest sites. Nest occupants other than bees were removed. Locations of nests within blocks, numbers of cells constructed, and dates of nest start and completion were recorded. Female bees were marked on the posterior section of their thoraxes (to avoid damaging their wings) with enamel paint (Testors, Vernon, IL, USA) of differing colours in order to track any individuals that constructed more than one nest within nesting blocks. To assess whether nesting cells had been parasitized by sapygids, straws were removed from nesting holes during observations, and small flaps were cut into each completed nest cell. Each individual nest cell was checked for presence of a sapygid larva or egg. Flaps were repaired with masking tape and the straws returned to their nest holes.

Initial methodology for this experiment constituted moving individual bee nest straws during the early morning or evening while female bees were dormant in the nests. This would have been performed to create artificial levels of nesting aggregation to assess its effect on parasitism rates in a more experimental context. Unfortunately, in the preliminary stages of the field experiment, it was found that if nest straws were moved to a new location while a female bee was constructing a nest, they would return to their initial nesting location and begin construction of a new nest, abandoning the original nest. This made manipulating nest aggregation impossible for this experiment and led to the use of natural variation in nesting density instead.

I used the naturally established variation in nesting aggregation to determine the effects of number of nearby active bees on rates of parasitism. When each nest cell was checked for the presence of a sapygid egg, the number of active bees/nests present within the nesting block during the day of cell construction was recorded. At the end of the study, all completed bee nests were collected and placed in incubators to allow offspring to emerge and be identified to species, and to identify any additional parasites that were not observed in the field. Three bee nests that were still active at the end of the study were left in the field, however the data collected from those nests was still included in the analyses.

Observation of Nesting Behaviour

The behaviour of nesting bees and sapygids was also observed at nesting blocks to determine a behavioural mechanism for density-related changes to the rates of parasitism. Thirty-three 45-minute visual observations of nesting blocks were conducted throughout the course of the nesting period of the bees, at times between 1100 and 1500 hrs when mason bees were the most active, on days in which there was no inclement weather to prevent bees from foraging. During each observation period the foraging behaviour of mason bees was recorded using a voice recorder, including the length of individual foraging bouts away from the nests and the type of foraging bout (collection of pollen for food provisioning or mud for construction of cell partitions). I also recorded the behaviour of sapygids around nesting blocks including length of time sapygid wasps were present at nesting blocks, and the number of mason bee nests entered or oviposited in by wasps during the observation periods.

Response behaviour of sapygids to mason bees was also observed. Sapygid responses to female bee presence at nesting blocks was classified into three categories: whether sapygids (1) left the nesting block entirely when female bees were present, (2) moved to the sides of the nesting blocks, or (3) did not react to the presence of bees at nests. Unfortunately, only two incursions by sapygids were observed at nesting blocks when behaviour was recorded, so little information about their behaviour was garnered from these observations, and these data were ultimately not analyzed.

Analysis

A comparison of generalized linear mixed effect models (GLMM) with binomial error distributions was conducted using the software package R with the package lmer to assess the effects of the measured variables on the proportion of nest cells parasitized by sapygids. Individual nest cells were the replicates in these models, and whether the nesting cell was parasitized or not was the dependent variable. A likelihood-ratio test was used to compare nested models and thereby assess the significance of each model term as a predictor of nest-cell parasitism. Number of active bee nests within a block at the date of cell construction, cell position within the nest (1^{st}, 2^{nd}, etc. with the 1^{st} cell being the innermost cell in the nest), the lifespan of the nest from start to completion in days, site location, and the cell-per-day rate of nest construction were included in the model as fixed effects. (Site was treated as a fixed effect because of the small number [3] of sites.) Identity of the bee constructing the nest, and nest identity were also included in the model as random effects. Because Spearman rank correlations among predictor variables showed that several of these were significantly correlated (Table 1), I tested for multicollinearity of the variables included in the model using variance inflation factors (VIF) with the R package USDM. I found that the removal of nest lifespan and nest identity from the GLMM was required to keep the VIF below a threshold of five for the remaining factors in the final model; lifespan was nearly redundant as a predictor when rate of nest construction was included in the model. I also ran an additional GLMM with a fixed categorical variable for cell position (outermost vs. not outermost) in lieu of numeric cell position (as in the previous model) to determine whether the last-constructed cell in a nest was more likely to have been parasitized.

A comparison of GLMMs was also conducted at the level of whole nests, with whether the nest was parasitized at least once by sapygids as the dependent variable. Number of cells constructed in a nest, cell-per-day rate of nest construction, site, and lifespan of the nest were included as fixed effects. Bee identity , block and nest ID were included as random effects. Calculated VIF values and Spearman rank correlations between fixed factors led to lifespan being removed from the final GLMM in this comparison to avoid multicollinearity similarly to the GLMM used for the individual cell level analysis.

An additional comparison of LMM was conducted with the length of foraging bouts by mason bees as the dependent variable. Type of foraging trip, site, and date were included as fixed effects, while Nest ID, and bee, were included in the models as random effects.

To determine whether nesting bees were more likely to select nesting blocks that were currently occupied by other bees or those that were empty of other active mason bee nests, Fisher’s method for combining independent p-values was utilized for both 2014 nesting data from this study and 2013 nesting data obtained from J. Forrest (unpublished data). The number of nesting blocks within a site currently occupied or unoccupied by mason bees was tabulated for the start date of each first-constructed nest by a bee at a nesting block. From this, the likelihood of each bee's observed first choice of nesting block (occupied or unoccupied) was calculated. These likelihoods were then combined using Fisher's method to generate a chi-square value with 386 degrees of freedom (2 x 193 independent observations of bee choices).

Floral-Resource Manipulation Experiment

To assess the effects of floral resource availability on parasitism rates in mason bees, another experiment was conducted at the Rocky Mountain Biological Laboratory concurrently to the nesting aggregation experiment, in which I attempted to manipulate the availability of floral resources adjacent to nesting locations on a short-term basis. Four meadow edge sites were selected adjacent to trembling aspen stands and five nesting blocks were attached to aspens at each site in the same manner as for the nesting aggregation study. Agricultural row covers (Johnny's Selected Seeds, Winslow, ME, USA) were used to cover flowering herbaceous plants in a 50 m diameter semicircle in the meadow adjacent to the nesting blocks at two of the four sites to prevent bees from having access to pollen resources close to their nesting sites. Nesting blocks were to be checked for occupancy and parasitism as in the aggregation experiment. Row covers were to be rotated between sites every two weeks to provide within-site controls, allowing all sites to experience both treatments (high and low pollen resources availability). Unfortunately, this experiment was ultimately unsuccessful due to a lack of occupancy in the nesting blocks within all sites.

CHAPTER 3: RESULTS

Nesting Statistics: A total of 78 mason bee nests were constructed within the three sites used for this experiment over the period 6 June to 21 July 2014: 62 in D1, three in D2, and 14 in D3. Within these nests 331 individual nest cells were completed with an average of 4.19±2.56 cells/nest (all measurements of variability presented are standard deviations). Of these cells, 260 were constructed on or after 27 June, the first date that an individual nest cell was parasitized by a sapygid wasp. Only these 260 nest cells were considered in the analyses. Overall, 30 nest cells were parasitized by sapygids (26 in site D1, 3 in D2 and 1 in D3), accounting for 9.1% of all cells constructed, and 11.5% of nest cells constructed after 27 June. Of the 78 nests constructed, 21 had at least one nest cell parasitized, accounting for 26.9% of nests (18 in D1, 2 in D2, 1 in D3) with a maximum of three cells parasitized within a single nest. Thirty-five individual bees were identified during the experiment, accounting for the construction of 67 of the 78 nests, with an average of 1.91±0.92 nests constructed per bee. The mothers of 11 nests could not be identified.

