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### Cover Page Footnote

Acknowledgements: I would like to thank Rufus Isaacs (Michigan State University) and Philip Starks (Tufts University) for their thoughtful discussion about these findings. Additionally, I would like to thank Thomas Wood (Michigan State University) for his identification of *Vespula germanica*. This project was funded in part by the USDA-NIFA (Award #: 2017-68004-26323).

## Heterospecific Hymenoptera Found Inside the Nests of *Bombus impatiens* (Hymenoptera: Apidae).

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### Abstract

The nests of social Hymenoptera are particularly attractive to species engaging in resource robbing due to their concentration of resources. Here, the identity of heterospecific intruders in *Bombus impatiens* Cresson (Hymenoptera: Apidae) nests are described, with a particular focus on intrusion by invasive species – *Vespula germanica* (Fab.) (Hymenoptera: Vespidae) and *Anthidium oblongatum* (Illiger) (Hymenoptera: Megachilidae). While *V. germanica* is well known as a resource robber, this is the first time *Anthidium* spp. have been documented entering the nest of a social heterospecific. Of 16 *B. impatiens* colonies placed in a field in Lansing, MI, eight had heterospecific intruders, including *Apis mellifera* L. (Hymenoptera: Apidae), *A. oblongatum*, and *V. germanica*. Motivations behind entering a foreign nest are discussed.

**Keywords:** *Anthidium*, *Vespula*, *Apis mellifera*, resource robbing

The nests of social Hymenoptera contain highly rewarding resources, such as stored nectar and pollen, making them particularly appealing targets for resource robbers. Thieves can range from conspecifics to vertebrate intruders (e.g. bears, honey badgers, humans) (Schneider and Blyther 1988, Gulati and Kaushik 2004). Localized brood also make colonies an appealing protein source for carnivorous species (e.g. the aphytrophagous lycaenid caterpillar, *Cigaritis acamas* Klug (Sanetra and Fiedler 1995)). It is therefore not surprising that social Hymenoptera have evolved effective ways to defend against nest intruders, with coordinated defensive mechanisms (Evans and Schmidt 1990). Yet still, foreign interlopers find their way inside social Hymenopteran nests. Here, I focus on heterospecific Hymenopteran intrusion into the colonies of *Bombus impatiens* Cresson, the common eastern bumble bee.

Both foreign conspecifics and heterospecific Hymenoptera have been documented entering the nests of social bees. Worker drift between conspecific colonies has been well documented in social species such as *Bombus* and *Apis*. “Drifting” can be a reproductive strategy, where workers will enter a foreign conspecific nest to lay male offspring, but may also occur due to orientation error, particularly when nests are in close proximity (Smith and Loope 2016). Workers entering a foreign nest can also be done with the goal of resource robbing (stealing stored nectar and pollen). Conspecific resource robbing behav-

ior is common in *Apis mellifera* L., particularly when resources are scarce (Free 1977). It is also suspected that *A. mellifera* will rob *Bombus* spp. when in close proximity, and it is recommended that managed bumble bee colonies be placed away from honey bee colonies for this reason (pers. comm. Koppert Biologicals).

Nest intrusion is also a strategy used by obligate or facultative nest parasites. Nest parasitism occurs in order to co-opt conspecifics or heterospecifics for rearing of young. Obligate parasites, such as the cuckoo bumble bees (*Bombus*, formally *Psithyrus*), are unable to provision their own nests, and must kill or suppress a host queen to reproduce (Fisher 1988). Facultative parasitism among conspecifics has been documented in bumble bees as well. Here, a conspecific, non-founding queen will enter a foreign nest and usurp the founding queen, using the host workers to raise her offspring (Sakagami 1976). (See Weislo 1987 for a full list of known Hymenopteran nest parasites).

Outside of Anthophila, Vespids have been documented entering *Apis* spp. colonies and can cause significant damage (Edwards 1980, Clapperton et al. 1989, Akre and Mayer 1994, Ono et al. 1995). Many Vespids will prey on adult *Apis*, though they usually target individuals outside the hive. However, *Vespa mandarinia japonica* Radoszkowski (Hymenoptera: Vespidae) attack the colony itself with large scale coordinated attacks between nestmates. These attacks can

**Table 1. Heterospecifics found in *Bombus impatiens* colonies, in East Lansing, MI, USA.**

Hive number	Date first discovered	Species
1	5 Sept 2017	1 male <i>Athidium oblongatum</i>
5	29 Aug 2017	1 <i>Anthidium</i> sp. (not recovered during dissection)
9	5 Sept 2017	1 female <i>A. oblongatum</i> , 2 male <i>A. oblongatum</i> , 4 female <i>Apis mellifera</i>
10	12 Sept 2017	3 female <i>A. mellifera</i>
11	5 Sept 2017	1 female <i>Vespula germanica</i>
12	(During dissection)	1 female <i>A. mellifera</i>
13	29 Aug 2017	3 female <i>A. mellifera</i>
15	5 Sept 2017	1 male <i>A. oblongatum</i> , 2 female <i>A. mellifera</i>

decimate a colony, as each wasp is able to kill up to 40 *A. mellifera* per minute (Ono et al. 1995). *Vespula germanica* (Fab.) and *vulgaris* (L.) have also been documented causing significant damage to honey bees, as they will both rob honey stores and kill adult *A. mellifera* (Clapperton et al. 1989). Much less is known about Vespids targeting *Bombus*, though they have been documented showing aggressive behavior towards *Bombus* when competing at the same floral resources (Thomson 1989). In this study, heterospecific Hymenoptera found inside the nests of commercially reared *B. impatiens* colonies are identified.

