

**Solitary bee genera differ in foraging activity timing and
temperature; Evidence of a seasonal dietary shift in
*Hoplitis fulgida***

Student: Jacob Ives – Undergraduate, University of Vermont

Mentor: Melanie Kazenel – PhD, Earlham College

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Abstract

Global warming, and exacerbated warming temperatures with humane activity, could impose novel conditions on the lives of many organisms. Of those, the behaviors and diets of solitary bees face adaptive stress as their optimal foraging conditions and preferred floral resources fluctuate. Trap nests make the perfect study system to investigate climatic consequences, because of their easily observable and accessible nature. Using this study system, we sought to answer two main questions: (1) How do foraging time of day and temperature differ among trap-nesting bee taxa? and (2) how does the pollen diet of *Hoplitis fulgida* vary seasonally?

Trap nests and their hosts, particularly pertaining to the questions being asked here, have been largely understudied, despite the possibility for broad inference on the temporal, thermal, and spatial differences in inter- and intraspecific solitary bee behaviors. Previous work has been done on the foraging activity of *Osmia*, though these studies haven't directly investigated niche partitioning in the presence of other taxa; similarly, there has been dietary study for solitary bees, but the literature lacks in study of *Hoplitis*. Our linear-mixed effect models revealed significant partitioning of both temporal and thermal periods between *Osmia* and *Hoplitis*. A combined principal component analysis and permanova distinguishes a notable degree of difference in the pollen diet of *Hoplitis fulgida* spatially. Visualization of *fulgida*'s seasonal diet across all sites reveals a significant degree of change over the course of the season. From our results, we surmise that *Osmia* and *Hoplitis* may face compressing or dividing pressures on their temporal/thermal segmentation, and that adverse nutritional and energetic consequences for *Hoplitis* could impact brood sizes, composition and survival.

Introduction

Increases in mean annual winter temperatures under climate change have led to earlier seasonal timing of snowmelt in temperate regions (Munson & Sher, 2015), resulting in earlier spring blooming of plants (Dunne et al, 2003). Early snowmelt can expose developing plants to late-season frost (Iler et al., 2019; Inouye, 2000; Inouye, 2008), which can cause loss of buds, and lower abundance of pollen and seeds (Inouye, 2022), and lessened late-season recruitment (Inouye, 2008). Changes in flowering timing can also be attributed to shifts in environmental cues including temperature, precipitation, and soil moisture (Stemkovski et al, 2020). Bees, the most important pollinators of many flowering plants, often use the same environmental cues for their phenology, but may differ in sensitivity to them (Stemkovski et al, 2020). Bees may also have specific overwintering developmental requirements that can influence their emergence (Stemkovski et al., 2020). These factors result in the potential for phenological desynchronization between the blooming of plants and the activity timing of pollinators (Kudo & Y Ida, 2013; Memmott et al., 2007; Peñuelas & Filella, 2007; Prather et al., 2023; Rafferty & Ives, 2012). Such phenological mismatch can alter plant and pollinator reproductive success, potentially cascading to influence the composition of plant-pollinator interactions and community composition. (Forrest et al., 2019; CaraDonna et al., 2014; Rafferty & Ives, 2012, Wolkovich & Donahue, 2021).

Climate change has the potential to alter and exacerbate both beneficial and malignant facets of bee livelihood. Phenological mismatches could produce catastrophic disruptions in resource

availability and species interactions (Lambert et al., 2010; Prather et al., 2023), particularly for plant-pollinator interactions. Beyond phenological mismatch, climate change can impact pollinators by altering physiology and rates of nest parasitism (Forrest & Chisholm, 2017). Warm temperatures could positively affect bee species, providing the optimal conditions required for insect flight, expanding opportunities for foraging and oviposition (Forrest & Chisholm, 2017), and increasing nest construction rates (Wong & Forrest, 2021); however, these high temperatures may promote parasitism, offsetting what good the heat brings, and greater nest availability does not correlate with heightened reproductive success (Wong & Forrest, 2021).

Bees may also respond to changing temperature conditions under climate warming by shifting their behavior (Forrest et al., 2019). Regarding the foraging behavior of solitary bees, studies document that pollen provisioning trips are mainly conducted in the morning for some species (Gonzalez et al., 2006), and their foraging ranges are more restricted than social bees (Gathmann & Tschardt, 2002). However, few studies have examined how both fluctuating daily temperatures and floral abundance influence the partitioning of time and resources among cohabiting bee species, combining consideration of behavioral and phenological responses to climate. Especially while dealing with the restructuring of floral communities. Resulting changes in the dietary preferences of a species over a season may in turn affect individual fitness and population success.

This study focuses on elucidating the effects of temperature and flowering phenology on the behavior of solitary bee taxa in the family *Megachilidae*. We aim to investigate the relationship between foraging activity and local climate conditions, documenting how time of day and associated temperature relate to foraging for pollen and nesting substrate in different bee species. We also aim to examine how changing seasonality alters plant-pollinator interactions. Specifically, we will address the following questions: (1) How do foraging time of day and temperature differ among trap-nesting bee taxa? and (2) how does the pollen diet of *Hoplitis fulgida* vary seasonally?

Methods

Study Sites

Fieldwork was conducted over a 10-week period in four subalpine sites located within the Rocky Mountain Biological Laboratory; located in Gothic, Colorado, USA. Sites were located at Judd Falls, South Gothic, Rosy Point, and within the Gothic townsite (insert figure). Each site consisted of a mix of 6-12 pre-established and newly established trap-nests drilled into (a) the trunks of live trees, (b) trunks of hard or soft snags, and (c) exterior frames of cabins in the Gothic townsite.

Study Taxon

Osmia & *Hoplitis* are genus of the family *Megachilidae*. A group of solitary bees differing from the more commonly thought of social bee groups. Solitary bees differ from social bees in many aspects of their behavior, physiology & life cycles (Forrest et al., 2011).

Osmia are found throughout North America, more commonly known as mason bees because of their tendency to construct nests of substrates comparable to masonry materials (mud, dirt, et cetera). *Osmia* are cavity-nesting bees, nesting in pre-existing holes or cavities, and are known for their efficient pollination services (Forrest et al., 2011). Certain species of *Osmia* can thermoregulate at impressively low temperatures (Stone and Willmer, 1989; Vicens and Bosch, 2000), enabling them to visit flowers to collect pollen and nectar at times and temperatures when many other bees are staying at home.

