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Parasite Hideouts: Preserving Native Hymenopterans Through Pollinator Hotel Management

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Abstract

As many insect species decline due to climate change, managing populations of native pollinator species is more important than ever. Pollinator hotels can provide a safe, protected space suited for many insects. However, these areas also attract parasites, including several species of parasitic wasps, which can multiply if hotels are not maintained. Pollinator hotels are usually filled with a variety of different materials, including cardboard, reeds, bamboo, wooden blocks, and native plant stems. This study sought to determine whether any materials used for pollinator hotels are more or less prone to parasitic infestation. We first conducted a survey to determine which species were present in the UNC Asheville pollinator hotel. Second, we collected samples of nests of different material types and reared them to observe any differences in parasite load. Third, we investigated overwintering boxes as a tool to facilitate cleaning and maintenance of pollinator hotels, which results in a reduced parasite burden. We observed that cardboard tubes were far more susceptible to parasite infection than other materials. Removal of these materials, coupled with regular maintenance, may help safeguard against parasitism. These results will provide guidance for selecting nest materials that safeguard pollinators and improve pollinator health in a wide variety of ecosystems.

Introduction

Pollination, and more specifically insect pollinators, are essential to healthy ecosystems and play a critical role in both crop production and the reproduction of many flowering plant species. In fact, nearly 70% of leading crop species experience increased production when pollinators are present (1). The European honeybee (*Apis mellifera*) often receives the most attention and recognition as a pollinator due to its wide distribution. However, *A. mellifera* is only one of over 20,000 species of bee documented worldwide (2). In contrast, native bees, such as bumble bees, mining bees, and mason bees, are both under-studied and under-appreciated, despite evidence that native pollinators are more effective than introduced bees at pollinating a number of flowering crop species (3-5). Native Hymenoptera exhibit a diverse array of life histories, ecological niches, and nesting habits. The majority (roughly 90%) of bee species are solitary (6) and build nests in soil (ground nesters) or dead wood, stems, or piles of brush (cavity nesters). While some native bees are generalists, willing to pollinate a wide array of flowering plant species, some are specialists, and will only collect nectar and pollen from a select few species (7). This intricate dependence on specific plant species and habitat structures makes native pollinators vulnerable to habitat loss and human disturbance.

One increasingly popular tool to help mitigate these problems for native pollinators is the pollinator hotel. While the majority of solitary bee species are ground nesters (approximately 70%), pollinator hotels can serve as habitat for cavity nesting species (8). These structures are more commonly called bee hotels, but they often host a variety of solitary wasp species as well as cavity-nesting bees (9). Mason bees (*Osmia* spp.) and leafcutter bees (*Megachile* spp.) are the two most common families reported utilizing pollinator hotels (9). Mason wasps (subfamily *Eumenidae*), grass carrying wasps (*Sphecidae* spp.), and square-headed wasps (*Crabronidae* spp.) are also frequently observed inhabiting the hotels (7). Aside from providing invaluable pollination services, these insects contribute to controlling pest insect populations. Pollinator hotels replicate these insects' natural nesting sites by offering access to hollow tubes, often made of bamboo, reeds, cardboard, drilled wooden blocks, or the stems of native plants. Providing the bees with a diverse array of materials may help to attract a wider range of bee species (10). However, each material type has its own unique attractants and drawbacks. Drilled blocks, for example, are known to be extremely attractive to both bee and wasp species but are difficult to maintain (11). Any old nests left in the blocks may contain diseases or parasites that are impossible to completely remove. Likewise, positioning the drilled holes too close to the edges of the block can make the nests susceptible to parasitism, especially by the *Leucospis affinis* wasp (12). Bamboo or reed tubes with too large a diameter may be either left completely unoccupied or preferentially host large non-native bees such as the giant resin bee (*Megachile sculpturalis*) (10). Cardboard tubes are commonly available in commercial nests, but very high rates of parasitism have been reported (13).

Because of these challenges, and especially the risk of parasitism, it is paramount that pollinator hotel installation and maintenance follow known best practices. There are data to support many best practices involved in installing pollinator hotels; for example, there is empirical evidence to support limiting the size of pollinator hotels to about 100 nest tunnels (14). The diameter and length of nest tunnels should also be tailored to the habits of bees known to nest in the area, as tunnels with narrow diameters may produce smaller offspring and a sex ratio skewed towards males (15-17). Successful pollinator hotels are also located close to sources of pollen and nectar, as many small solitary bee species have limited flight ranges (18). Some pollinator hotel maintenance practices are less well-supported. Emergence boxes are small, mostly enclosed containers in which nest materials can be placed to provide a transitional area where the bees can emerge. The old, used nesting materials can then be removed and cleaned, while the bees are free to make nests in fresh materials in the main hotel. These boxes often come with commercial pollinator hotel kits, but there is little to no empirical evidence that demonstrates whether the boxes are an effective tool for pollinator hotel maintenance. This lack of data matters because cleaning pollinator hotels is an essential maintenance task. Chalkbrood and parasitic mites can linger in old nests and spread throughout the pollinator hotel without regular cleaning (13). Other parasites, such as Houdini flies, beetle larvae, and parasitic wasps may also accumulate in pollinator hotels without regular maintenance.

Parasitic wasps are a common secondary inhabitant of pollinator hotels. Common parasites include *Monodontomerus* spp., *L. affinis*, and *Chrysididae* spp. *Monodontomerus* wasps are tiny parasites that lay their eggs in *Osmia* and *Megachile* nests (13). *Leucospis affinis* is also known to parasitize the larvae of *Osmia* and *Megachile*, along with some species of solitary wasps (19). Members of the *Chrysididae* family are also known to parasitize pollinator hotel residents. There is some indication that parasitism may be more common in pollinator hotels than in dispersed nesting sites, and that this increase may correlate with the higher density of nest sites that pollinator hotels encourage (20-22). The parasitism problem in pollinator hotels has also been shown to disproportionately affect the native bee residents of the hotel, rather than introduced bees or either native or introduced wasp species (23). Reducing parasitism in pollinator hotels would dramatically increase the efficiency of these hotels as both a tool for ecological conservation and education.

In this study, we investigated the parasite burden present in four different pollinator hotels and whether emergence boxes could be implemented as an effective maintenance and cleaning tool. We first conducted a biodiversity study of the pollinator hotel located on the campus of the University of North Carolina Asheville. Secondly, we surveyed the parasite burden across four pollinator hotels. We hypothesized that the parasite burden would be highest in cardboard nest tubes, as previously described in the literature, but that nests made of reeds would also have a higher parasite load than bamboo tubes because of the relative thinness of the reed tube walls. Lastly, we tested the efficacy of emergence boxes as a maintenance tool. We hypothesized that bees placed into the nest boxes would be able to hatch out and return to the hotel, rather than returning to nest inside the box itself. In half of the emergence boxes, we implemented a one-way valve that we hypothesized would further reduce the rate of bees re-entering the emergence boxes.