### Materials and Methods

On 15 August 2017, 16 commercial *Bombus impatiens* colonies (Biobest U.S.A. Inc., Leamington, Ontario) were placed in an open field in Lansing, MI (Location: 42.691383 N, -84.496945 W). For another study, these colonies were being monitored for growth over a six-week period. Growth was determined through weekly, nighttime weighing. During weighing, colonies were visually checked to note any dead individuals, or emergence of reproductives. At this time, presence of heterospecifics inside the nests were also recorded. All *B. impatiens* colonies were frozen after six weeks (27 September 2017), and later dissected. During dissections, any heterospecifics found in the nests were collected and identified.

### Results

In the third week of *B. impatiens* colony placement (29 Aug 2017), a dead *Anthidium* was found inside one of the *B. impatiens* colonies (Table 1). In another colony, three *A. mellifera* workers were also found. In the following weeks, additional dead *Anthidium* and *A. mellifera* were found, as well as a Vespid wasp. Heterospecifics inside the colonies were always located along the outer edges of the hive box (plastic box located inside card-

board housing) away from comb and brood. In the fourth week, an *Anthidium* was also found outside the plastic hive box, between the hive box and the sugar water reservoir located below. Heterospecific specimens were not collected until colony dissections, so as to minimize disruption of the *B. impatiens* colonies (as colonies were being used for another study).

Five *Anthidium* spp. were recovered from the *B. impatiens* colonies during dissections. All five *Anthidium* were *A. oblongatum* (Illiger) (Hoebeke and Wheeler Jr. 1999, Miller et al. 2002, Romankova 2003, Gonzalez and Griswold 2013). Four males, and one female (Table 1). One *Anthidium* was not recovered. The one wasp was identified as *V. germanica* (Buck 2008).

### Discussion

Two Old World species of *Anthidium* have been introduced to the eastern United States. *Anthidium manicatum* L. was first discovered in upstate New York in the early 1960s (Severinghaus et al. 1981), but has since rapidly expanded its range across the continent (Miller et al. 2002, Gibbs and Sheffield 2009, Maier 2009, Strange et al. 2011, Graham and MacLean 2018). *A. oblongatum* was first discovered in eastern Pennsylvania in 1995, but is now found throughout the Northeast, and northern Midwest states (Hoebeke and Wheeler Jr. 1999, Miller et al. 2002, Maier 2009, O'Brien et al. 2012). The introduction of *A. manicatum* has been particularly noteworthy due to its aggressive behavior towards native bees while defending floral resources (Severinghaus et al. 1981, Starks and Reeve 1999). Within a defended floral territory, male *A. manicatum* discourage foraging by heterospecific pollinators through direct attacks that often result in severe injury or death to the encroaching pollinator (Wirtz et al. 1988). Comparatively, we know significantly less about *A. oblongatum*'s interactions with heterospecifics. This study finds a unique behavior for *A. oblonga-*

*tum*, heterospecific nest intrusion that has not been recorded in any other congeners.

For *Anthidium*, no instances of resource robbing have ever been recorded (to the best of my knowledge). Therefore, it is more likely that *A. oblongatum* are entering *B. impatiens* hives for alternative reasons. *A. oblongatum* is known to fly into August/September (Hoebeke and Wheeler Jr. 1999, Maier 2009), with this experiment marking the end of *A. oblongatum*'s activity. The nights around the first incidence of *A. oblongatum* discovery marked several unseasonably cold nights, where temperatures dropped below 4°C (measured by temperature probes inside the *B. impatiens* nests). These conditions continued into the following week prior to the other *A. oblongatum* discoveries. Therefore, it is possible that *A. oblongatum* were searching for a warm place to spend the night. They were then either killed by *B. impatiens* workers defending the nest, or died from natural causes.

However, resource robbing as an alternative explanation cannot be discarded. Floral resources were particularly scarce in the landscape during that time, with very few late summer flowering species found in close proximity. For *A. mellifera* and *V. germanica*, resource robbing is a more likely explanation for nest intrusion, as both of these species have previously shown robbing behavior (Free 1977, Edwards 1980). However, additional work would need to be done to confirm this.

While the underlying motivations behind entering a heterospecifics nest are still highly speculative, they are certainly interesting in light of the invasive status of *A. oblongatum* and *V. germanica*. If they are indeed resource robbing, it would indicate an additional negative impact on native bees. There is still a lot left unknown about heterospecific nest intrusion in managed *Bombus* colonies.

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I would like to thank Rufus Isaacs (Michigan State University) and Philip Starks (Tufts University) for their thoughtful discussion about these findings. Additionally, I would like to thank Thomas Wood (Michigan State University) for his identification of *Vespula germanica*. This project was funded in part by the USDA-NIFA (Award #: 2017-68004-26323).

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