Hoplitis fulgida is a species of bee in the genus *Hoplitis*. Found in mountainous regions of North America, this solitary bee species nests in pre-existing cavities in dead trees, live trees and snags/stumps (Tepedino & Parker, 1984). Females typically emerge starting in mid-June (Wong, 2017) and promptly begin searching for suitable nesting spaces. Their nests are comprised of a series of brood cells neatly organized, separated by partitions of nesting substrates collected from sources such as mud, leaves and other material ((Tepedino & Parker, 1984). Each cell contains a pollen provision and nectar, where when complete an egg is laid by the nesting female (Tepedino & Parker, 1984).

The pollen diet of *Hoplitis fulgida* has been studied in the context of the species' nesting behavior and biology, but relatively little is known about the specific pollen sourcing of the species. (Müller, 2018) found that the species exhibits a strong preference for the pollen of *Potentilla* (Rosaceae). But the species has been found to exhibit polylectic behavior in other studies conducted at the Rocky Mountain Biological Laboratory (Wong, 2017).

Trap nests can be used to attract solitary bees due to their ability to provide suitable nesting sites and simulate the preferred natural nesting environment of solitary bee species. Trap nests allow for the easy observation and quantification of multiple trophic interactions between the species that may use them solely or collectively, as well as their pollen provisions and nesting substrates (Staab et al., 2018). Furthermore, trap nests can be used to observe the foraging activity of trap-nesting solitary bees, whereby their comings and goings can be used to access information regarding specific individual's temporal niches, temperature preferences, and distinct individualistic behavior.

Foraging Observations

Trap Nests were monitored at three of the study sites (Judd Falls, Gothic Townsite & South Gothic) for foraging observations. Each of these sites was monitored every other two-three days, Monday-Friday, excluding periods where observations weren't performed due to weather events or low occupancy. Trap nests were arranged in blocks consisting of two trap nests each; except in the townsite, where blocks were singularly located. Blocks consisted of two nests drilled into opposing sides of the same tree, facing the same direction. There were four and three blocks of trap nests at Judd Falls and in South Gothic, respectively, and 8 traps located within the Gothic Townsite, with 2 of those traps stacked. Each block was monitored for an interval of one hour on its assigned day. Blocks were observed in consistent order, but time of day for that order was randomly chosen; excluding the first observation block, which consistently occurred at time of arrival on site.

Blocks were observed from 1 meter away. When an insect was observed entering or exiting the nest, the following was recorded: Site, Tube ID (block letter & hole number). Date, Time, Taxon, Parental Status, Entering or Exiting, whether the insect was carrying pollen, or whether it was carrying nest construction substrates. Foraging distance was also noted in rare circumstances, when the insect stayed within observable range.

Nest Occupancy

All four sites were monitored 1-2 times a week to record their occupancy status (find new nests and monitor the progress of existing ones). Straws were pulled with hemostat pliers and held up to a light source to observe the progress of the mother, quantified through the number of cells created. The outermost provision or outermost completed wall of each nest was marked with a vertical line and the current date. For new nests, the top of each straw was marked at the nest entrance to ensure that straws were extracted and returned at all points thereafter in the correct orientation.

The number of cells and the completion of incomplete cells was marked to the nearest 0.1 cell each time sites were visited. Number of completed cells was not recorded for nest occupants other than *Osmia* or *Hoplitis* (e.g. wasps). A cell was considered complete if observations could not see through the wall or if the occupant had laid an egg in the cell. A cell was considered still in progress if there was some amount of pollen in front of the wall, but no egg. Total cells were observed as 0 if there was a mother present, but no pollen provisions.

When possible, all nesting *Osmia* and *Hoplitis* individuals were uniquely marked using permanent paint. Markings served as a tracking mechanism for the successful reproductive output and offspring success of individuals (Wong & Forrest, 2017). Markings also served to note whether nests had been abandoned and reoccupied, instances of co-visitation and conspecific/heterospecific interactions (i.e interaction of members of the same/different species in the same tube). Bees were caught using bee squeezers, which were held or positioned over the entrance of a bee's nest. Once the bee had come out, the bee was trapped between the mesh of the bee squeezer and the rapidly inserted foam component of the squeezer. A blade of grass was dipped in Testors paint, and a tiny dot of paint (or combinations of dots) was placed on the bee's thorax, making sure to avoid heavy volume or dotting near the wing bases. Bees were released as soon as possible so that the paint did not lock their wings in place. Immediate flight may also work to dry the paint faster. Color combinations were often necessary as multiple individuals of the same site were often present at each site. The total number of brood cells were counted once nests had been completed.

Pollen Provision Sampling

For all *Hoplitis fulgida* nests, each completed brood cell was opened and sampled for the pollen therein. All instruments used in the pollen sampling process were sterilized using ethanol and kimwipes before use. Perpendicular incisions were made into the tube encircling each completed brood cell using a scalpel. Then, a minute "window" (1mm x 2mm) was made using a pair of microscissors; first a cut was made parallel to the tube and perpendicular to the incision line, then a secondary cut was made from the end of the second cut and parallel to the first cut. This

“window” was then folded upwards using a pair of forceps, and the pollen inside was lightly sampled using one end of the forceps. Cuts were made closer to the innermost wall of the cell to avoid slicing the egg or larva that is deposited on each pollen provision.

Pollen samples were deposited on microscope slides. Labeled with the nest ID (site, tube ID, cell #) & current date. A tiny cube of fuchsin gel was placed on a microscope slide, where the collected pollen was then deposited by rubbing lightly onto the gel. The gel was melted using an ethanol burner or lighter, until a bubble had formed over the sample. After melting had ceased, a coverslip was placed on top of the sample. Bubbling/intense melting was avoided to not burn the sample. Following pollen sampling, the windows of the straw were closed in tape and returned to the proper position in the trap nest.

Pollen was also sampled from the innermost and outermost cells of *Osmia* nests to aid in species identifications, and for use in taxon dietary comparisons.

Flower Counts & Sampling

During each site visit to Judd Falls, South Gothic, and Rosy Point, all open flowers located within five fixed 1-meter by 1-meter quadrats at each site were counted. For traps located within the Gothic townsite, the species identities were noted of all plants blooming within 50 meters of each nest. Each time a new species was observed in bloom, a pollen sample was collected from it by placing a cube of fuchsin gel on an insect pin, and gently brushing the pin against the anthers of each flower. The pollen-containing gel was later mounted on a microscope slide using the methods described above.

Chosen flower species were sampled at multiple sites to compare how the pollen varied across location.

Microscopy & Pollen Identification

Pollen samples were imaged using a Leica DM750 compound microscope, with imaging occurring at magnification of 20/40x. The imaging software used was the Leica Application Suite V4.12, the camera model was the Leica DM750. One representative photo was taken of each pollen sample (i.e. a representative area with the maximum pollen diversity present). Since observation of all pollen present was improbable, it is possible that all pollen species present may not have been captured. For each pollen sample taken from flowering species, a single photo was taken with an ample quantity of pollen cells. Brightness & infrared were adjusted on a per-image basis to obtain the sharpest image possible, though settings were consistent at large.