Materials and Methods

To assess and describe the extent of the parasite problem, three different studies were conducted on a total of four artificial Hymenoptera nest sites (pollinator hotels) between the summer of 2022 and the spring of 2024. The first study (Study 1), conducted in the summer of 2022, concerned only the pollinator hotel located on the University of North Carolina Asheville campus, and examined the family-level diversity present within the hotel.

The second (Study 2) and third (Study 3) studies were conducted from February 2023 to March 2024 on both the UNC Asheville hotel and three smaller pollinator hotels on the campus of North Carolina State University in Raleigh, North Carolina (Table 1). These three hotels were denoted Centennial, Court of the Carolinas, and Kilgore, respectively, based on the area of NC State Campus in which they were located. All four pollinator hotels were located in similar settings near areas of frequent travel, had all been established for several years prior to the study, and contained cardboard tubes, reeds, bamboo, and wooden blocks for the insects to nest in. The UNC Asheville pollinator hotel also utilized hollow sticks, drilled logs, and clay blocks as nesting materials, but these three material types were excluded from all but the biodiversity survey due to lack of observed nests. While the NC State pollinator hotels were all small and uniform in size, the UNC Asheville pollinator hotel is much larger.

Table 1. Local information and treatments present at each of the four pollinator hotels included in this study

Name of Hotel	Coordinates	Treatment. (Emergence Box)	Distance from Garden (m)	Notable Flowering Plant Species
UNCA	35.617N, 82.563W	Control Valve	1	*Solanum carolinense, Rudbeckia hirta, Lactuca spp., Choreopsis spp., Salvia lyrata, Helianthus angustifolius, Geranium spp., etc.*
Centennial	35.766N, 78.676W	Control Valve	2-3	*Callicarpa americana, Salvia spp., Helianthus angustifolius*
Court of Carolinas	35.786N, 78.666W	Control Half-valve	2-3	*Narcissus spp., Asteraceae spp.*
Kilgore	35.788N, 78.672W	Control Half-valve	1.5-2	*Cercis canadensis, Hibiscus spp., salvia spp., Asteraceae spp., Ipheion uniflorum*

Study 1: Biodiversity Survey

Site Characteristics:

The pollinator hotel located on the campus of the University of North Carolina Asheville was established in 2016. It is located in a semi-urban environment approximately 5-10 meters from areas of high foot traffic. The hotel is also between 1 and 5 meters from two separate pollinator gardens that contain a range of native flowering plant species, and it is backed by a small, wooded area (Table 1). The hotel had not been ideally maintained for several years before the date of this study. Before beginning collections, the pollinator hotel was divided into twelve equally sized boxes in three rows of four, numbered one through twelve from the top right to the bottom left (Figure 1). All samples gathered were kept separated by box number. Collected materials were transported to the Natural Enemy Management and Applications (NEMA) lab and stored for evaluation.

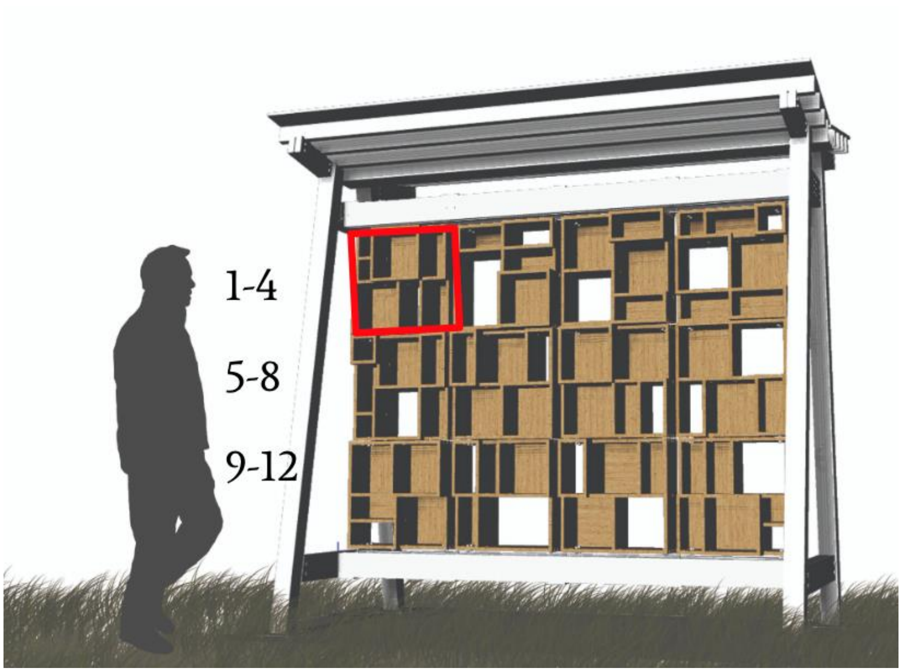

Figure 1. Model of the UNC Asheville pollinator hotel drawn to scale. This pollinator hotel was subdivided into twelve equally sized boxes for the purpose of these experiments. The first box is outlined in red.

Sample Collection and Analysis:

Adult insects were randomly collected from in and around the pollinator hotel using aspirators. These collections were done on three separate days in June, July, and August 2022. Adult insects from different sub-boxes within the hotel were collected in separate aspirators, euthanized separately using 95% ethanol vapor, and stored frozen in separate containers. All adult insects were thawed, pinned according to museum specifications, and identified to family using a microscope and the dichotomous key from Borror and Delong's *Introduction to the Study of Insects* (7th addition, 2004).

On a separate day in July, nesting materials were randomly collected from each of the twelve boxes within the hotel (Figure 1). This pollinator hotel utilized paper tubes, reeds, hollow sticks, bamboo, drilled logs, and clay blocks as nesting sites. Clay blocks were later excluded from the sample as there were no nest sites evident in any of the clay samples collected. The types of nest materials were not distributed equally across all twelve boxes, but a sample of every material present in each box was collected. In total, between five and twenty nest tubes were gathered from each pollinator hotel subsection and frozen. These nests were opened by using an Exacto knife to make a small, precise cut at the front end. Paper tubes were then unwound along the seam, and reeds and bamboo were carefully cracked open. All adults found within these nests were pinned and identified to family using the same dichotomous key. All nest materials were opened, and any cocoons discovered inside were characterized by material and color.

Data Analysis:

Heatmaps and proportions of parasites versus non parasites in each section of the pollinator hotel were created using raw count data in Google Sheets.