Correlations: Spearman rank correlations among variables describing individual nest cells were low (Table 1), with the highest magnitude coefficient between rate of cell construction and lifespan of nests ($\rho=-0.49$). Correlations were higher among whole-nest variables, with the correlation between number of cells in a nest and lifespan of the nest being the highest ($\rho=0.7186$).

Number of Active Bees Within a Nesting Block: A maximum of four bees were observed constructing nests within a block at one time. Nest cells constructed with one bee present at the block (the mother of that nest) constituted the largest proportion of nest cells constructed (109 cells, 41.9%; Figure 1). Fewer cells were constructed with more active bees present, down to a minimum of 13.8% of cells (36 cells) constructed with four active bees present (Figure 1). As the number of active bees at a block increased, the proportion of nest cells parasitized by sapygids also increased (Figure 2), from a low of 5.5% among cells constructed with one bee present, to a high of 25% among cells constructed with four bees present. Number of active bees was a significant predictor of nest-cell parasitism by sapygids (Table 3).

Rate of Construction and Lifespan of Bee Nests: The lifespan of individual nests varied between 2 and 19 days, with an average lifespan of 6.46±3.97 days. Rate of nest construction varied between 0.16 and 2.0 cells per nest per day with an average of 0.77±0.44 cells constructed per day. The highest incidence of parasitism was observed at low rates of nest construction (Figure 3); however, variation in rates of parasitism was quite large among these slow nests, and rate of nest construction was not a significant predictor of parasitism of individual nest cells (Table 3).

Cell Position within a Nest: The number of nest cells constructed within a single nest varied between one and 13 cells. Most nests were small, with few nests having more than eight cells (Figure 4). Parasitism rate showed little noticeable trend with cell number, save for the single cell #13 being parasitized by a sapygid (Figure 3). Cell number was a significant predictor of parasitism rate in individual cells (χ^2=4.01, df=*1*, *p*=0.045); however, when cell #13 was removed from the data, cell number ceased to be a significant factor (χ^2=2.83, df=*1*, *p*=0.09). When both cell number and whether a particular cell was the last constructed were both included as factors in the model, neither was significant (last cell: χ^2=2.02, df=*1*, *p*=0.15, Cell Position: χ^2=0.47, df=*1*, *p*=0.48). However, if cell number was removed, last cell became a significant predictor of cell parasitism (Table 3) with the 21.2% of last cells within nests being parasitized by sapygids, compared to only 8.24% of other cells (Figure 5).

Other Factors and Parasitism of Individual Nests: Among the other factors included in the analysis (site, nest ID and identity of mother bee) only site had a significant effect on whether individual cells were parasitized by sapygid wasps (Table 3). In addition, site was the only factor among the variables included in the nest level analysis (site, identity of mother bee, number of cells constructed and rate of nest construction) that was a significant predictor of sapygid parasitism at the nest level (Table 3).

Nesting Choice of Individual Bees: On their first choice of a nesting hole, slightly more bees chose already-occupied nest-blocks compared to the compiled expected values (Figure 6); however, the difference from expected values was not significant (Fisher's method, $\chi^2 = 182.99$, df = 386, $p = 1$).

Foraging Trip Times: Foraging trip durations were significantly longer for bees collecting pollen compared to those collecting mud, with an average of 701±68 seconds for pollen-collecting trips and 201±34 seconds for mud-collecting trips (Figure 7). Trip type was a significant predictor of the length of a foraging trip ($\chi^2 = 24.26$, $df = 1$ $p = 8.4\times10^{-7}$). None of the other factors included in the model (nest ID, bee, site, date) were significant predictors of foraging-trip duration.

CHAPTER 4: DISCUSSION

The overall goal of this study was to elucidate the effects of both nesting aggregation and resource availability on vulnerability of mason bees to brood parasitism by sapygid wasps. The effect of pollen resource availability could not be evaluated due to a lack of mason bee occupancy in the nesting block sites established for that experiment. However, I observed a strong positive effect of nesting aggregation on brood parasitism. I also found that the last cell within mason bee nests was significantly more likely to be parasitized than inner nest cells, suggesting that bees may choose to terminate nests after brood parasite attack. Mason bees do not appear to avoid nesting near other *Osmia*, despite the increased risk of parasitism this entails; as I explain below, nest termination after parasite incursion may be a defensive mechanism against these natural enemies.

Parasitism of Individual Cells—Effect of Aggregation

This study clearly indicates a significant positive effect of nesting aggregation on the likelihood of mason bees suffering from brood parasitism by sapygid wasps, with 25% of cells being parasitized at the highest aggregation level compared to only 5.5% for the lowest (Figure 1). These results support the hypothesis that the vulnerability of mason bees to brood parasitism tends to increase with increased nesting aggregation. They also show that this vulnerability increases quite quickly with increases in aggregation, with significant brood parasitism already occurring with small numbers of bees (a maximum of four active bees within a nest block in this study).

Parasitism of Individual Cells—Cell Position

Although cell position was a significant factor within the initial GLMM, this effect was due only to a single data point with high leverage. However, whether a cell was the last cell constructed in a nest was a significant predictor of parasitism, with nearly half of parasitized cells being the last ones constructed in their respective nests. This result may reflect mason bee responses to parasitism, suggesting that bees may abandon nests once they detect sapygid parasitism, choosing to cease construction of a nest and seal the entrance then absconding. Although it could be argued that the more outer nest cells may simply be more vulnerable to parasitism, the construction of the thicker nest cap by the bee when finishing a nest likely protects cells from further parasitism once a nest has been completed.

If abandoning nests that are being parasitized by sapygids reduces the probability of further exploitation by these parasites, this could represent a potential defensive response of bees to brood parasitism. Continuing to construct a nest that has suffered from brood parasitism or is vulnerable to subsequent parasitism has significant fitness costs to an organism (Guigeuno and Sealy 2011, Polidori and Andrietti 2006). Nest abandonment is a strategy that has been observed in multiple groups for avoiding continued costs from brood parasitism, including sphecid wasps attacked by cuckoo wasps (Polidori and Andrietti 2006) and songbirds attacked by brown-headed cowbirds (Guigeuno and Sealy 2011a, Guigeuno and Sealy 2011b). If mason bees do abandon their nests more readily in response to brood parasitism, it suggests that they have the ability to recognize oviposition by sapygid wasps and deny them additional opportunities to oviposit in their nests.

Parasitism of Individual Cells-Site

None of the other factors in the original model were significant predictors of the likelihood of individual cells suffering sapygid parasitism aside from site location (Table 1). There was significant among-site variation in the numbers of mason bee nests (with site D1 containing 67.3% of constructed cells and 86.6% of parasitized nests). As well, previous observations of sapygid parasitism in mason bee populations have found significant variations in victimization rates, ranging from 15% to 56% of cells being parasitized (J. Forrest unpublished data) among sites. Variation in parasite risk based on location/habitat variation has also been observed in other groups including great reed warblers parasitized by cuckoos (Moskat and Honza 2000) and caterpillars parasitized by *Trichogramma* wasps (Romeis *et al.* 2005).

Parasitism of Individual Nests

Site was the only factor studied that had a significant effect on the likelihood of an individual nest being parasitized at least once over the course of its construction. It appears that aside from variation based on site, vulnerability to parasitism is a more transient risk to mason bees. Brood parasitism appears to fluctuate based upon more temporary exterior factors such as the number of nearby active bees, as opposed to risks inherent to a particular nest or the bees constructing it (such as the rate of cell construction). These more static factors were not significant predictors of parasitism in this study (Table 3). However, attributes of the nest location, such as insolation or local vegetation characteristics, which were not examined in this study, may influence nest-level parasitism.