Pollen samples taken from flowering species were compared side-by-side to pollen samples taken from *Hoplitis* nests to best match which flowering species were present in each sample. No characterization of pollen morphology was performed. A maximum of three pollen species were identified per sample.

Data Analysis

Rstudio Version 2024.04.02+764 was used with the following packages: stringr, dplyr, tidyr, ggplot2, lme4, emmeans, lubridate, data.table, car, piecewiseSEM, knitr, kableExtra, gridExtra, grid, broom.mixed, RcolorBrewer, stats, reshape2, tibble, vegan, igraph, ggraph, ape, rotl, and taxize.

Foraging Activity

Details of the foraging timing of observed taxa were read into Rstudio as a .csv file. As were temperature data from HOBO loggers that had been attached to the bottom of our trap nest blocks in Judd Falls and South Gothic, and at four traps across a longitudinal gradient in the Gothic Townsite. Temperature data was extrapolated from the nearest logger for traps that didn't have a logger directly attached.

We performed an Analysis of Variance (ANOVA) and a linear mixed effects model (LME) to investigate when each taxon was active. Specifically, that meant looking at the effect of "taxon" on "time_into_day". Secondly, we performed Tukey's Honest Significant Difference (HSD) test to make pairwise comparisons between "taxon", so we could identify which specific groups differ from each other. Lastly, our linear-mixed-effects model refined the analysis done by the HSD test by factoring in random effects for variables such as "site", "date", and "site_trap_nest".

To determine the effect of temperature on foraging activity, we first performed an ANOVA to the response of temperature ("temp_c") to the predictor "taxon". We then performed a Tukey's HSD test to compare the different levels of "taxon" to each other. And then fit in random effects ("site", "date", "site_trap_nest") through a linear mixed effects model.

We further examined the foraging activity of taxon's pollen and substrate collection timing. For both pollen and substrate collecting activity, we ran an ANOVA to evaluate whether there were significant differences in pollen/substrate collection times ("time") across taxa ("taxon"). Again, a linear-mixed-effects-model was fitted the data to account for random effects (aforementioned). An anova was then ran on the mixed-effect model to test whether mean pollen/substrate collection timing differed between taxa.

Pollen Diet

Details of the pollen composition of all the *Hoplitis* cells that we sampled were read into Rstudio as a .csv file. As were the reports of our flower surveys and the final nest occupancies for all sites. We then filtered out cells that were not taken from *Hoplitis* nests, using the occupancy data.

We used ggplot2 to graph and understand the relationships of cell composition over the season, primary provision identity across cells of the same nest, diversity of total pollen provisions between months, and species abundances and resourcing across space and time.

To investigate the relationship between pollen provision identity and location, we ran several tests. First performing a principal component analysis on a matrix of pollen data to reduce the dimensionality of the data while preserving variance; and allow us to observe overlap of the

identity of pollen provisions between sites. We then performed a Permutational Multivariate Analysis of Variance (PERMANOVA) on the matrix generated for the principal component analysis to test for significant differences in species composition between sites. We then performed a broader investigation using a Chi-Square test into species identity across sites, encompassing all of the data we collected.

We further used Rstudio to perform a taxonomic classification for our full list of surveyed plant species, and then filtered this data to only include species whom we identified in our cell-pollen samples.

Results

Dataset: We recorded data on the foraging activity of a total of 114 individuals representing four taxonomic groups during a 35-day span in the summer of 2024. Species were distributed across three solitary bee taxon (*Hoplitis*, *Osmia*, and *Megachile*) and included a collective grouping for Wasps. Our dataset mostly consisted of *Hoplitis* observations (~64%), followed by *Osmia* (~29%), Wasps (~6%), *Megachile* (~0.6%). Of these observations, all the *Hoplitis* individuals belonged to the species *fulgida*, while individuals from *Osmia*, *Megachile*, and Wasp were not identified down to the species level.

Foraging Activity Timing: Temporal variation of foraging activity was found to differ greatly between taxa ($Df = 3$, $F = 23.75$, $p = 6.7e-15$; Table 2). *Hoplitis* tended to forage later in the day than *Osmia* (estimate = 0.443, $df = 883.7$, $p = 0.0112$; Table 1). Visual inspection of histograms (Figure 4) shows that *Osmia* activity peaked in the morning and *Hoplitis* activity peaked in the afternoon. There was no significant difference in foraging activity timing between *Hoplitis* and *Megachile* (estimate = 1.344, $df = 62.7$, $p = 0.5991$; Table 1). No significant difference was found between *Hoplitis* and Wasp individuals (estimate = -0.129, $df = 1002.6$, $p = 0.9079$; Table 1). *Megachile* and *Osmia* also did not differ significantly in foraging activity timing (estimate = 1.787, $df = 65.1$, $p = 0.3550$; Table 1). Similarly, no significant difference was seen between *Megachile* and Wasp (estimate = 1.214, $df = 65.1$, $p = 0.6814$; Table 1). Lastly, a significant difference was found between *Osmia* and Wasp, with *Osmia* showing a lower estimated value (estimate = -0.572, $df = 965.5$, $p = 0.0261$; Table 1).

Foraging Activity Temperature: Thermal variation of foraging activity was found to differ greatly between taxa ($Df = 3$, $F = 4.62$, $p = 0.00328$; Table 3). But this overall significance only carried down to two pairwise relationships between taxa. A highly significant difference in foraging activity temperature was found between *Hoplitis* & *Osmia* (estimate = 1.351, $df = 755.0$, $p = 0.0001$; Table 4). There was no significant difference observed in foraging activity temperature between *Hoplitis* and *Osmia* (estimate = -1.033, $df = 43.0$, $p = 0.9909$; Table 4). No significant difference was detected between *Hoplitis* and Wasp individuals (estimate = 0.181, $df = 745.1$, $p = 0.9668$; Table 4). Similarly, *Megachile* did not have significant differences in foraging activity timing with either individuals of *Osmia* or Wasp (estimate = 2.382, $df = 43.0$, $p = 0.9037$; estimate = 0.852, $df = 43.6$, $p = 0.9949$; Table 4). But a significant difference was found between *Osmia* and Wasp individuals (estimate = -1.532, $df = 747.4$, $p = 0.0020$; Table 4).