Study 2: Parasitic Contamination and Distribution

Site characteristics:

This study utilized both the UNC Asheville and NC State pollinator hotels, which were established in 2018 (Table 1). To ensure the data were not skewed by previous contamination with parasites, bundles of new, unused nesting materials were created using rubber bands. Each bundle contained one paper tube with a diameter of 8mm, one paper tube with a diameter of 6mm, one paper tube with a diameter of 4mm, one reed with a diameter of 8mm, one reed with a diameter of 6mm, and one stick of bamboo with diameters ranging from 10mm-6mm. The paper tubes and reeds were sourced from Crown Bees (Woodinville, WA). All materials had one open and one closed end and were between six and eight inches in length.

Sample Collection and Evaluation:

Four to six of the nest material bundles were randomly placed in each of the four pollinator hotels on the second weekend of the month in February, April, and June 2023. The bundles remained in the pollinator hotels for approximately two months and were collected for analysis on the second weekend of April or June, or the third weekend of August, respectively. All bundles were found and removed from the hotels at each collection period with the exception of the June collection from the Centennial pollinator hotel, where several bundles, along with a substantial portion of non-study materials, were removed from the hotel without authorization.

The samples were moved back to Asheville, North Carolina, and processed within a week of collection. They were kept separated by month, pollinator hotel, and material type (reed, paper tube, or bamboo). Processing involved carefully opening each individual nest site with an Exacto knife using the same procedure detailed in the biodiversity survey and analyzing the contents. Any already-hatched insects were euthanized with 95% ethanol and identified to the family using the dichotomous key found in Borror and Delong (7th addition). Any intact cocoons were placed into well plates whose lids had been punctured to facilitate airflow. The well plates were kept in an incubator initially set to 1 ^{o}C to simulate natural winter conditions and facilitate the insects' growth and development. At the end of February, the temperature was set to the average of the weekly temperatures of Raleigh and Asheville. This incubator also contained a dish of water to increase humidity.

The incubator was checked every other day for insect hatching. All insects that hatched within the well plates were removed and identified to family, using the dichotomous key noted above. The hatching dates, material type, month, and location of origin of the hatched insect were recorded. Some small parasitic wasps escaped from the well plates through the airflow holes. These insects were collected, euthanized, and identified to family. The date of their escape was also recorded. Due to their extremely small size, Eulophid wasps were especially prone to escape. To reduce the risk of cross-contamination with this parasite, any well containing Eulophidae wasps was immediately taped over.

Data Analysis:

All data were analyzed using the R Statistical Programming Language (version 4.5.1) and RStudio (version 2025.09.1+401) (24, 25). The following packages were also used: *car* (26), *readxl* (27), *tidyverse* (28), and *emmeans* (29). Raw parasite count numbers were evaluated for effects of family against month, location, and material type using generalized linear models with Poisson distribution, followed by an examination of the pairwise comparisons using *emmeans*. All test results were evaluated for significance at α = 0.05.

Study 3: Emergence Boxes

Site Characteristics:

This experiment used all four pollinator hotels to test the efficacy of overwintering boxes. In February of 2023, 80-85 filled nest materials were collected from each pollinator hotel. These nest materials contained all nest types and diameters and were taken from all different parts of the pollinator hotel. The materials were kept separated by pollinator hotel of origin through this process.

Rectangular overwintering boxes were constructed out of yellow pine (Figure 2). All panels were attached with a nail gun as well as screws and wood glue. A front panel was also attached with slight overhang on the left and right sides, and a hole was drilled in the center with diameter 0.5 inches (Figure 2). The back panel was cut but not attached until all materials were inside. All gaps except the front entry hole were filled in with a mixture of wood glue and sawdust. The tops of these boxes were covered with Vycor Pro Fully Adhered Butyl Flashing Tape (GCP Applied Technologies, Alpharetta, Georgia) to increase waterproofing and prevent rain incursion. A small piece of PVC conduit was attached to the outside of the entry hole with hot glue (Figure 2). The PVC conduit pieces were left empty on half the overwintering boxes, while those on the other half contained a one-way valve apparatus constructed from interlocking false eyelashes (Kiss USA, Port Washington, NY) and attached with hot glue (Figure 3).

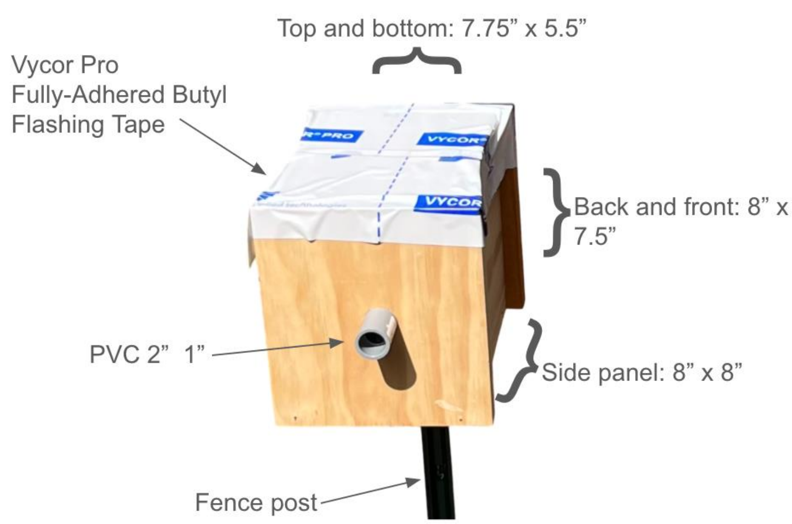

Figure 2. Schematic of overwintering boxes constructed from 1” thick boards of yellow pine.

Figure 3. PVC pipe (2” length by 1” diameter) one-way valve constructed from false eyelashes and hot glue.

Sample Collection and Analysis:

Once in the lab, the diameter, material type, number of holes (indicative of previous parasite infestation), and nest cap material of each nest tube were recorded. Nest caps were sorted into brown mud, fiber, grey mud, grass, leaf pulp, orange mud, resin, or yellow mud. We randomly assigned each nest tube to be either reared out, placed in a control box, or placed in a treatment box. Nests assigned to either the control or treatment box were bound together with rubber bands with a longer stick so that the open ends would not fall against the side of the box and prevent hatching. These nests were then sealed inside the overwintering boxes with screws and a piece of wood identical to the front section.

One control box and one treatment box were assigned to each pollinator hotel. Two of the treatment emergence boxes (located at Court of the Carolinas and Kilgore) lost their valves approximately halfway through the study period in June. These two emergence boxes have therefore been reclassified as belonging to a third treatment (Half-Valve). After this reclassification, there were four boxes belonging to the Control group, two boxes belonging to the Valve group, and two in the Half-Valve group. All nests were placed inside the overwintering box which corresponded to their pollinator hotel of origin. These overwintering boxes were then placed on metal fence posts approximately three to four feet off the ground and one to two meters away from each individual pollinator hotel and left in place until March 2024.