Mechanism for Increases in Parasitism

Since behavioural observation periods were unsuccessful in discerning changes in the behaviour of bees and sapygids in response to changes in nesting density, the mechanism for the observed difference in parasitism rate due to differing levels of aggregation remains unknown. This lack of results unfortunately made it impossible to test the prediction of an increase in sapygid incursions in response to aggregations of mason bees in this field experiment. Although this specific effect was not observed in this field study, rate of nest construction can be ruled out as a mechanism for increased brood parasitism. A slower rate of cell construction might leave individual cells vulnerable to being exploited by a sapygid for a longer period of time. This is similar to the predictions of the resource-availability hypothesis, with low pollen availability slowing construction of nest cells and increasing their vulnerability to brood parasitism as bees are required to spend more time foraging. However, while rate of nest construction shows a weak negative relationship with the likelihood of brood parasitism, (Figure 3), it is not a significant predictor of parasitism. Given the lack of a significant effect of rate of nest construction, it appears that bee industriousness does not affect the likelihood of brood parasitism.

Choice of Nesting Block

In terms of the likelihood of bees forming aggregations, the largest number of nest cells were constructed in circumstances where there was only one mother bee nesting within blocks. Progressively smaller numbers of cells were constructed as the number of active bees increased, while these larger aggregations had larger proportions of nest cells being oviposited in by sapygids. This result suggests that mason bee are more vulnerable to brood parasitism when nesting in higher densities, but that many nest cells in the population avoid parasitism by virtue of not occurring in aggregations.

Mason bees showed no preference in regards to choosing to construct their first nest in blocks that contained other *Osmia* or not. The choice of the location of a first nest did not deviate from what would be expected from a random selection of nesting blocks with no regard to the presence or absence of other bees. It appears that the behaviour of individual mason bees does not promote or prevent the formation of aggregations of nesting individuals, and does not reflect attempts to avoid parasitism. It is important to note that this analysis included all *Osmia* species within blocks; it is possible that if individual species were distinguished, evidence of aggregation avoidance may be observable. Nevertheless, these results are in contrast to the observed tendency of certain species of mason bees (specifically *O. lignaria*) to nest gregariously in single nesting locations (Torchio 1984).

Both these results suggest a lack of selective pressure to develop behaviours to avoid nesting aggregation, despite the potential risks of increased parasitism. As the largest proportion of nest cells in my study were constructed when there was only one bee present, most bees were not at high risk of density-related parasitism. It is possible that in natural settings, bee nests tend to be highly dispersed in space, so there is no necessity for avoidance of aggregation. An alternative explanation is that if nesting sites are highly limiting in nature, then aggregations are inevitable and bees have no choice but to nest in high densities. However, these results also suggest that mason bees are likely to be vulnerable to parasitism if proper nesting sites are limited or if populations are particularly large, which could be the case in managed populations in agricultural settings.

Nesting Aggregations and Sociality

The results of the nesting aggregation experiment are consistent with previous findings of increased exploitation of solitary Hymenoptera by their parasites when nesting in high densities (Freeman 1982, Rosenheim 1989, Rosenheim 1990). It is also in accordance with studies of other typically solitary Hymenoptera that found that multiple nesting individuals within a location did not provide increased defence against natural enemies compared to individuals nesting in solitary conditions (Prager 2014).

My results provide evidence for positive density-dependent parasitism in mason bee populations. They contrast the findings of inverse density-dependent parasitism in some groups of Hymenoptera (Antonini *et al*. 2003, Wcislo 1984), as large groups of nesting individuals clearly do not deter sapygid individuals from exploiting mason bee populations. This suggests that aggregations do not provide a defence against parasitism rates as has been considered in other solitary Hymenoptera (Wcislo 1984, Antonini *et al.* 2003). Intriguingly, many observed instance of inverse density-dependent parasitism are in circumstances where the parasites are species of Diptera (Wcislo 1984, Antonini *et al.* 2003). In contrast, in instances of positive density-dependent parasitism, wasps are typically the parasites (Rosenheim 1989, Rosenheim 1990). This is consistent with the findings of this study, suggesting that different parasite taxa respond differently to host density.

The transient risk of brood parasitism observed in this field study suggests that sapygid parasitism is likely responsive to aggregations in nesting individuals on a short-term basis. My results suggest that sapygid parasites do not locate and continually exploit single locations over long periods of time, which has been considered a possible mechanism for positive density-dependent parasitism (Rosenheim 1989, Rosenheim 1990). Instead, it is possible that nesting aggregations represent more easily locatable opportunities for sapygids in a more short-term manner, with sapygids using active nesting individuals as indicators of opportunities to oviposit and aggregating in these locations during the day (Rosenheim 1989). In order to more rigorously assess this assertion, behavioural observations of individually marked sapygids would have to be conducted over the course of multiple days. If numbers of sapygid wasps in proximity to mason bee nests responded to bee aggregation levels on a day to day basis compared to building up over time, it would support the notion of a short-term response of parasites to host density.

One of the potential benefits of animals developing sociality is increased defense against natural enemies, the presence of multiple individuals in a location deterring natural enemies or reducing their effectiveness (Jeschke and Tollrian 2007), or increasing the defense against them

(Hamilton 1971). However, the formation of nest aggregations in mason bees clearly does not provide this benefit, at least in respect to sapygid parasitism in this natural setting. Aggregations instead increase exploitation by these natural enemies of the mason bees observed in this study, suggesting that aggregation behaviour is not beneficial to these bees. This study provides evidence that increased defence against natural enemies may not be a means for social behaviour to be favoured in mason bees, as parasite-mediated selection would be against the formation of aggregations. Thus it is unlikely that the precursor behaviours to more advanced sociality, such as progressive feeding and nest sharing (West-Eberhard 2005, Flores-Prado 2012), will develop in this group as aggregations are not favoured in mason bee nesting.

Although mason bees have been observed to develop aggregations (Torchio 1984, Freeman 1982, Rosenheim 1989), bees in this study did not tend to form them by choice, leaving the explanation for the formation of aggregations in previous studies unknown. However, limitations in desirable nesting locations provide a potential explanation for the formation of aggregations of nesting individuals. Aggregations would inevitably develop if multiple bees are required to nest in the few available areas where conditions are appropriate for nest construction.

Population-Level Consequences

Increased brood parasitism in response to increased nesting aggregation suggests a form of population regulation by top-down control on mason bee populations, especially considering the large proportions of nest cells being parasitized. Large populations of mason bees will likely lead to the formation of nesting aggregations, if mason bees inhabit large proportions of available nesting locations, leading to increased exploitation by parasites, depressing their populations. Patterns of brood parasitism observed in this study suggest that parasites could have a stabilizing effect on mason bee populations, as has been observed in other systems (Berryman 2001).

In addition, the results of this study suggest that nest availability (a bottom-up factor) could affect rates of brood parasitism by sapygids (a top- down factor). A limitation in nesting locations will not only limit the numbers of mason bees that are able to reproduce, but also interact with top-down influences, leading to increases in brood parasitism if bees are forced to aggregate in the few available nesting locations. This highlights the interconnectedness inherent in factors affecting animal populations (Berryman 2001, Freckleton *et al*. 2006, Moreau *et al.* 2006), and the importance of understanding how these factors influence one another in natural conditions.

