Documenting Activity Levels of Discovered Seasonal Native *Bombus* Nests

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ABSTRACT

Bumblebees are ecologically and economically important pollinators in North America. Many species have experienced decline over the past few decades, making them a popular subject of conservation efforts. Yet , there is still a large knowledge gap surrounding their wild nesting ecology, primarily due to the difficulty of locating nests in the field. This study aimed to fill that gap by documenting nest activity of discovered bumblebee nests throughout the colony's duration. Each nest was observed for approximately six hours each week, distributed from 6:00 a.m. to 8:00 p.m. Nest traffic, bees entering or exiting the nest, was documented. Nest traffic over the course of the day was analyzed. We found that nest activity was highly variable across the four nests. Activity of three out of the four nests showed no obvious within-day activity structure. One nest exhibited a notable decrease in activity in the middle of the day, and an increase in the afternoon/evening. Our results are in contrast to other literature on bumblebee activity, where bees were observed to not only have a clear diurnal pattern, but a clear peak in activity towards the middle of the day - however, most studies use lab-reared colonies as opposed to wild nests. More research should be conducted on wild bumblebee nesting ecology before any conclusions are reached, but factors like colony size (where smaller colonies, particularly those in subalpine environments, have more variable activity), UV index, or nest architecture could all contribute to a wild colony's activity over the day.

INTRODUCTION

Bumble bees are important native pollinators throughout North America. Their ecological and economic importance makes bumble bees a priority for conservation (Velthuis & Van Doorn, 2006), especially considering so many native species are threatened (Goulson et al., 2008). Bumble bees have a unique life cycle, in which colonies only last a single season. Nests are established by queens that have emerged from hibernation from the previous season. These nests usually use pre-existing cavities, such as underground rodent burrows or holes in trees. The queens then create a female worker caste to assist with foraging and nest-keeping. As more food is foraged, the colony grows in size (more workers). Eventually, when the colony is of sufficient size, the queen will start producing reproductives: males, who leave the nest in search of queens to mate with, and gynes (potential future queens) who live at the nest for longer to gain mass before hibernation. At the end of the summer season, gynes will leave and mate with males from other colonies, and then hibernate until the following spring, when they will emerge and attempt to establish their own colonies (Cardé & Resh, 2012).

Properly understanding each portion of the bumblebee life cycle is crucial for conservation purposes, particularly the more sensitive parts like colony establishment. Unfortunately, bumble bee nests are notoriously difficult to find, and bumble bee nest survey studies usually rely on reported sightings in human areas (Boone et al., 2022), or dedicating large amounts of time and manpower to locate and observe nests (Pugesek & Crone, 2021). As a result, nests and their activity are severely understudied (Liczner & Colla, 2019), particularly wild colonies in natural areas.

Although many studies use foraging as a method for surveying bumble bee activity, this is fundamentally different from examining activity at the level of the colony. Colonies of the same species of bumble bee may forage on different flowers or in different areas, have different activity levels throughout the day, or collect pollen and nectar at different times or different rates. These potential differences between colonies are difficult to discern if the researcher is studying at the level of the greater population or species.

In this study, traffic of wild bumble bee nests was observed from the date of nest discovery until nest senescence. Specifically, we asked: How does bumble bee nest traffic change over time and compare to other nests? We hypothesized that, if traffic is correlated with time of day, then bumble bees will show peak activity levels in early afternoon, when temperature is ideal and floral resources are most abundant, as well as decreased levels in the morning and evening.

METHODOLOGIES

FIELD METHODS

Four bumble bee nests were discovered over the course of the season: One *Bombus bifarius* nest at our Gothic Spires site (near the 401-trailhead parking lot), and three *Bombus* *appositus* nests in the Research Meadow. No specific methods were used to discover the nests, rather researchers found nests haphazardly while completing work for other projects.

The *B. bifarius* nest was placed at the very edge of the aspen tree line next to a subalpine meadow, while the three *B. appositus* nests were all directly in the subalpine meadow. Upon post-senescence excavation, all nests were discovered to be utilizing abandoned rodent burrows, indicated by either the structure of the nest or the presence of rodent duff. Notably, one *B. appositus* nest, although utilizing an abandoned rodent burrow, was located in a tussock. The others were all at the end of pre-dug tunnels descending into the topsoil.

Each nest received approximately six hours of observation each week, spread throughout the day. Because some nests were discovered later in the season than others, not all nests received equal observations throughout the season.

Each observation session was conducted for half an hour, and was contained within an hour block (ex. 10:05 a.m. – 10:35 a.m., not 9:53 a.m. – 10:23 a.m.). These sessions usually took place between 9:00 a.m. – 6:00 p.m., although occasionally as early as 6:00 a.m. or as late as 8:00 p.m., in order to capture the full scope of daily activity. During these observation sessions, observers sat at least a meter away from the nest entrance, to preserve the microhabitat and not obscure the nest from arriving bees.