To assess the residents of these nest materials, a small portion of the samples were brought back to the NEMA lab on the UNC Asheville campus to be reared out. These nests were kept separated by the hotel of origin and opened within a week of collection, using the same methodology described in Study 1. Their contents were placed in well plates with holes in the lids for airflow. The well plates were kept in an incubator using the same temperature settings and humidity control as the parasitic contamination study (Study 2). Any insects that hatched under these conditions were removed from the incubator, euthanized with 95% ethanol vapor, and identified to family using the dichotomous key from Borror and Delong (7th edition). The hotel of origin of each hatched insect was also recorded.

In early March 2024, all overwintering boxes were removed and brought back to the UNC Asheville campus. They were opened within a week of removal, and the contents were analyzed. Any adult insects present within the box were recorded as well as box conditions (mold, debris, presence of spider webs, etc.). All data gathered at the beginning of the study (on nest cap material, holes present in the sides, etc.) were regathered to check for changes that would indicate parasitism or re-nesting. All nest materials within the boxes were then opened, and if any intact cocoons were present, they were removed and reared in well plates in an incubator (same conditions in Study 2).

Data analysis:

All data were analyzed using the R Statistical Programming Language (version 4.5.1) and RStudio (version 2025.09.1+401) (24, 25). The following packages were also used: *ggplot2* (30), *dplyr* (31), *tidyverse* (29), *ggsignif* (32), *scales* (33), *pscl* (34), *viridis* (35), *lme4* (36), *ggsankey* (37), *ggalluvial* (38), and *ggpubr* (39). Count data of parasitoid holes was evaluated against different predictors using a zero-inflated negative binomial model in the package lme4. Changes in cap material (which would indicate a re-nesting event) during the study were evaluated using a generalized linear model with a binomial distribution. Correlations between cap material and the species of hatching insects were done by hand.

Results

Study 1: Biodiversity Survey

The total number of insects observed in each of the twelve sub-boxes of the pollinator hotel ranged from 3 to 234 (Figure 4). However, all sub-boxes except Box 7 contained between 3 and 100 insects. The outlier in Box 7 (234 insects) was caused by a large nest of Formicidae found inside some of the nest materials (Figure 4). The total number of families found inhabiting each of the sub-boxes ranged from 3 to 8 (Figure 4). Box 7 also contained the joint highest number of families observed, along with Box 5. The sub-boxes with the greatest number of families (5, 7, 9, and 10) are all clustered around the left-most boxes of the second and third rows, which is where the morning sun falls on the hotels (Figure 4). In total, 10 different Hymenoptera families and over 600 adult insects were seen inhabiting the pollinator hotel during this study.

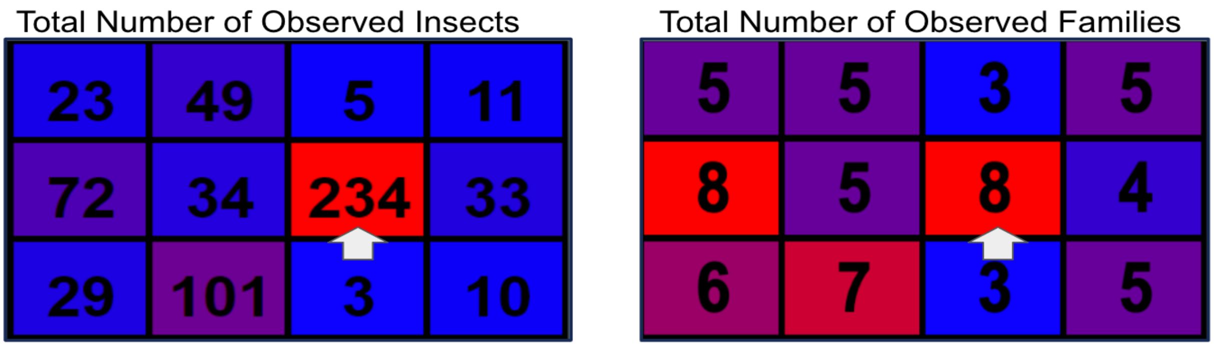

Figure 4. Heatmap of the total number of individual insects (left) and of the total number of insect families (right) observed in each of the twelve sub-boxes at the UNC Asheville pollinator hotel. The outlier nest of Formicidae in Box 7 is indicated by a white arrow on both maps.

There were more total insects observed in bamboo clusters than in logs, but sample size was too small to make further inferences about relative insect abundances within the other material groups (Figure 5). This survey found that parasitoid insects were widespread across all material types in the pollinator hotel, and in some instances (bamboo, logs, paper tubes, and sticks) (Figure 5).

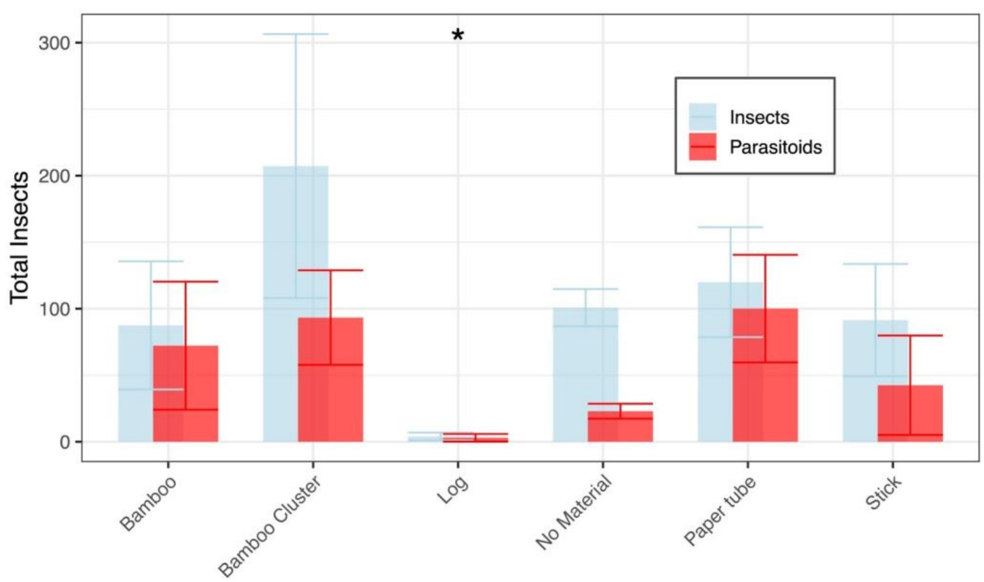

Figure 5. Mean ± standard deviation of the number of parasitoid (red) and non-parasitoid (blue) insects found in each of the five material types examined. Insects labeled as “no material” were collected while either perched on the outside of the nest materials in a particular box or flying from box to box.