Substrate-Collection Timing: Overall timing of substrate collection events between all taxa varied greatly ($Df = 2$, $F = 0.00081$; Table 6). No significant relationships were found between any of the pairwise comparisons of substrate-collecting taxa in our dataset. *Megachile* and *Osmia* had the most significant relationship (estimate = 10312, $df = 50.0$, $p = 0.1298$; Table 5). *Hoplitis* and *Osmia* had the next most significant relationship (estimate = 2861, $df = 39.7$, $p = 0.2248$; Table 5). *Hoplitis* and *Megachile* had the least significant relationship for substrate-collection timing (estimate = -7451, $df = 48.4$, $p = 0.3449$; Table 5).

Pollen -Collection Timing: Timing of pollen collection did not vary greatly between taxon ($Df = 2$, $F = 2.437$, $p = 0.0896$; Table 8). Moreover, no significant relationships were found between any of the pairwise comparisons of pollen-collecting taxa in our dataset. *Hoplitis* and *Osmia* had the most significant relationship (estimate = 1524, $df = 134$, $p = 0.4137$; Table 7). *Osmia* and Wasp followed in terms of significance (estimate = -3376, $df = 197$, $p = 0.5214$; Table 7). *Hoplitis* and Wasp had the least significant relationship for timing of pollen-collection (estimate = -1851, $df = 190$, $p = 0.8294$; Table 7).

Pollen Diet

Results

Dataset: We collected pollen samples from 165 *Hoplitis fulgida* nests over a forty-one-day period in the summer of 2024. Nests were collected from four field sites, and three of those four sites had associated floral resource data collected on the same day, or ± 2 days. Our pollen samples were ~35% from Judd Falls. ~27% each from Gothic Townsite and South Gothic, and ~10% from Rosy Point. Our floral surveys observed the emergence of sixty unique flowering species across all sites over the forty-one-day span. Of those 60, 50 were observed at our plots around the Gothic townsite, 22 in South Gothic, 18 at Judd Falls, and 7 at Rosy Point. The flowers we surveyed belong to 42 genera and 14 families (Table 10) while the pollen samples we collected belonged to 18 of the genera, and 8 of those families (Table 9). We found pollen sourced from 26 unique flowers in our sampled *Hoplitis* nests; with 17 of those being from the Gothic Townsite, 15 from Judd Falls and South Gothic each, and 11 from Rosy Point. Visual examination of Figure 10 reveals the disparity of floral abundance and diversification across our sites, with the Gothic Townsite holding both higher species richness and abundance, followed sequentially by South Gothic, Judd Falls, and Rosy Point.

Seasonal Change: Visual observation of Figure 14 & Table 11 show that there is a notable difference in the pollen composition of *Hoplitis fulgida* nests throughout the summer season. When categorized by month, we observed that *Hoplitis* nests were composed of ~35% *Aconitum columbianum*, ~14% *Boechera stricta*, ~24% *Delphinium nuttallianum*, ~4% *Eriogonum umbellatum*, ~17% *Lathyrus lanszwertii* var. *leucanthus*, and ~7% *Senecio integerrimus* in June. Before rapidly diversifying into an amalgamation of 25 species in July, with the top five by percent makeup being *Aconitum columbianum* (~22%), *Eriogonum umbellatum* (~17%), *Helianthella quinquenervis* (~8%), *Linum*

lewisii (~8%), and *Delphinium barbeyi* (~5%). Pollen composition varied across sites as well, with Judd Falls reporting a June pollen diet of 50% *Lathyrus lanszwertii* var. *leucanthus* and 50% *Aconitum columbianum*, the Gothic townsite reporting a June pollen composition of 67% *Delphinium nuttallianum* and 33% *Lathyrus lanszwertii* var. *leucanthus*, Rosy Point split three ways between *Aconitum columbianum*, *Eriogonum umbellatum*, and *Lathyrus lanszwertii* var. *leucanthus*, and South Gothic reported 38% *Aconitum columbianum*, 19% *Boechea stricta*, 24% *Delphinium nuttallianum*, 10% *Lathyrus lanszwertii* var. *leucanthus*, and 10% *Senecio integerrimus*. The most utilized floral resources for July also differed across sites: Judd Falls was mostly *Aconitum columbianum* (~17%), *Erigeron speciosus* (~17%), and *Eriogonum umbellatum*, (~20%), the townsite was 27% *Eriogonum umbellatum*, 27% *Aconitum columbianum*, and 8% *Linum lewisii*. South Gothic's primary triad was 26% *Aconitum columbianum*, 10% ERSO, and 10% *Eriogonum umbellatum*; and Rosy Point's major sources were *Linum lewisii* (~21%), *Erysimum capitatum* (~17%) and *Helianthella quinquenervis* (~17%). These findings are consistent with visualization of this data (Figure 16 & 18), where the breadth of dietary diversification is clear. Moreover, Figure 15 demonstrates two seasonal peaks in the pollen-collection/nest-construction of *Hoplitis fulgida*, a primary peak in early July, and then a much larger, secondary peak in the tail end of July.

Nest Overlap: A principal component analysis was run to examine the overlap of the identity of pollen collected between sites, disregarding seasonality. Visual observation of Figure 13 reveals a notable degree of overlap between all sites. First, a chi-square test was performed on the entire dataset to assess the distribution of cell-pollen composition across different sites, where significant variation was found (X-squared 237.47, df = 75, $p < 2.2e-16$; Table 12). However, test assumptions were violated due to small sample sizes and lower than expected frequencies, so we performed a secondary analysis. Our Permanova analysis of the aggregated pca data reveals that sites differed significantly in the pollen composition of *Hoplitis* nests (Df = 3, F = 2.5495, $p = 0.001$; Table 13) across the season.

Discussion

Foraging Activity

We found that *Hoplitis* foraged later in the day than *Osmia* on average. Differences in the timeliness of foraging activity could arise through the unique activities of each taxon throughout the day (Drummond, 2016). Though we collected data on the substrate and pollen-collecting activities of the bees, this data does not reflect timely aspects of these bouts that should be considered:

(1) This temporal differentiation could be attributed to competition for shared resources shaping the biological rhythms of these taxa (Gao et al., 2020). *Hoplitis fulgida* is polylectic species, whereas the *Osmia* genus is partially oligolectic and polylectic (Cripps, 1989). This overlap may create an isolating barrier between species whose floral preferences don't or only partially

overlap. Whereby the unique flowering species that oligolectic versus polylectic species forage on experience peak pollen production at different times of the day (Percival, 1955).

(2) Frequency and duration of individual foraging trips rapidly increased when *Hoplitis* individuals were collecting substrates, particularly when sealing their nest. For analyses between taxa, this activity has the potential to skew results including those substrate-collecting trips, where foraging behavior of this kind was not noticed in *Osmia* individuals. One explanation is that their physiology may allow them to complete fewer trips for the same amount of substrate. Various studies have demonstrated *Osmia*'s efficient foraging habits (Bosch, 2021; Vicens & Bosch, 2007).