While research on factors that influence parasitism rates in nesting Hymenoptera and other groups is still limited , their potential impact on populations should not be discounted. Given the importance of mason bees as pollinators and as managed agricultural species (Fliszkiewicz *et al.* 2011, Gruber *et al.* 2011, Jauker *et al.* 2012, Schindler and Peters 2011, Ward *et al*. 2010), understanding factors affecting their populations is of considerable importance for future work in this field.

Management Implications

This study's finding that higher levels of nesting aggregation lead to increased levels of brood parasitism in mason bees has significant implications for their usage as agricultural pollinators (Fliszkiewicz *et al.* 2011, Gruber *et al.* 2011, Jauker *et al.* 2012, Schindler and Peters 2011, Ward *et al*. 2010). This increase in parasitism represents a potential problem if low numbers of large, artificial nest structures are used for cultivating mason bees. Grouping nesting blocks in single locations will favour nest aggregation, especially in conjunction with the fact that agricultural nests are typically seeded with bee cocoons at the start of the seasons. The risk of deleteriously large nesting aggregations is increased if, as observed here, mason bees do not have behaviours for avoiding aggregations. If artificial nest structures in agricultural field are large and small in number and natural nesting locations are lacking, mason bees are likely to be vulnerable to exploitation in these settings if brood parasite populations are present.

Large losses of bee larvae stemming from higher vulnerability of bees to parasitism will reduce the sustainability of populations of managed mason bees. These losses will likely lead to reductions in mason bees' effectiveness in providing pollination services to crops over multiple seasons, as agriculture requires consistently high levels of pollination (Winfree *et al*., 2007, Burgett *et al*. 2010). Loss of larvae will also incur increases in the costs of using mason bees as managed pollinators, as high larval mortality will reduce the numbers of individuals available as pollinators in subsequent seasons, necessitating purchases of additional bees to maintain sufficient pollination service.

The nesting habits of mason bees make the maintenance of their populations in single locations a more significant concern compared to honey bees. Honey bee hives are often transported to multiple locations over the course of a season, depending on the demand for pollination service, while mason bees do not possess that level of flexibility in transport. This makes maintaining viable mason bee populations within fields an attractive option, especially given the high costs for the use of managed pollinators in agriculture (vanEngelsdorp and Meixner 2011).

The results of this study suggest that an overhaul to the use of artificial bee nesting structures may be beneficial to their usage as pollinators. This is also reinforced by existing literature calling into question the overall beneficial nature of bee structures for enhancing their population growths, with native bees suffering increased parasitism within artificial nest structures compared to introduced species (Maclvor and Packer 2015). It is likely that the use of multiple smaller nesting blocks in agriculture will help to avoid increases in brood parasitism in areas where sapygid parasitism is a risk. This is especially relevant given the significance of sapygids as pests in agricultural populations of mason bees (Torchio 1979). This study is also in accordance with the findings that mason bees retain nests for longer periods of time when they are able to nest in larger numbers of smaller nest boxes (Artz *et al*. 2013), reinforcing the benefits of the availability of large numbers of potential nesting locations for mason bee pollinators. It would also be beneficial to support artificial nesting structures with more availability of natural nesting locations to further offset the likelihood of aggregations and reduce mason bee vulnerability to brood parasitism.

Foraging Behaviour and Floral Resources

Trip type was a significant predictor of the length of mason bee foraging bouts, with the average length of pollen/nectar foraging bouts lasting three times longer than average mud collecting bouts (Figure 7). Given that bees foraging for pollen will have to travel to multiple flowers over a larger distance to collect sufficient pollen or nectar to provision their nesting cells compared to typically being able to collect mud from a single location close to their nest (as observed in this field study; this may not always be the case), this result is not surprising. Trips for collecting mud were also more numerous than those for collecting pollen.

In regards to the resource availability experiment, the lack of success of this experiment leaves the effect of this factor in a field context unknown. The potential of proximity to pollen resources to affect vulnerability of mason bees to parasitism is still poorly understood (Goodell 2003), and is of considerable importance given the ephemeral nature of pollen availability in agricultural settings (Jauker *et al.* 2012). The mechanism for the higher levels of parasitism with lower floral resource availability observed in the study by Goodell (2003) is still uncertain and the effect remains unstudied outside of a cage experiment. Additional study is needed to further understand this factor's impact on brood parasitism.

Conducting this experiment in an agricultural setting may allow for a greater variation in nesting densities to better elucidate changes in rates of brood parasitism. An experimental agricultural field will by nature have a more defined margin where floral resources are not present and allow for more effective covering of flowers to ensure a removal of pollen availability during the course of the experiment. In addition, an agricultural setting will more accurately represent the conditions in which this research has practical applications.

Conclusions and Future Directions

This study provides evidence for the hypothesis that increased nesting aggregation leads to increases in brood parasitism in solitary mason bees. This was supported by the significant increase in the proportions of individual nest cells being oviposited in by sapygid wasps in nesting blocks with multiple active mason bees, this high effect size, in combination with the statistical significance of these results, leading to a finding that I am significantly confident in. While specific mechanisms for these increases could not be elucidated by behavioural observations, these results nevertheless demonstrate the importance of outside factors on brood parasitism of mason bees. This study also revealed a potential defensive response of mason bees to sapygid parasitism, with a large proportion of parasitized nest cells being the last ones constructed in their respective nests. Abandonment of the nest could represent a means for mason bees to avoid further exploitation by brood parasites.

Future study is needed to more fully understand the effect of nest aggregation on brood parasitism in mason bees, and the most pertinent next step would be identify the specific mechanisms for the changes in victimization rates of mason bees to sapygid parasitism with increase nesting density. While this study was able to discern an effect of nest density on brood parasitism from a mostly observational approach, the inability to artificially manipulate nesting aggregation is a definite drawback. Although manipulation of nesting aggregations in a natural setting proved unfeasible for this experiment, attempting to create differing levels of aggregation artificially is not without merit. A more controlled approach to this question, especially making use of field cage experimentation, would be an excellent supplement to this study. The smaller spatial scope and ability to control more external variables would allow one to discern potential mechanisms for the increased vulnerability to parasitism found at higher levels of nesting density and more closely observe mason bee responses to sapygids. In addition, although there is a possibility that in a field cage setting sapygids would be able to locate all mason bee nests regardless of nesting density, it would also likely allow for more consistent observation and tracking of both sapygid wasp and mason bee behaviour.

In addition, this sort of approach would be extremely useful in further evaluating the tendency of mason bees to abandon nests in response to brood parasitism, as the specific behaviours of mason bees in response to brood parasitism would be more easily observed within field cages. In addition, smaller scale experiments may allow for more exact observations of the timing of sapygid parasitism and whether mason bees responded with nest abandonment.

Further research is also needed to elucidate the effect of nesting density on brood parasitism in a more agricultural setting. Making use of multiple potential designs for the size and positioning of artificial nest structures for use in management will allow for the evaluation of the optimal designs for minimizing brood parasitism. A multi-year approach to this research could also allow for monitoring of both mason bee and parasite populations. This could be conducted to determine how nest parasitism rates change over longer time periods with regards to differing management strategies of mason bees, and to see if parasite populations are favoured by more aggregated host nest distributions.

Overall, this study enhances our understanding of the effect of external factors on rates of parasitism in mason bee populations and the potential responses of these organisms to parasitism by sapygid wasps. In addition it highlights the potential significance of these factors on management practices of mason bees for use as pollinators of agricultural systems.

TABLES

Table 1. Spearman rank correlations between all fixed factors (cell position within a nest, number of active bees at the time of cell construction, date of cell construction, lifespan of nest and rate of nest construction) in the initial generalized linear mixed model (GLMM) for parasitism of individual nest cells.