Study 2: Parasitic Contamination and Distribution

Four families of parasitic insects were observed in our results: Ichnumonidae, Drosophilidae, Eulophidae, and Torymidae. Of these four, Ichnumonidae and Drosophilidae had only 2-3 occurrences and could not be statistically evaluated. Results are therefore focused on the families Eulophidae and Torymidae. Eulophid burden was significantly different across all three material types examined (Paper/bamboo: SE = 0.0299, df = Inf, z = -11.445, $p < 0.0001$. Paper/reed: SE = 1.2600, df = Inf, z = 25.783, $p < 0.0001$. Bamboo/reed: SE = 0.4770, df = Inf, z = 7.233, $p < 0.0001$) (Figure 6). Eulophid burden was highest in paper tubes, lowest in reeds, and intermediate in bamboo (Figure 6). Paper tubes had far, far higher levels of Eulophidae observed than the other two material types, and this trend was still observed when a large statistical outlier of over 1,500 individual Eulophid wasps (originating from the paper tubes taken from the UNC Asheville pollinator hotel in August) was removed. Torymid wasp burden was also highest in paper tubes (Bamboo/paper: SE = 0.1090, df = Inf, z = -3.210, p = 0.0038. Paper/reed: SE = 0.3980, df = Inf, z = 2.945, p = 0.0091) (Figure 6). However, there was no significant difference observed between Torymid wasp burden in bamboo versus reeds (SE = 0.2610, df = Inf, z = -0.283, p = 0.9569) (Figure 6).

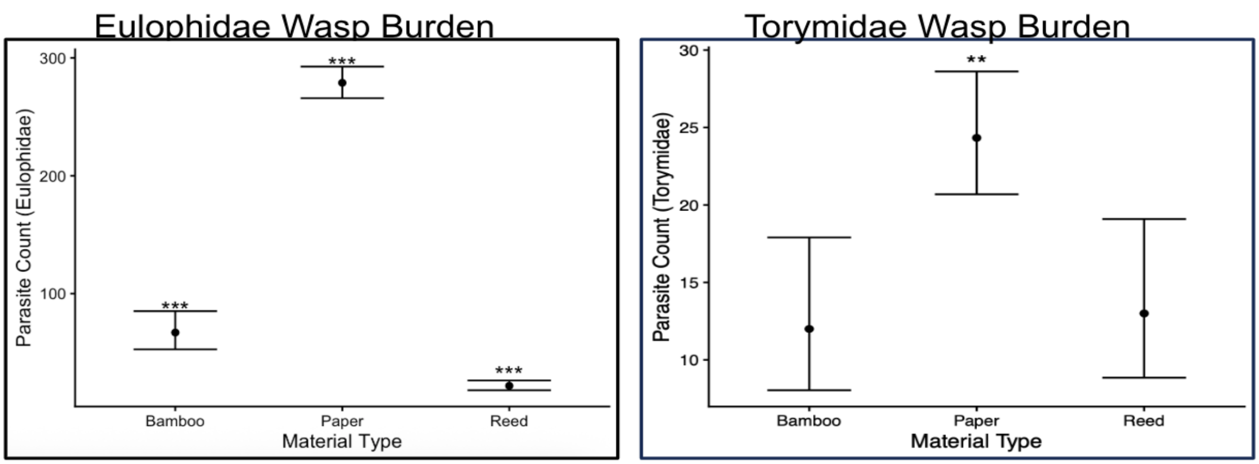

Figure 6. Mean and 95% confidence interval plot of Eulophid wasp burden (left) and Torymid wasp burden (right) across all three material types.

The amount of Eulophid and Torymid wasps also varied widely by their hotel of origin. Eulophid wasps appeared in far greater numbers at the UNC Asheville pollinator hotel than any of the other three hotels (SE = 0.00364, df = Inf, z = -28.362, $p < 0.0001$) (Figure 7). Eulophid wasps were also significantly more prevalent at the Centennial and Kilgore hotels than at the Court of the Carolinas hotel (Centennial: SE = 6.47000, df = Inf, z = 4.917, $p < 0.0001$. Kilgore: SE = 0.02300, df = Inf, z = -6.029, $p < 0.001$), and more prevalent at the Kilgore hotel than the Centennial hotel (SE = 0.09920, df = Inf, z = -3.279, p = 0.0057) (Figure 7). These trends held even when the Eulophid wasp outlier (UNCA, paper tubes, August) was removed. Torymid wasps displayed nearly the exact opposite pattern. Torymid wasps were significantly less prevalent at the UNC Asheville hotel than any of the other hotels (Centennial: SE = 8.64000, df = Inf, z = 8.827, p < 0.000. Court of the Carolinas: SE = 5.90000, df = Inf, z = 8.192. Kilgore: SE = 1.63000, df = Inf, z = 3.396, p = 0.0038). The Kilgore hotel had the second lowest levels of Torymidae present. The Torymid burden at the Kilgore hotel was significantly lower than either the Centennial or Court of the Carolinas hotels (Centennial: SE = 1.63000, df = Inf, z = 6.583, p<0.0001. Court of the Carolinas: SE = 1.08000, df = Inf, z = 5.717, p<0.0001). Torymid burdens were not significantly different between the Centennial and Court of the Carolinas hotels (SE = 0.23200, df= Inf, z = 2.077, p = 0.1606).

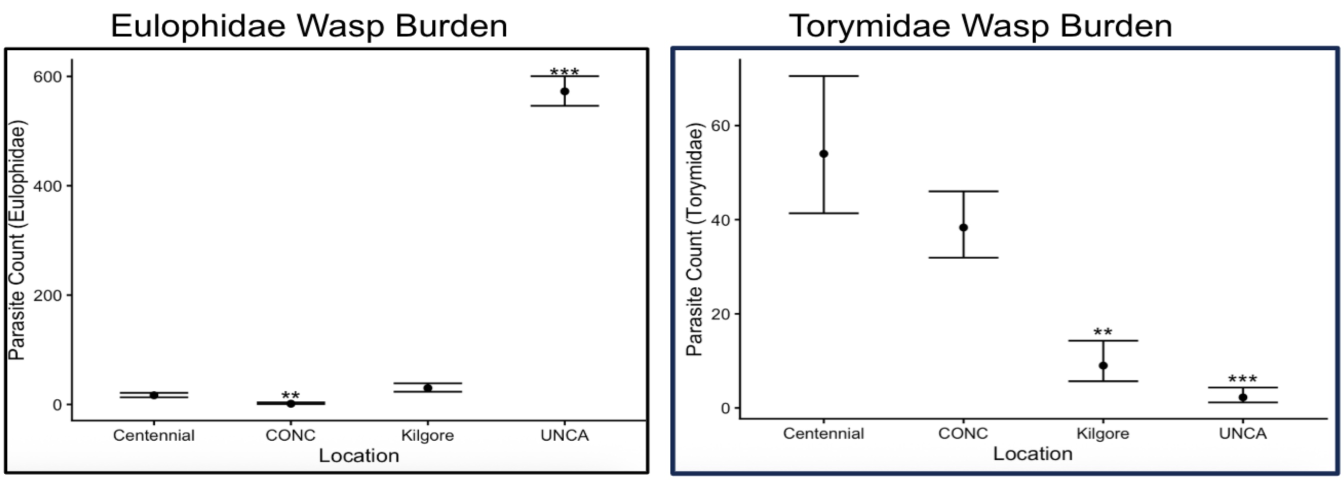

Figure 7. Mean and confidence interval plot of Eulophid wasp burden (left) and Torymid wasp burden (right) at all four pollinator hotels studied.