(3) Differentiation could also arise through species-specific exploitation of peak nectar production during floral blooming, which has been demonstrated in nectar-feeding birds (Wolf, 1975). Solitary bees generally collect their energy and nutrient requirements at earlier or later times in the day (Gottlieb, 2005) after taking advantage of optimal foraging conditions; however, there was an abundance of trips within bouts where neither pollen nor substrate were noted to be held by an individual. These occurrences could be attributed to nectar-feeding trips, intermixed with pollen and substrate-carrying events (Leonhardt, 2007) so individuals can maximize the total number of cells that can be constructed in a day.

(4) Contrast in foraging timing between *Hoplitis* and *Osmia* could arise through differential responses to increasing noise in the system throughout the day. Taxa such as flies and mosquitoes may experience peak activity at different optimal temperature, light, and humidity conditions than *Hoplitis* and *Osmia* (Ginsberg, 1983). Anecdotally these external taxa entered our study system when diurnal solar radiation peaked. Delayed diurnal emergence into the system may create a behavioral tendency in *Osmia* to complete most of their bouts in the morning, to avoid the noise in the afternoon. Whereas *Hoplitis* individuals may be indifferent to increasing "noise" throughout the day. Or simply bear through it out of necessity.

(5) Foraging activity timing may also be affected by abiotic factors in the environment such as humidity, light period, and temperature (Gonzalez, 2006). Many pollinators at high elevations exhibit bimodal activity patterns to avoid the midday heat (Xu et al., 2021). This tendency aligns with our data (Figure 3), though we also stress that the midday depression our analyses revealed is not fully studied as there is a deficiency of observation periods during this allotted time. Visual observation of Figure 19 reveals slopes on either side of this depression in activity, indicating that there is a decrease in bee activity to some extent. The evolution of dim-light foraging in bees (Wcislo & Tierney, 2009) provides an alternative explanation for a midday nap or rest event. These behaviors are often associated with optical and neurobiological adaptations, aiding competitive release and risk/enemy aversion (Wcislo & Tierney, 2009); this finding both supports the hypothesis of a midday break in activity, where overhead light is overwhelming, and our finding of temporal heterogeneity between taxa, suggesting *Osmia* may prefer dim-light conditions in the morning, and *Hoplitis*, the afternoon.

The temporal differentiation we found aligns with the spring emergence trends between these genera, where *Osmia* tends to emerge from diapause earlier than *Hoplitis* (Stemkovski et al., 2020). This could reflect an adaptation to wetter, more precipitous conditions by *Osmia*.

(Keaveny & Dillon, 2022), who may be better equipped to handle moist foraging conditions. This hypothesis corresponds with the substrate-collection timing of *Osmia*, where the median collection time occurs in the morning, under more saturated conditions (Figure 1); compared to *Hoplitis*, whose peak substrate-collecting time was in the afternoon, after the soil has absorbed the peak solar radiation that comes midday (Puente-Sánchez et al., 2016).

Hoplitis and *Megachile* had overlapping foraging periods, with *Megachile* emerging and foraging exclusively in the afternoon. One rationale for this coincidence is that *Megachile* is a genus of leafcutting bees, that use leaves as a nesting substrate; *Megachile*'s temporal foraging habits may arise from a behavioral tendency to only use dry leaves in the construction of their nests (Sabino & Antonini, 2011; Royauté et al., 2018), making their primary substrate consistently unavailable until the afternoon. *Megachile* species range from broad generalists to narrow specialists (Muller, 1996; Kunjwal et al., 2020), making an overlap with the polylectic *Hoplitis* likely. However, visual observation of Figure 4 indicates that *Megachile* emerges and begins foraging activities much later in the day than *Hoplitis*, after *Hoplitis* activity has slowed down for the day. These two genera are therefore unlikely to have to contend with each other for resources.

Hoplitis and *Megachile* both share foraging periods with Wasps, though Wasps are consistently active throughout the day (Figure 4). Though *Hoplitis* activity and density were lower at sites with higher Wasp abundances (unpublished data). In our study system, *Megachile* and Wasp species tended to inhabit nests of the same size (anecdotal/unpublished data). Cohabiting the same spatial niche in our trap nests likely leads to low co-occurrence of nesting at any given trap nest by these two taxa. Though our analyses suggest that these taxa forage during the same temporal periods, results could be different if they were seen sharing a spatial niche (i.e. Wasps and *Megachile* living in the same trap) instead of it seeming to be a system of priority-based competition where occupation of one nest in a trap by Wasp or *Megachile* claims the trap for that taxon.

This competition for nesting space between bees of *Megachilidae* and Wasps has been seen with wool-carder bees and wasps, where wool-carder bees will place plant secretions in their nests to protect against wasp parasitism (Eltz et al., 2015), implying competition between wool-carders and wasps. *Osmia* and Wasp individuals did temporally differentiate foraging activity, though this likely has less to do with interspecific intricacies between *Osmia* and Wasp individuals and is more likely an indirect effect of both taxon's interactions with other organisms. This presumption stems from observations of unwavering *Osmia* activity amidst fluctuations in Wasp activity, while the behavior of *Hoplitis* is substantially more affected by Wasp activity.

Megachile and *Osmia* may have temporal overlap due to shared preferences for the most abundant flowering species in a local area, when taxa with shared preferences target peak pollen-production at the same time (Barônio & Torezan-Silingardi, 2016; Minckley et al., 1994). Contradictory to this finding is our observation of late-day emergence by *Megachilid* species, who emerge at a time presumably after peak pollen production for the flowering species that *Osmia* sources from. *Osmia* and *Megachile* species are consequently unlikely to have shared preferences at our field sites. Secondary grounds for this temporal overlap could simply be that

these genera have little agonistic tendencies with each other. So, a cooperative airspace is feasible.

The significance of temperature-dependent foraging activity mirrored that of time-dependent foraging activity, with all significant and non-significant relationships seen again. This is consistent with what we would expect given that analyses based on time are inherently going to have temperature data tied to them. An organism's activity patterns (temporal niche) are going to be closely tied to fluxes in temperature and expose the associated thermal niche (Tracy & Christian, 1986). Discerning which factor is more influential is therefore complicated. There are caveats to this entwined relationship that can convolute whether a taxon is valuing time over temperature or vice versa: Precipitation events can dampen flowers and even if post-precipitation temperatures are consistent with those found on dry days, bee activity is likely to be sparse (Hilário et al., 2007). Other events likely to disrupt an organism's diurnal cycle (or alter its temporal/thermal niche) include heatwaves, capricious weather events, and cloud cover (Dai et al., 1999).