The following variables were recorded: time of activity, caste of individual, direction of activity (in or out of the nest), pollen load, pollen color, pollen load size, temperature, and cloud cover.

Pollen load was defined as the existence of visible pollen grains on the back of the rearmost legs, within the pollen basket of females (except *B. insularis* females). Pollen color was defined categorically, typically ranging from white to bright orange. Pollen load size was defined as either large or small. Temperature was measured every hour prior to starting observations using a Kestrel, and taken at both the ground level of the nest as well as approximately five feet into the air, in order to capture the temperatures bees would experience leaving the nest as well as flying above the nest. Cloud cover was calculated using the judgment of the observer from where they were positioned on the ground.

STATISTICAL ANALYSIS

Activity data were sorted into chunks according to the hour of observation (ex. Data entries taken at 12:05, 12:18, and 12:23 were all sorted into hour block 12). Activity data were then transformed into proportion data to account for fluctuating nest size over the course of the season, with each hour chunk describing the proportion of entrances and exits in that hour block out of the entire day. Hourly data was then sorted into one of three bins: morning, mid-day, and afternoon. For each nest, hourly observations were used to create dot plots for visual purposes, while the binned data were used to make box plots for official analysis.

For official analysis, we used a binomial generalized linear model on each nest to compare the proportion of entrances to the time-of-day bin, weighted by the total number of entrances in an hour chunk. Then, we used an ANOVA on the generalized linear model to obtain a final p-value and chi-square value for each nest. Lastly, for post-hoc analysis, we compared pairs of boxplots together to determine which time bin, if any, differed most from the others for each nest, using the emmeans package in RStudio.

RESULTS

Nest activity varied throughout the day, for all four nests. There was no significant effect of time of day on nest traffic for three out of the four nests. Activity of the sole *B. bifarius* nest ($\chi^2 = 2.9101$, $p = 0.2334$) did not have an observable trend in the dot plot, and although the boxplot appeared to reveal a slight decrease in activity from mid-day to afternoon which was confirmed by the post-hoc pairwise analysis, such a decrease was not significant. The first two *B. appositus* nests ($\chi^2 = 1.0296$, $p = 0.5976$; $\chi^2 = 0.97933$, $p = 0.6128$) also lacked an observable dot plot trend, with points within the same day jumping and dropping rapidly in activity levels every hour, or remaining at similar levels for long stretches of time. Post-hoc analysis did not reveal if any time chunk was even slightly different from the others. The last *B. appositus* nest ($\chi^2 = 8.7299$, $p = .01272$) showed that time of day had a significant effect on nest traffic, with mid-day having significantly less nest entrances than the morning, and notably less than the afternoon.

DISCUSSION

Contrary to our expectations, the activity of most nests were highly variable. Nest activity was not significantly impacted by time of day for the other three out of four nests. It is possible that the one *appositus* nest that was significantly impacted by time of day was confounded by low sample size, since it had the least total observations out of all the nests. These results are in contrast with other studies on nest traffic. Traffic is usually observed to follow a clear diurnal pattern, with activity levels increasing throughout the morning, peaking in midday, and slowly declining into the evening. No activity is observed at nighttime (Shelly et al., 1991; Stelzer & Chittka, 2010). We did not observe any such pattern during our study. Certain nests seemed to experience an increase in activity from 9:00 a.m. to 11:00 a.m., but others saw a decrease in activity.

It is important to note that other studies on nesting activity typically use *Bombus* species that have large population numbers relative to subalpine bumble bees in Colorado (e.g., *B. bifarius* and *B. appositus*). In (Stelzer & Chittka, 2010), colonies with smaller amounts of consistently foraging workers had a less-obvious diurnal rhythm, and did not always see a clear peak in activity during the day. It is possible that colony size directly affects nest traffic, where workers in smaller colonies may have to divide more of their time between foraging and rearing, as opposed to larger colonies.

It is also crucial to note that other studies on nesting activity tend to use lab-reared colonies, as opposed to wild colonies. This does allow for easier monitoring of activity and even monitoring of individual bees (Stelzer & Chittka, 2010), but prevents proper examination of nesting ecology. It is possible that wild colonies have more variable activity rates over the day, as opposed to lab-reared colonies.

Although abiotic variables like temperature and cloud cover were recorded, they were not examined for statistical analysis at this point in time. Comparing the traffic of nests under sunny, warm conditions to nest traffic under cooler, cloudy conditions could also potentially provide an explanation for the lack of pattern we observed. The Colorado region, particularly in high montane areas, experiences variable weather during the summer season due to monsoons. These monsoons involve shifts in temperature, cloud cover, and potentially UV index (Calbó et al., 2005). The bumblebee circadian rhythm responds strongly to UV levels (Chittka et al., 2013), which posits comparing the nest traffic of these wild species to weather conditions, particularly UV index, as a worthwhile exploration.