Study 3: Emergence Boxes

There were no significant differences in the amount of parasitoid holes present at the start of the study with the initial three groups (Control, Valve, and Rearing) (Estimate = 0.08375, Std. Error = 0.17759, z = 9.425, p = 0.6372). However, after the separation of the Half-Valve group, Half-Valve emergence boxes had significantly more parasitoid holes at the beginning of the study (Estimate = 0.33103, Std. Error = 0.16423, z = 2.016, p = 0.0438) (Figure 8). By chance, the two boxes that lost the valves midway through the study had nest tubes inside with more parasitoid holes.

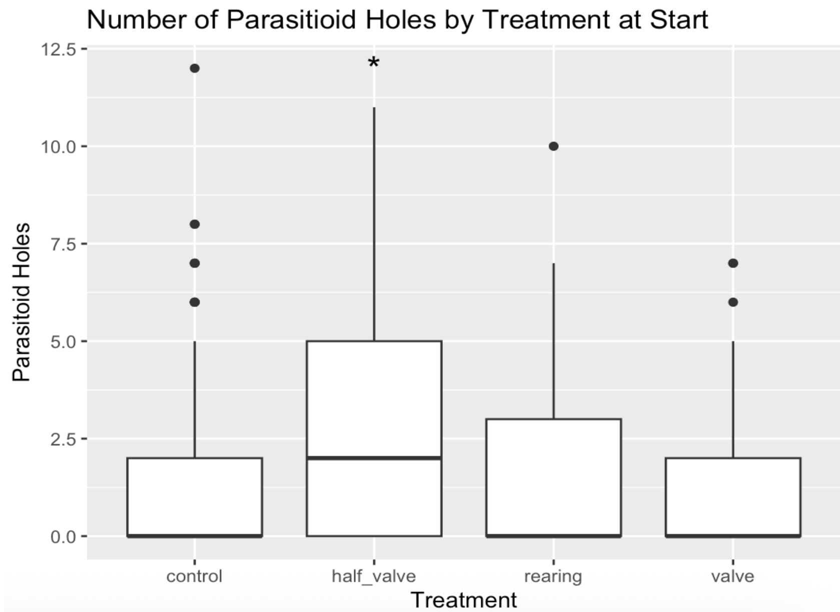

Figure 8. Nest materials in the half valve treatment group had significantly more parasitoid holes than the other three treatments.

There were no significant differences between the treatment groups in the number of additional parasitoid holes observed at the end of the study (Estimate = -0.2464, Std. Error = 0.6417, z = -0.465, p = 0.6661) (Figure 9). Extremely low levels of additional parasitoid holes were observed after a year in the emergence boxes across all three emergence box treatments, with the vast majority of nest materials gaining no additional parasitoid holes (Figure 9).

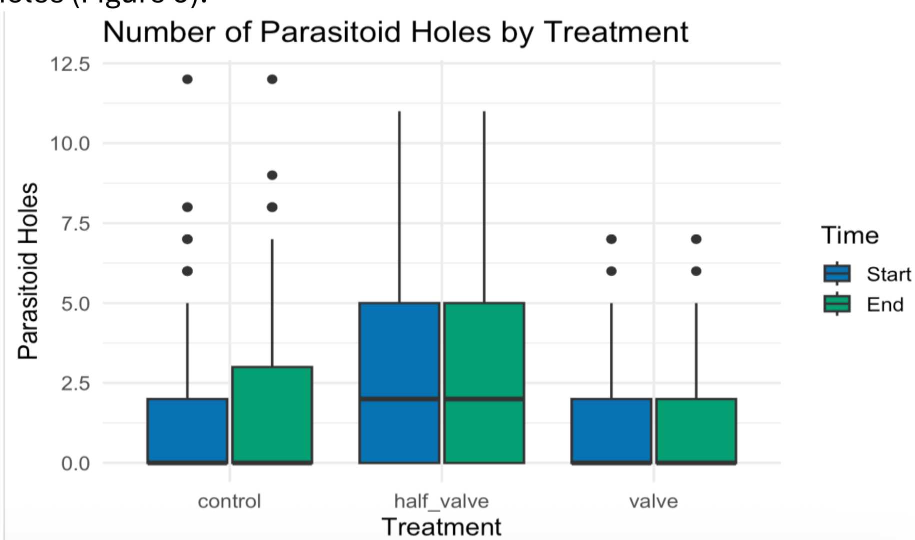

Figure 9. Box and whisker plot of the number of parasitoid holes at the start (blue) and end (green) of the experiment across all three experimental treatments.

The proportion of nest materials with evidence of re-nesting was low across all three treatments (Figure 10). Out of 261 total observations, there were 41 instances of either cap material change or transition from a hole in the cap to no hole in the cap of the nest that indicated re-nesting behavior had taken place. While the Control and Valve treatments both had a re-nesting proportion of approximately 0.13, the Half-Valve treatment had significantly lower levels (Estimate = -1.5563, Std. Error = 0.7625, z = -2.041, p = 0.0413) (Figure 10). The most common nest cap transition observed was between different types of mud caps (and within this type, the largest category was a shift from brown mud to orange mud), but every cap material was observed going through at least one transition (Figure 11).