Alternatively, an organism's thermal niche could be just one facet of the overarching temporal niche (Tracy & Christian, 1986). Nonetheless, the determinants of an organism's thermal niche are just as important as those of a temporal niche. Temperature, and thereby incoming solar radiation, mainly interact with the body size of solitary bees, where it has been seen elsewhere that body size has a positive correlation with critical thermal maxima in female solitary desert bees (Barrett et al., 2023). Moreover, larger female solitary bees emerge and forage at lower temperatures than smaller females (Stone, 1994), this is because larger solitary bees are better able to thermoregulate and achieve flight at low ambient temperatures (Stone, 1993). All this suggests that larger female solitary bees have thermal tolerances that are more extreme than smaller female solitary bees. Interestingly, this doesn't seem to reflect in our data; visual observation of Figure 5 shows that *Hoplitis* individuals were seen foraging at greater or equal extremes than larger solitary bee groups. The underlying presumption was that the sizing of *Hoplitis*, *Osmia*, & *Megachile* individuals was consistent. Without measuring these taxa consistently however, we cannot comment on the interspecific differences in thermoregulation pertaining to body size.

As mentioned before, the pairwise relationships that were significant for timing-dependent foraging activity were also significant for temperature-dependent foraging activity. And likewise for insignificance. This trend supports the “temporal houses thermal” niche segmentation, as this correlation indicates that thermal differentiation and/or thermal niche determination is being affected by many of the same indirect consequences of a taxon's temporal niche determination.

Some taxa may live by certain biological rhythms, which reflects their adaptation to a certain temporal environment (Yerushalmi & Green, 2009). However, such taxa may exhibit short-term plasticity to disruptions in their temporal environment (De Lisle et al., 2021). One of the ways in which this is reflected is through alterations to an organism's thermal niche, with the parallel alterations to an organism's temporal niche being incongruent with the thermal response (Catatayud et al., 2020). The relative importance of thermal versus temporal niche partitioning should thus be further investigated in this study system.

The scale at which we measure temporal and thermal niches is thus important. Measurements across different scales could therefore reflect the potential malleability of an organism's temporal and thermal niches across those scales. With solitary bees, overlaying data across different scales could inform knowledge on the plasticity of diet, reproductivity, and behavior. Monthly measurements could reveal seasonal shifts in both temporal and thermal niches. Weekly measurements could mirror shifts in the reproductive rate of an individual female and how they consequently shift their niche. Daily measurements could reveal short-term plastic responses to unforeseen weather, hourly discloses the "normal" niche, or what we would expect the average day of a bee to look like, and how it might be differentiating from normal. With anything smaller, we could anticipate gaining knowledge of the behavior of individual bees, and how individual coping methods to thermal flux vary. Data interacts across scales, and taking a layered approach in this system could give rise to an understanding of the plasticity of solitary bees across time scales.

Other studies have suggested that substrate-collection timing may differ among solitary bee taxa due to their reliance on resources from different habitats within a mosaic landscape (Williams & Kremen, 2007). This finding could pertain to our system, where taxon preferences could give rise to the diurnal difference in substrate-collection timing noted in Figure 1. *Hoplitis* could prefer much dryer nesting substrates than *Osmia* based on the concentrated times of days that these taxa collect substrate. Alternatively, since *Hoplitis* nests tend to have more cells than *Osmia* nests (unpublished data), *Hoplitis* may be constructing more cells per day than *Osmia*. A supposed continual effort throughout the day demonstrated by both general foraging trip timing and substrate and pollen collection timing (Figures 3, 1 & 2), suggests that substrate-collection timing being concentrated later in the day for *Hoplitis* may be a byproduct of extended general foraging activity, rather than any startling rationalization behind this result. Strangely, this trend aligns with that seen for overall foraging activity, with *Osmia* peaking in activity at an earlier time than *Hoplitis*.

Pollen-collection timing (Figure 2) reflects the composition of foraging bouts to be generally pollen-comprising (personal observation). Visual observation of Figure 2 shows that median peak pollen-collection timing is approximately the same for both *Hoplitis* & *Osmia*. Indicating a probability that the overlap between the oligolectic *Hoplitis* and polylectic *Osmia* may be prominent, with proximate peaks suggesting that peak blooming for these taxa's key floral resources may overlap. If not one and the same.

Megachile and Wasp were removed from visualization of substrate/pollen collecting timing because of a lack of data or because data was not collected for the carrying habits of that taxa. *Megachile* was included in the substrate-collection analysis because certain individuals were noted carrying masticated leaf material. Wasps were included in the pollen-collection timing because certain individuals were noted to be constructing cell-like structures with pollen. However, more data is needed on the relative timing of pollen and substrate collecting trips, and larger sample sizes are required for *Megachile* and Wasps at large to make more specific conclusions on their foraging activity.

The occupancy of similar thermal niches on average (Figure 6) by *Hoplitis* and *Osmia* indicates that these taxa could have similar responses to warming temperatures. However, the spread of

temperature-dependent foraging activity demonstrated by *Hoplitis* (Figure 5) indicates that a similar response may not be as likely as the median prompts. A dissimilar response would be in line with past studies, where higher temperatures and greater temperature fluctuations were found to decrease body and cell size (Kierat et al., 2017); and quantity of resources and temperature were found to be the most pivotal determinants of body size, rather than pollen diversity (Radmacher & Strohm, 2010). Implying that smaller solitary bee species such as *Hoplitis* will be favored under drastic climate scenarios. However, climate change could expand the number of foraging hours available each day for foraging (Forrest & Chisholm, 2017); if malevolent pressures such as wasp parasitism, are also favored under drastic climate scenarios (Forrest & Chisholm, 2017; Nouhuys & Ehrnsten, 2004) and extreme weather events (Moore et al., 2015) don't offset what good the heat may bring.

The shared foraging space of *Osmia* and *Hoplitis* has scarcely been studied, but work within the *Osmia* genus has revealed comparative behavior to honeybees when spatially cooperating (Yokoi & Kandori, 2016), and tendencies to visit flowers in low temperatures, strong winds and reduced solar radiation (Matsumoto & Maejima, 2010). Moreover, genus-specific studies on *Hoplitis* are scarce.

However, previous studies on other solitary bee taxon have found results pointing to similar conclusions as ours. A prior study in the Andes found that the foraging activity of *Anthophora walteri* is constrained by temperature, with foraging times between 7am and 5pm (Gonzalez, 2006). Likewise, the stingless bee *Scaptotrigona depilis* forages in reaction to climatic factors such as temperature, humidity and photovolume (Figueiredo-Mecca et al., 2013). Other studies have similarly found that the activity of bees in the genus *Melipona* decreases and is affected by temperature and humidity (Silva et al., 2011; Maia-Silva et al., 2014; Macías-Macías et al., 2016; Maia-Silva et al., 2020). However, deeper research into the mechanics behind these findings is untapped, and more work is necessary to understand how these bees are changing their behavior. Not just why.