	Cell Position	Active Bees	Date	Lifespan	Rate
Cell Position	1.00	-	-	-	-
Active Bees	-0.12	1.00	-	-	-
Date	0.09	**-0.31**	1.00	-	-
Lifespan	**0.37**[1]	**-0.21**	**-0.13**	1.00	-
Rate	**0.19**	0.01	0.08	**-0.49**	1.00

1-Bold coefficients represent significant correlations (p<0.05).

Table 2. Spearman rank correlations between all fixed factors (number of cells constructed in a nest, lifespan of nest and rate of nest construction) in the initial generalized linear mixed model (GLMM) for parasitism of individual nests.

	Cells	Lifespan	Rate
Cells	1.00	-	-
Lifespan	**0.71**[1]	1.00	-
Rate	**0.27**	**-0.41**	1.00

1-Bold coefficients represent significant correlations ($p<0.05$).

Table 3. Likelihood ratio test results for models of sapygid parasitism at the level of a) individual nest cells (n=260) and b) entire nests (n=65). Models were generalized linear mixed models with binomial error distributions, and significance of each term was assessed by comparing the reduced model (without that term) to the full model. Variables in the models for a) individual nest cells: number of active bees, rate of nest construction, site the nest was constructed in, mother bee, whether a cell was the last in a nest and the date of construction, for b) individual nests: rate of nest construction, mother bee, number of cells constructed within the nest, and the site the nest was constructed in.

Factor[1]	Coefficient	df	χ^2	Pr(>χ^2)[2]
a)Nest Cells				
Active Bees	0.6309	1	7.3745	**0.0066**
Rate	-0.0739	1	0.0146	0.9040
Bee	0.8743	1	2.6109	0.1061
Site	1.1472	1	6.5466	**0.0378**
Last Cell	0.9870	1	4.7112	**0.0299**
Date	0.0002	1	0.0000	0.9965
b)Nests				
Rate	-0.4431	1	0.2391	0.6249
Bee	$1.3x10^{-10}$	1	0.0000	1.0000
Cells	-0.0050	1	0.0018	0.9663
Site	1.1883	1	6.3027	**0.0428**

1-Italicized factors denote random variables within the model

2-P values in bold denote significance (<0.05)

FIGURES

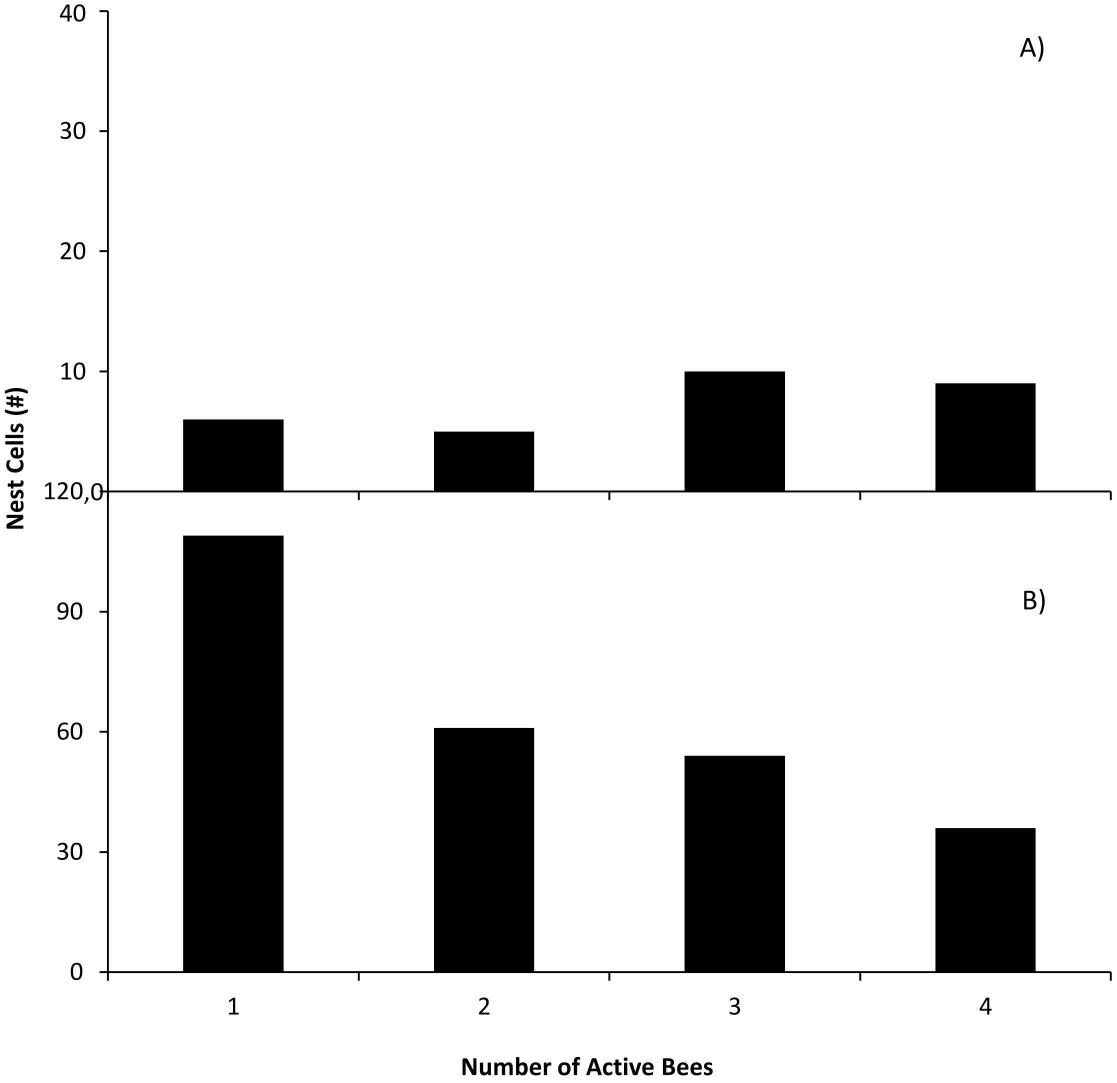

Figure 1. Number of individual *Osmia* nest cells A) parasitized by sapygids and B) constructed by bees versus the number of active bees within a nesting block during construction of the cell (n=260 cells). Note that the scale for y-axes differ for panels A) and B)