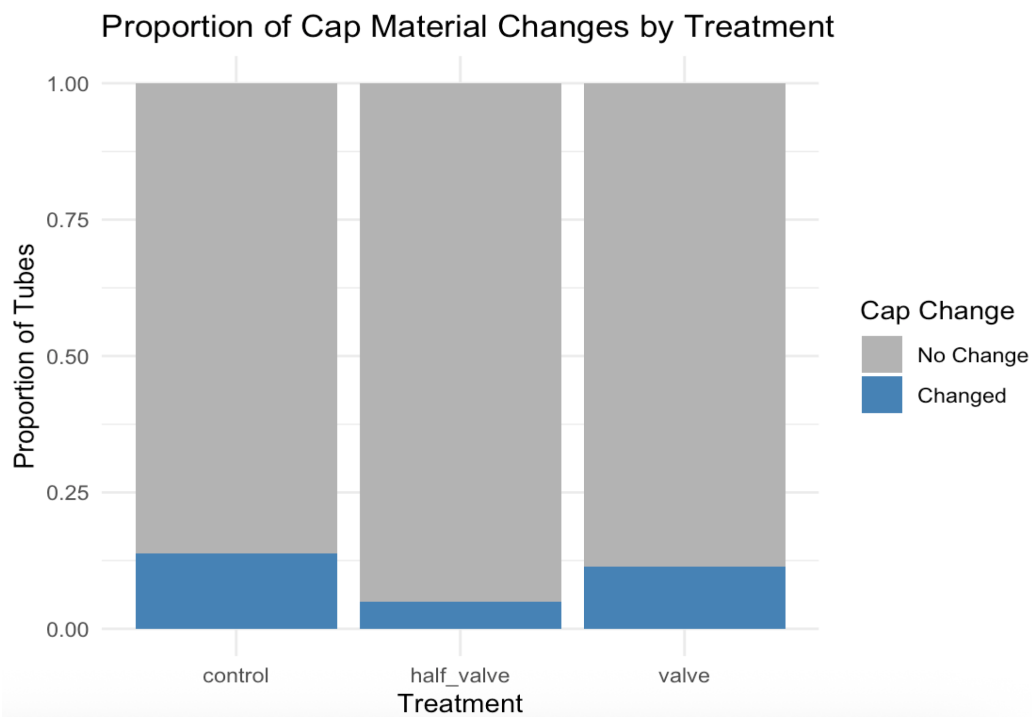

Figure 10. Proportion of nest materials that experienced a change in cap material or a change in the presence of a hole in the nest cap indicative of re-nesting over the course of a year in the emergence boxes.

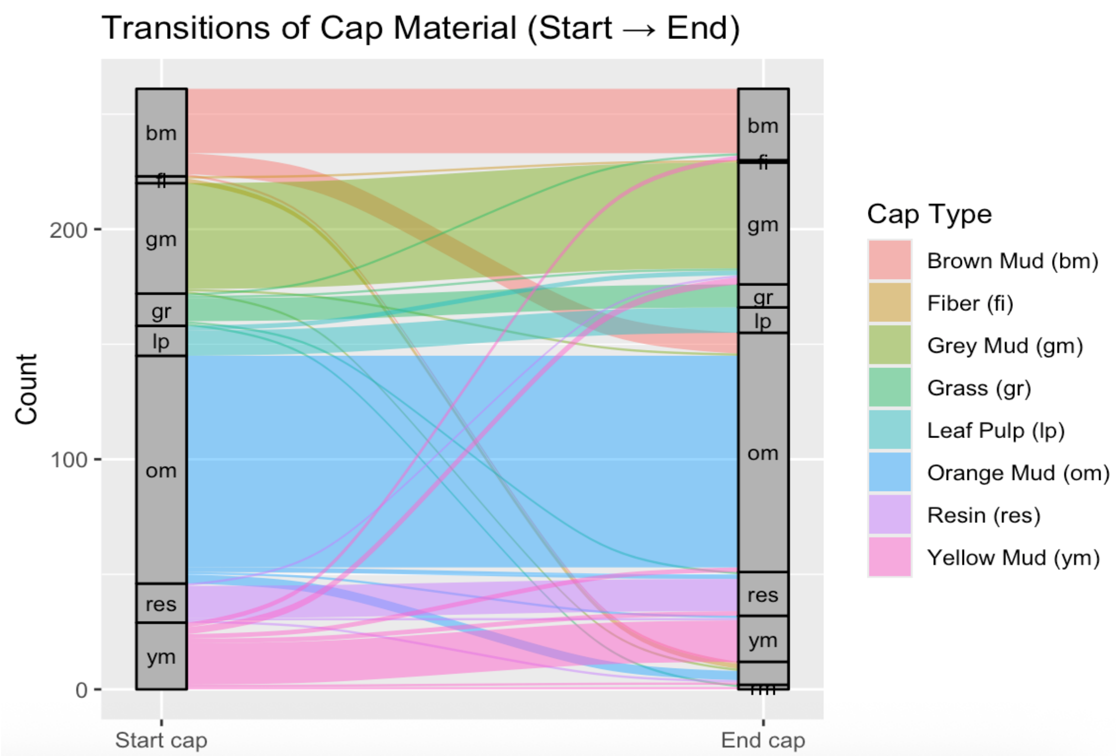

Figure 11. Sankey diagram of cap material changes from the start to the end of the emergence box study.

Discussion

The results of the biodiversity survey (Study 1) show that the UNC Asheville pollinator hotel is home to a wide variety of bee and wasp species, but that that diversity is unevenly distributed across the hotel. The highest numbers of both observed insects and observed families were concentrated in sub-boxes 5, 7, 9, and 10. These sub-boxes are all located in the middle to bottom left of the hotel, while the upper row and right side had both consistently fewer insects and less diversity across families. Due to the south-eastern orientation of the pollinator hotel, the upper row of the hotel (sub-boxes 1-4) is shaded by the roof for several hours after the bottom rows have begun to receive sun. This may help to explain the lower densities of insects and lesser diversity found in the upper boxes. Orienting pollinator hotels towards the southeast is standard practice in North America and Europe, and nests with exposure to the morning sun are thought to be more attractive to bees (12). However, the left and right side of the pollinator hotel receive sunlight at approximately the same time in the mornings and evenings, so orientation cannot explain all of the trends evident in the data. Bees have previously been shown to also prefer nests that are sheltered from prevailing winds (40). Boxes in the left-most corner of the pollinator hotel may offer some protection from the wind, or there may have simply been a greater concentration of suitable nest materials in those sub-boxes than in the ones on the right side.

These results also indicate that parasites are indeed a problem in the pollinator hotel. Several of the material types surveyed, including paper tubes and bamboo, had nearly the same number of parasitoid insects as non-parasitoid insects found inside. Previous research has found that paper tubes are particularly vulnerable to parasitoids, especially the parasitic wasp *Monodontomerus obscurus* (13). Beyond parasitic wasps, birds and rodents have also been observed dismantling bee nests housed in paper tubes, consuming the larvae, and using the paper itself for their own nests (13). Bamboo and reeds have previously been considered safer for bees, and the thicker walls of these materials can offer some protection from other common parasites like *Leucospis affinis* (12). However, the high presence of parasitoids in these materials in the biodiversity survey indicates an underlying problem. Before the start of this research in 2022, the UNC Asheville pollinator hotel had not been properly maintained or cleaned since its construction in 2016. Regular maintenance is necessary to prevent the build up of parasites and diseases in pollinator hotels, and the absence of this maintenance likely explains the high volume of parasites observed. These results, while striking, likely over estimate the number of parasitoid insects present in the pollinator hotel, as only adult insects were included in the data set. Unparasitized cocoons containing healthy larvae were therefore likely observed but not counted.