Furthermore, studies of this kind would benefit from extended monitoring/observing of these behaviors, through cameras, radio-tracking, or other methods. Improved methods could give researchers a better idea of the spread and representation of foraging activity across a diel periods, instead of brief subsets of those periods.

Pollen Diet

We found that the pollen diet of *Hoplitis fulgida* notably changed throughout our forty-one-day sampling period. With visual observation of Figure 16 demonstrating a clear increase in diet diversification at all sites between June and July. This is understandable, given that pollen diets are expected to undergo change due to differences in phenological trends based on seasonality and nesting location (Dorian et al., 2022). This factor may explain site-specific differences in pollen diet (Figure 13); plant phenology has been shown to vary across locations (Wolkovich et al., 2014; Fitchett et al., 2015), due to both intraspecific responses to climatic variance and differences in community composition (Diez et al., 2012). Mismatches in site richness and abundances could influence individual foraging decision make-ups (i.e. the percent preference of foraging trips to local flowering species). The conspicuous cause for this observation is that

fulgida's pollen diet undergoes changes due to the seasonal availability of local flowering species (Wood et al., 2018). Nonetheless, there are numerous other factors that could explain the observed trends:

Previously, pollen amalgams by solitary bees have been predicted to be a fitness strategy to complement nutrient imbalances or mitigate harmful secondary metabolites in certain pollen provisions (Eckhardt et al., 2014). This hypothesis may account for the variation we see in pollen composition across the course of a season, where *Hoplitis* individuals may primarily be changing the floral sourcing of their primary pollen provisions, and then adapting their choices for secondary and tertiary provisions to complement this primary switch.

The pollen diet of solitary bees has also been found to change throughout the summer to provide sex-specific nutritional requirements for their offspring (Filipiak, 2019). But this hypothesis confounds with the known laying of female offspring in inner brood cells and males in outer cells within the same nest (Kim, 1992). Sex partitioning is specific to nest, rather than seasonal timing, which may explain the variation reported in the identity of primary pollen provisions between the former and latter cells of individuals nests (Figure 9). Analysis of primary provision identity is preliminary however, and more investigation is required into this supposed nutritional-partitioning.

Similarly, seasonal flux in the nutrient composition of pollen has been found to align with the shifting nutritional needs of honeybee colonies throughout the year (DeGrandi-Hoffman et al., 2018); this mirrors shifts in sex-specific nutritional requirements, and hold true for shifts, or constants, in the nutritional requirements of *Hoplitis* mothers themselves. There may be disparities between the nutritional value of pollen and nectar belonging to the same flower; it could be that shifts in the pollen diet of *Hoplitis* throughout the season not only reflect shifts in the quality and availability of pollen, but conspecific nectar as well. For instance, a *Hoplitis* mother may change primary provision preference from one flowering species to another not due to changes in the former's pollen holdings, but its nectar holdings. In effect maximizing the efficiency of their foraging bouts by ensuring they can meet their own fitness needs while continuing to construct their nest at a consistent pace.

This theory requires more investigation, as other studies have found solitary bees and honeybees forage mostly before sunrise and sunset when nectar production in their food plants is highest (Gottlieb et al., 2005, Schaffer et al., 1979). *Hoplitis fulgida* and more broadly, all pollen-collecting solitary bees, may face a climatic bottleneck as rising temperatures constrain the hours where pollen production is optimal and/or when specific thermal requirements are met (Hamblin et al., 2017). Climate change imposes two principal tradeoffs here:

- (1) acquisition of pollen vs nectar, where pollen-production decreases under increasing heat (Prasad et al., 1999; Paupière et al., 2014, Müller & Rieu, 2016), limiting the hours where solitary bees can find preferred provisions; consequentially stretching out the length of foraging bouts and narrowing pre-existing windows where these taxa feed on nectar. Previous work on the thermal-dependent foraging activity of nectar-feeding insects has found that foraging is largely determined by thermal inhibitions, as foragers must be cognoscente of

potential overheating (Willmer, 1983), nectar rewards were an afterthought when influencing foraging patterns (Willmer, 1983).

(2) Climate change alters nectar quality (Descamps et al., 2021), reducing the quantity available to local pollinators and congesting their nutritional and energy supply. Under climate change, this could have disastrous consequences as pollinators have to weigh the value of staying healthy versus collecting pollen and substrate. Consequences could be decreased foraging activity, increased mortality, and decreased brood sizes.

Other studies have noticed certain solitary bees exhibit seasonal variation in sex allocation, with a female bias earlier in the season, and a preference for male ovipositing later in the season (Martins et al., 1999; Torchio & Tepedino, 1980; Frohlich & Tepedino, 1986). Though plausible, confirmation of this finding within our study system would require site-specific nutritional analyses of those flower species preferred at various points throughout the season. Namely, are June dominants (*Aconitum columbianum* and *Delphinium nuttallianum*), nutritionally superior to July dominants (*Aconitum columbianum*, *Eriogonum umbellatum* and *Helianthella quinquenervis*)?

Here, consistent findings of *Aconitum columbianum* and *Eriogonum umbellatum* put this hypothesis in dispute. The pollen morphology of these two species complicated identification as their characteristics could be applied to varying observable characteristics in our cell-pollen samples. Furthermore, pollen of *Eriogonum umbellatum* was alike the pollen of numerous other flower species but was applied to all similar pollen samples to be consistent. Characterization of the morphological characteristics of pollen samples taken from floral resources is likely needed for a more thorough and exact identification between flower-pollen samples and those sampled from *Hoplitis* nests.

Request for characterization is supported by the lack of overlap consistently found between cells with *Aconitum columbianum* and *Eriogonum umbellatum* present; and those flowering species surveyed locally at the same time.

Interestingly, the bimodal peaks observed in the pollen provision counts of *Hoplitis* throughout the summer (Figure 15) seem to indicate that more provisions are made during a male-biased ovipositing period. However, solitary bees do not necessarily lay more males than females, with variation observed in sex ratios (Johnson, 1988). This could do with a tendency for solitary bees to produce more males when provision masses are lighter (Johnson, 1988), which could be a consequence of increasing drought and temperature during the summer months reducing pollen and nectar production; as well as the protein and nutritional composition of these produce (Descamps et al., 2021; Descamps et al., 2021; Waser & Price, 2016). This offspring preference is intriguing, considering that the alternative to producing a male under these conditions is producing smaller, less viable, female offspring (Bosch, 2008).