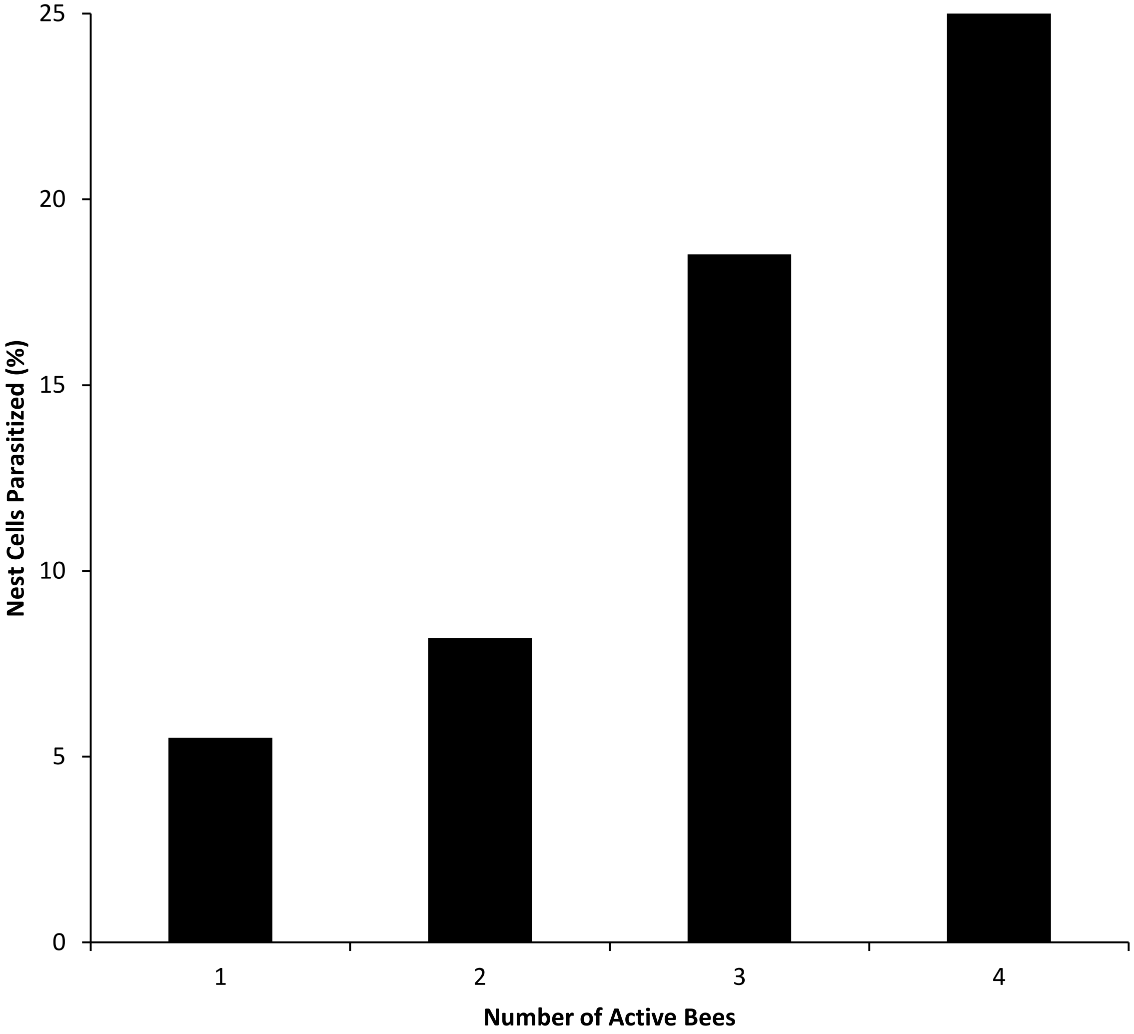

Figure 2. Percentage of individual *Osmia* nest cells parasitized by sapygid wasps versus the number of active bees within a nesting block during the construction of the cell (n=260 cells).

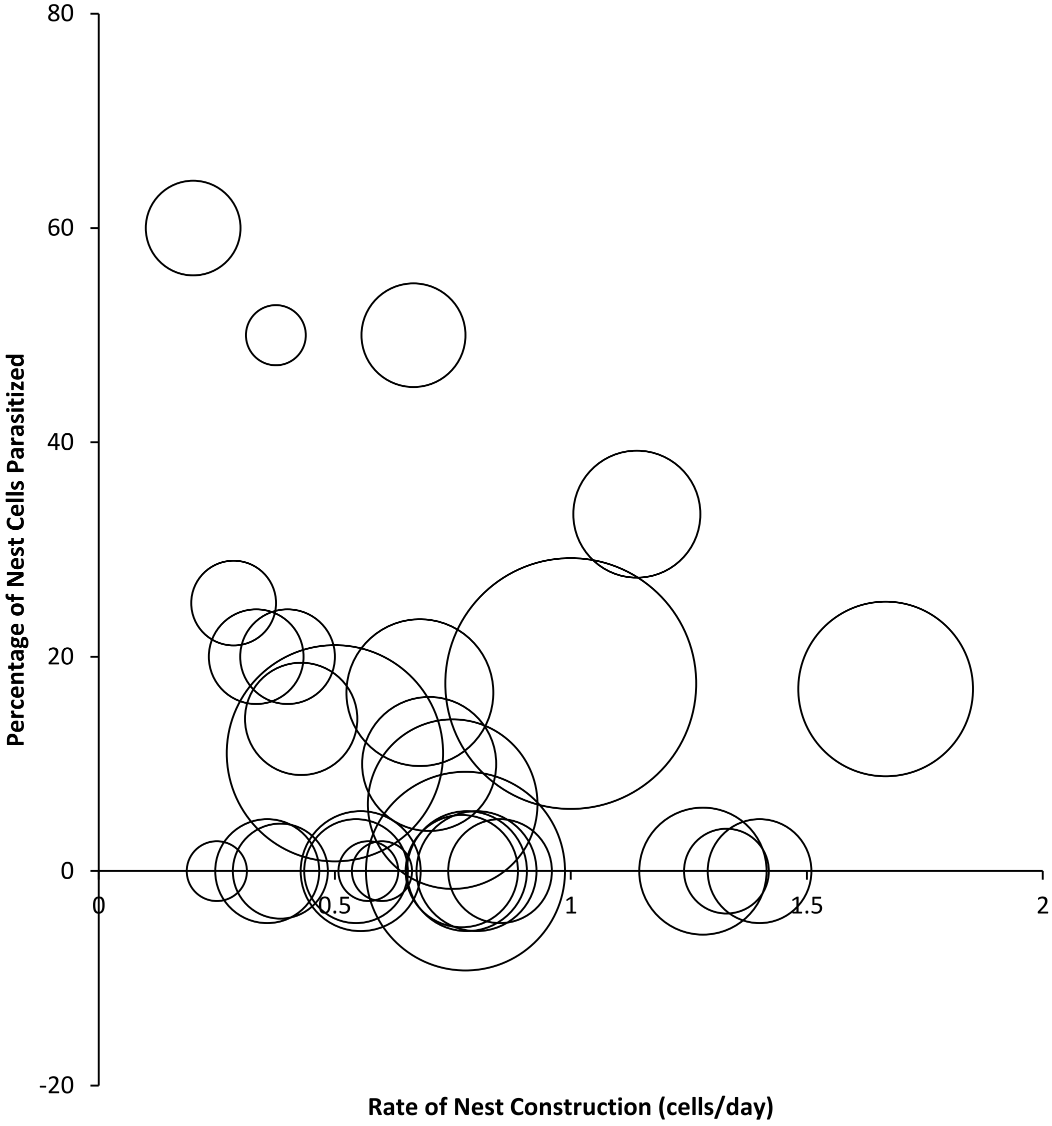

Figure 3. Percentage of individual *Osmia* nest cells parasitized by sapygid wasps versus the rate of construction of the nest in which a cell was constructed (cells/day) (n=260 cells). The size of each data point corresponds to the number of individual nest cells constructed in nests with a particular rate of construction and level of parasitism (number of cells represented by a point range from 2 to 26).