Lastly, microhabitat and other physical nest characteristics should be considered when considering nest traffic, particularly because nesting habitat is so variable for bumblebees (Antoine & Forrest, 2021). Importantly, the one *B. appositus* nest for which time of day did have an effect on traffic was located inside the grass tussock. Although the main chamber was insulated with rodent duff, the chamber was noticeably closer to the surface than the other nests. It is possible that, with its unique architecture, this nest was more susceptible to the increase in temperature throughout the day. However, it is impossible to be sure without knowing the average temperature inside all of the nests.

In a study of four subalpine bumblebee nests, we observed high variability in daytime activity levels. Given the general absence of information on the nesting ecology of bumblebees for many species, especially in the Rocky Mountains, this work provides useful natural history information on an important group of taxa.

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FIGURES

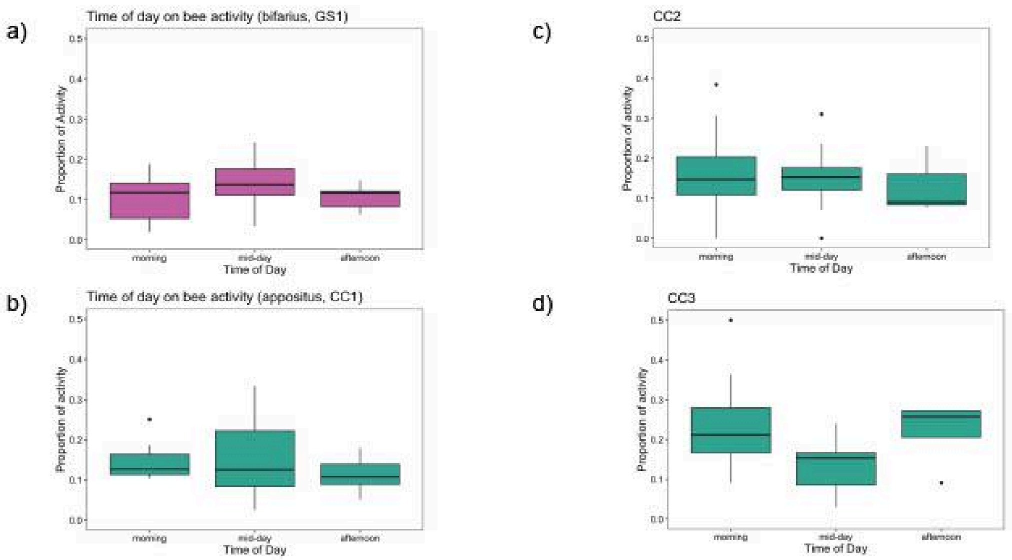

Fig 1. Boxplots of time of day (binned) on proportion of entrance activity for: a) *B. bifarius* nest GS1, b) *B. appositus* nest CC1, c) *B. appositus* nest CC2, d) *B. appositus* nest CC3

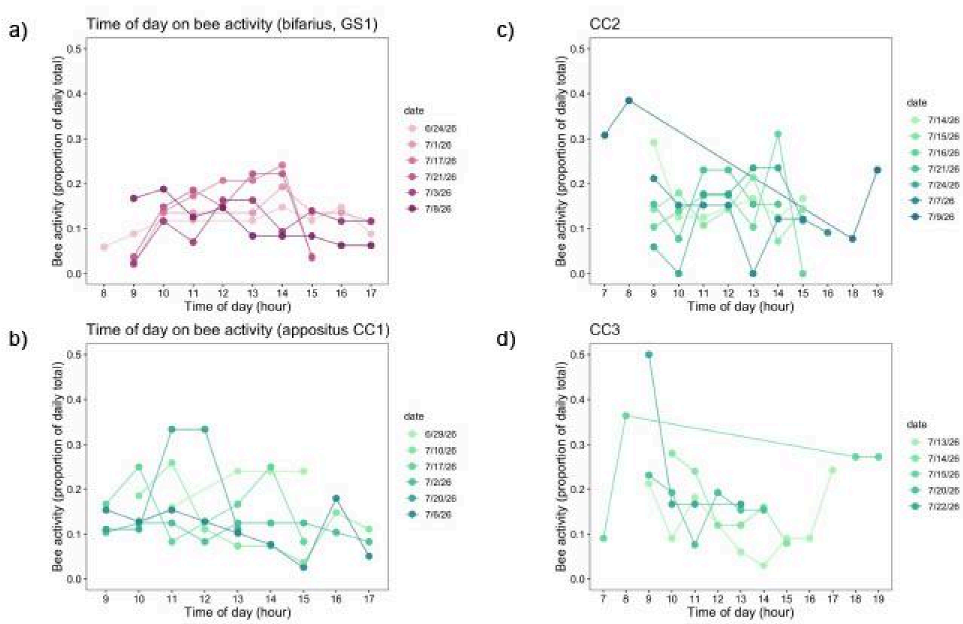

Fig 2. Dotplots of time of day (hourly) on proportion of entrance activity, sorted by date for: a) *B. bifarius* nest GS1, b) *B. appositus* nest CC1, c) *B. appositus* nest CC2, d) *B. appositus* nest CC3