The four families of parasites represented in the parasitic contamination and distribution study (Study 2) were somewhat different from the parasite families documented in the first study. Ichnumonid wasps were not documented in the first study at all, while significant levels of *L. affinis* wasps and members of the Chrysididae family were observed that were not then documented in the second study. Leucospid and Chrysidid wasps were observed again in the emergence box study (Study 3), though in low numbers (one individual each). The discrepancies in the identified families between the three studies could be due in part to the sampling method. The second study used only nests made in new, undamaged nest materials that had only been in the pollinator hotel for two months. It is possible that members of the Leucospid and Chrysidid families are more prone to parasitizing damaged materials, or that the positioning of the new tubes in bundles made it more difficult for the wasps to penetrate them. Leucospid wasps, specifically, rely on ovipositors to penetrate up to ¾ of an inch into the outside of a nest to reach the bee's cocoon (12). The materials located towards the inside of our material bundles would likely have been inaccessible to the wasps, which may have influenced the types of parasites seen in the study.

The patterns of parasite burden observed across the Eulophid and Torymid species were more expected. The far higher abundance of parasites observed in paper tubes as compared to bamboo and reeds fits both with our hypotheses and with previous research that paper tubes are more susceptible to parasitism (13). Eulophid wasps were also more common in bamboo nests than reed ones. The susceptibility of bamboo to parasitoids in pollinator hotels is much less well studied than the susceptibility of paper tubes. Further, we are prevented from making determinations about the reasons behind the greater burden of Eulophid wasps in bamboo tubes by the obscure nature of the wasp itself. The species of wasp observed at the pollinator hotel was identified as belonging to the family Eulophidae, genus Melittobia, by the Plant Disease and Insect Clinic (NC State University).

However, the species was unable to be determined, and the life history and host(s) of this wasp are unknown.

The patterns of parasite burden across pollinator hotel locations were less expected. Torymid and Eulophid parasites displayed nearly opposite trends across the four pollinator hotels surveyed. Levels of Eulophidae parasites were so much lower in the three NC State hotels than the UNC Asheville hotel that it may be incubator cross-contamination, rather than an actual representation of Eulophid parasites from bee nests at NC State. The Eulophid parasites were so small that they could easily fit through the air holes in the lids of the well-plates, and though any escaped wasps were captured within 24 hours, cross-contamination at low levels may have occurred. Torymid wasps, more specifically the wasp *Monodontomerus obscurus*, which occurred in significantly greater numbers in all three NC State pollinator hotels, are known parasitoids of *Osmia* and *Megachile* bees (13). These genera were found at all four pollinator hotel locations. At the UNC Asheville hotel, Eulophid wasps may have outcompeted the Torymid wasps for hosts and resulted in reduced populations. Varying population levels of suitable hosts may also be the distinguishing factor between the differing burden of Torymid wasp parasites among the three NC State hotels.

Identification of insects from the parasitoid study was only conducted at the family level, but if genus or species-level identifications were made, the three hotels would likely have differing population levels of susceptible bees. The populations of the bees themselves were likely affected by the differing environments of the hotels themselves; while all three NC State pollinator hotels were roughly the same size and contained a similar spread of material types and diameters, the environments and flowers provided close to the hotel differed widely among the three locations (Table 1). Overall, the results of the second study suggest that both environmental differences among hotel sites and competition among parasitoid species may have jointly influenced the observed variation in parasitoid prevalence across pollinator hotels.

One of the primary concerns with emergence boxes as a tool to reduce parasite burden was the risk of confining healthy, unparasitized nest cells in the same small space as parasitized cocoons. If the parasitoids were able to hatch before most of the bees, the box itself might serve as an accelerator of parasitism, since it increases population density even more than in the hotels itself. The increased population density of pollinator hotels has been previously linked to increased risk of parasitism (20). However, small numbers of new parasitoid holes were observed across all three treatments at the end of the emergence box study, which indicates that parasitoid wasps cannot hatch and parasitize bee cocoons before the bees themselves are able to leave the boxes. While some nest tubes did increase parasitoid holes over the study, the data were heavily skewed towards zero levels, indicating that additional parasitism inside the emergence boxes was uncommon. This bodes well for the success of emergence boxes as a maintenance tool.

The other main concern with emergence boxes is the potential for bees to re-nest inside them. While an emergence box is in use, the rest of the materials in the hotel can be cleaned and replaced as needed to reduce the buildup of parasitoid wasps, mites, and fungal pathogens (7). Cavity nesting bees seek protected cavities to nest in, and, if the bee has just hatched out of an emergence box, it may decide to return to the box to search for a place to make its new nest. In the third study, while the proportion of bees that re-nested in the emergence box was not zero, it was low (at or below 12.5%) across all three treatments. This indicates that the great majority of bees that hatch out of the emergence boxes found another area (presumably the cleaned and refreshed pollinator hotel nearby) to make their nests in. The vast majority of these re-nesting events consisted of transitions between different types of mud caps, rather than transitions from resin, leaf pulp, or grass. This indicates that mason bees, rather than leafcutter bees, resin bees, or grass wasps return to nest in the emergence boxes most frequently. However, because every type of material underwent at least one re-nesting event, no definitive conclusions can be drawn about which families of bees are most likely to re-nest in an emergence box. Future studies should focus on this re-nesting phenomenon and whether or not particular families, genera, or species of bees are more likely than others to return to nest in the emergence boxes. Given these data, the risk of installing an emergence box could be evaluated on a case by case basis, based on the composition of that hotel's bee population.

Surprisingly, the attachment of a one-way valve apparatus to the entrance of the hotel resulted in no obvious reduction in the proportion of bees that re-nested in the hotel. The treatment that showed the lowest rate of re-nesting was the half-valve treatment. While this could be due to an initial preventative effect from the one-way valve, the lack of any difference between the re-nesting rates of the control and valve treatment groups suggests other factors may be in play. The half-valve group also had significantly more parasitoid holes at the beginning of the study than the other two groups, indicating that these boxes contained more parasitized nests than the other treatments. Parasitoids are highly lethal to bees inside affected nests, and so the half-valve treatment group may have had a higher proportion of nests at the onset that were not viable. Fewer viable nests may have led to fewer bees hatching from these boxes, and therefore fewer bees returned to re-nest in the emergence boxes.

Taken together, the results of these three studies highlight both the ecological complexity and management challenges of artificial nesting habitats for solitary bees. Patterns of biodiversity and parasitism at the UNC Asheville pollinator hotel reveal how environmental orientation, maintenance history, and material choice shape community composition and parasite load. The differences in parasitoid prevalence and family across the four hotel locations underscore the strong influence of site-specific factors and potential interactions among parasitoid species. Finally, the emergence box experiments demonstrate that these tools can effectively allow for maintenance and parasite reduction without significantly increasing parasitism risk or encouraging extensive re-nesting within the boxes themselves. While certain behaviors, such as limited re-nesting, merit further study, the overall findings suggest that careful placement, regular cleaning, and the strategic use of emergence boxes can enhance the long-term health and sustainability of managed pollinator populations.

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