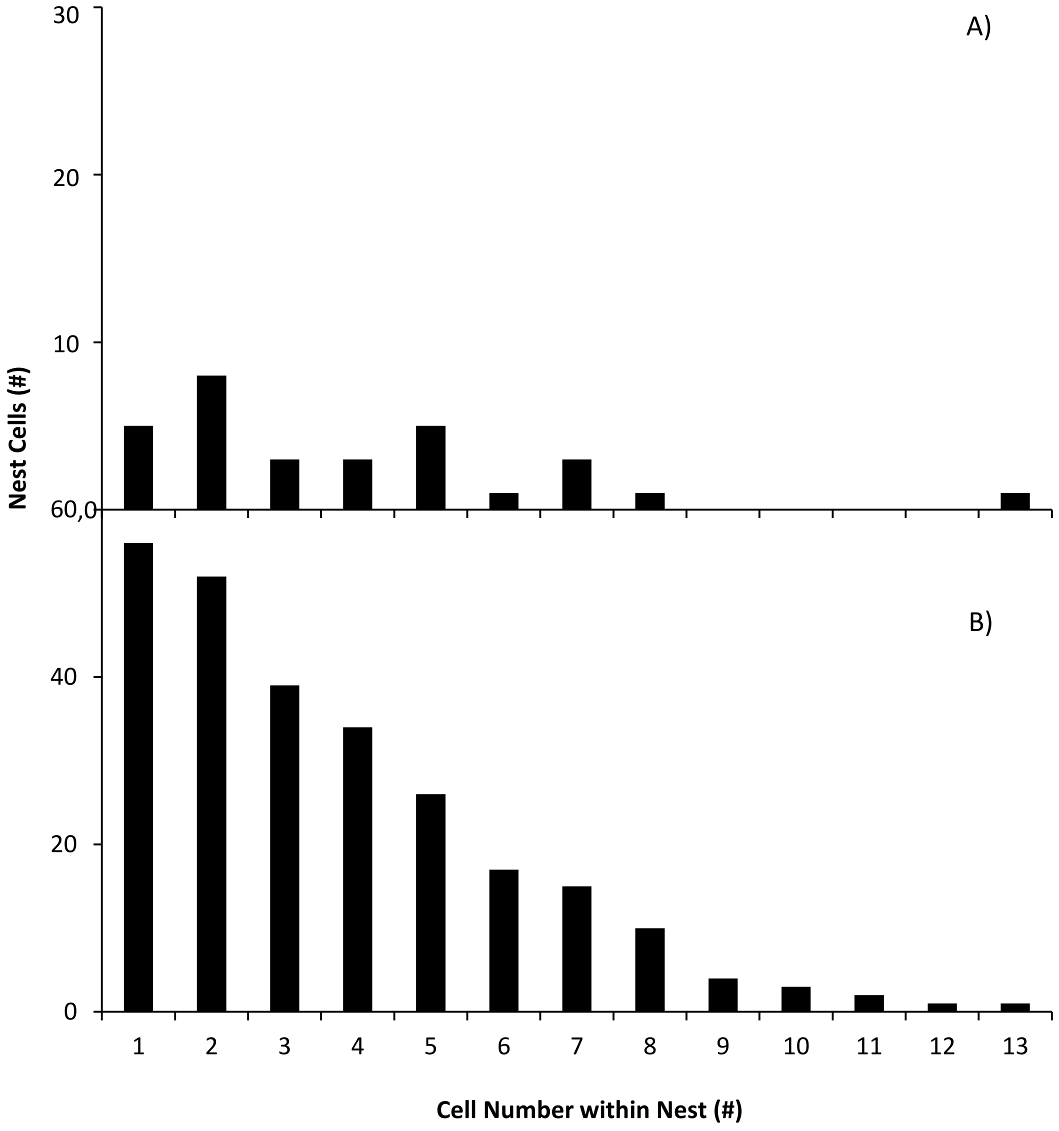

Figure 4. Number of individual *Osmia* nest cells parasitized by sapygid wasps A) and total constructed B) for each cell position within a nest (n=260 cells). Cell #1 is the innermost cell. Note that the scale for y-axes differ for panels A) and B).

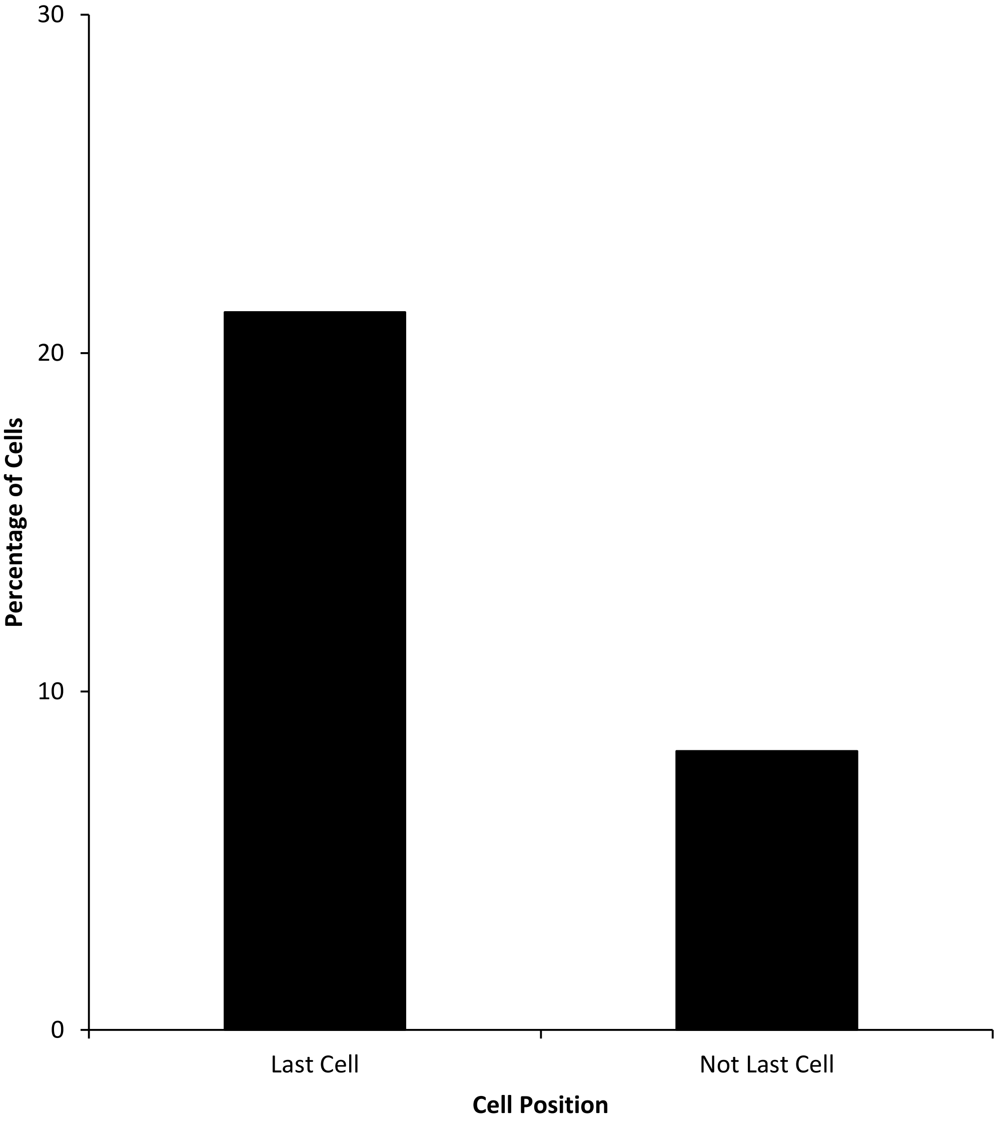

Figure 5. Percentage of nest cells parasitized by sapygids as a function of cell position within the nest (whether the last (outermost) cell (n=66 cells) or not (n=194 cells)).

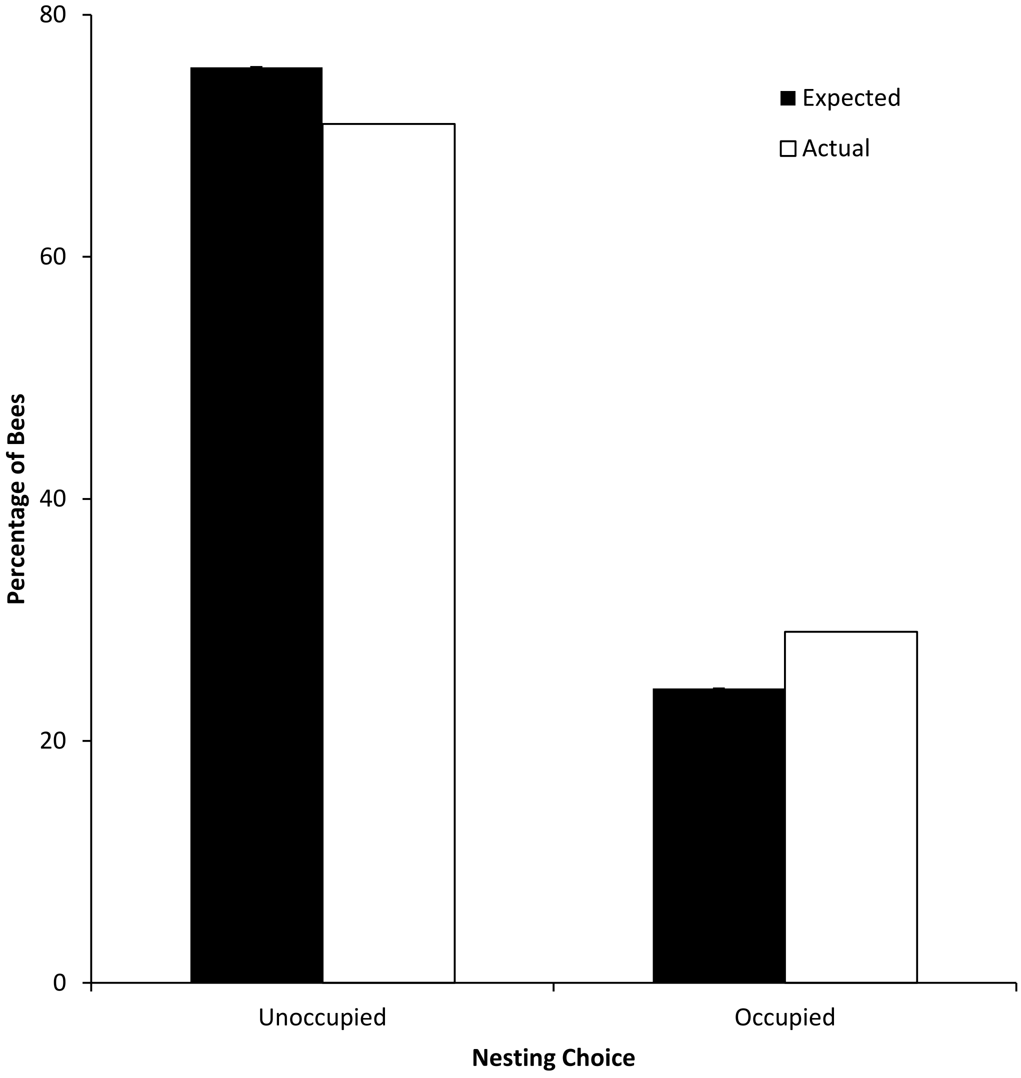

Figure 6. Expected and actual percentages of first nesting block choice of *Osmia* compared to occupation status of the block from both 2013 and 2014 nesting data (n=193 nest choices). Error bars for expected values represent one standard deviation in the likelihood of a choice of nest by a bee due to varying occupancy rates through time. The SD was not used for testing the difference between observed and expected values.

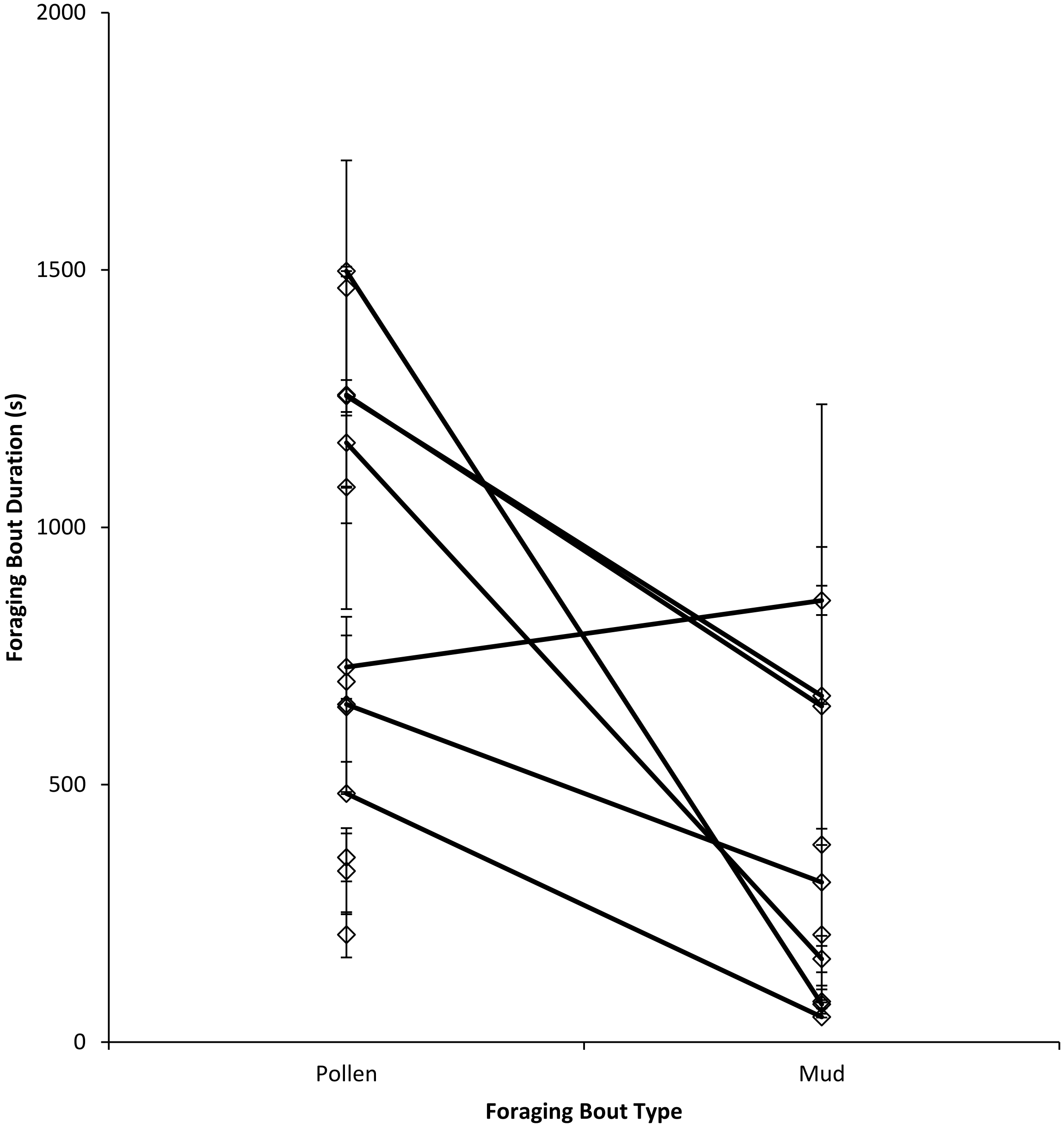

Figure 7. Mean duration (±SE) of *Osmia* foraging bouts for 17 individually observed bees for each type of material collected by bees during foraging bouts (n=147 bouts). Each pair of points and connecting line represent an individual bee. Some connecting lines and error bars are not distinguishable due to the density of data points. “Pollen” trips likely included nectar-foraging as well.

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