Consideration of results should contemplate two factors:

(1) Monthly variance may not be a perfect cut-off for measuring changes in the diversity/composition of pollen diets. This is because June sampling was limited, with sampling beginning in the latter end of the month, and at a lesser rate than would occur later in the season. Furthermore, June sampling occurred shortly after *Hoplitis* emergence, and may not be an exact reflection of true foraging and pollen-collecting behavior. For instance, solitary bees have been shown to experience seasonal declines in lipid reserves and oocyte sizing following spring emergence (O'Neill et al., 2015).

(2) Consistent findings of *Aconitum columbianum* and *Eriogonum umbellatum* remain largely unexplained. However, much of the same conclusions can be made even if their consistent presence in our pollen samples is subtracted from analysis. Additionally, studies of this kind could benefit from efforts to characterize the morphology of sampled and surveyed pollen, to best obtain exact matches.

This study system holds promise for investigation of further questions regarding the composition of *Hoplitis* nests across temporal and spatial scales; as well as how variation in seasonality/phenology may be reflected in pollen acquisition, and how inferences about fitness decisions may be made from analysis of pollen diets

Conclusion

Hoplitis and *Osmia* differ in foraging activity, both temporally and thermally. Furthermore, the pollen diet of *Hoplitis fulgida* was seen to change remarkably throughout the summer. Further examination and corroboration of these findings could determine inferences on the behavioral and dietary capacities of these taxa to survive under potential climate change. This study was limited by observational capacity (one-person, manual monitoring) and an inability to characterize pollen comparisons due to time constraints. With consideration of these limitations, our findings still represent segmentation in the foraging activity of *Osmia* and *Hoplitis*, with potential to clarify niche-partitioning existence between taxa and extrapolate to similar study systems. Moreover, studies of the resource-acquisition tendencies of *Hoplitis*, and more broadly, solitary bees, could shed light on temporal, spatial, nutritional and fitness-depending differentiation in the intra and interspecific diets of taxa. Future studies would benefit from efforts to obtain as much information as possible on these study systems. For instance, full diel observations with exact accounts of pollen and substrate carrying events, and precise data on the times, sequencing (pollen preference, counts) and sourcing (which flower? from where?) of pollen provisioning.

Appendix

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Figures & Tables

Pairwise Comparisons Results

contrast	estimate	SE	df	t_ratio	p_value
Hoplitis - Megachile	-1.344	1.077	62.7	-1.248	0.5991
Hoplitis - Osmia	0.443	0.143	883.7	3.087	0.0112
Hoplitis - Wasp	-0.129	0.193	1002.6	-0.671	0.9079
Megachile - Osmia	1.787	1.078	62.9	1.657	0.3550
Megachile - Wasp	1.214	1.089	65.1	1.115	0.6814
Osmia - Wasp	-0.572	0.204	965.5	-2.808	0.0261

Table 1 (above): Results of Linear-Mixed Effect Model for Timing Effect on Taxon. Estimate demonstrates difference in predicted values. Standard error indicates the precision of the estimated difference, df reflects the amount of data used in the pairwise comparison, t_ratio shows how many standard errors the estimate is away from zero. P_value < 0.05 indicates significance, shown in *Hoplitis - Osmia* and *Osmia - Wasp*.

Table 2 (below): ANOVA Results for Timing Effect on Foraging Activity. Highly significant effect of taxon on foraging activity shown, F value of 23.75 and small p-value indicate that the differences seen in foraging activity are not due to random chance.

ANOVA Results for Timing Effect on Foraging Activity

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
taxon	3	150.4	50.14	23.75	6.7e-15 ***
Residuals	1112	2347.7	2.11	NA	

ANOVA Results for Temperature Effect on Foraging Activity

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
taxon	3	178	59.34	4.62	0.00328
Residuals	760	9762	12.85	NA	NA

Table 3 (above): ANOVA Results for Temperature Effect on Foraging Activity. Highly significant effect of taxon on foraging activity shown. Differences not due to random chance, temperature plays a significant role in influencing foraging activity.

Table 4 (right): Results of Linear-Mixed Effect Model for Temperature Effect on Taxon. P-value < 0.05 indicates significance, shown in *Hoplitis-Osmia* and *Osmia-Wasp*.

Pairwise Comparisons Results

Contrast	Estimate	SE	DF	T-ratio	P-value
Hoplitis - Megachile	-1.033	3.497	43.0	-0.295	0.9909
Hoplitis - Osmia	1.351	0.313	755.0	4.310	0.0001
Hoplitis - Wasp	-0.181	0.390	745.1	-0.464	0.9668
Megachile - Osmia	2.383	3.500	43.0	0.681	0.9037
Megachile - Wasp	0.852	3.512	43.6	0.242	0.9949
Osmia - Wasp	-1.532	0.427	747.4	-3.584	0.0020

Pairwise Comparisons Results

Contrast	Estimate	SE	DF	T-ratio	P-value
Hoplitis - Megachile	-7451	5293	48.4	-1.408	0.3449
Hoplitis - Osmia	2861	1702	39.7	1.682	0.2248
Megachile - Osmia	10312	5230	50.0	1.972	0.1298

Table 5 (top left): Results of Linear-Mixed Effect Model for Timing Effect on Substrate Collection. No significant relationships shown.

Table 6: bottom left): ANOVA Results for Timing of Substrate Collection. Highly significant effect of timing on substrate collection across taxon. Differences not due to random chance, timing plays a significant role in influencing substrate-collection timing.

ANOVA results for Timing of Substrate Collection

Term	Df	Sum Sq	Mean Sq	F value	Pr(>F)
taxon	2	3.437e+08	171846235	7.323	0.00081
Residuals	253	5.937e+09	23466580	NA	NA

Table 7 (top right): Results of Linear-Mixed Effect Model for Timing Effect on Pollen Collection. No significant relationships shown.

Table 8 (bottom right): ANOVA Results for Timing of Pollen Collection. No significant relationship found in the pollen collection timing between taxon. Results not due to random chance, timing does not play a significant role in influencing pollen-collection timing.

Pairwise Comparisons Results

Contrast	Estimate	SE	DF	T-ratio	P-value
Hoplitis - Osmia	1525	1200	134	1.272	0.4137
Hoplitis - Wasp	-1851	3175	190	-0.583	0.8294
Osmia - Wasp	-3376	3097	197	-1.090	0.5214

ANOVA Results for Timing of Pollen Collection

Term	Df	Sum Sq	Mean Sq	F value	Pr(>F)
taxon	2	1.475e+08	73763959	2.437	0.0896
Residuals	242	7.326e+09	30272127	NA	NA

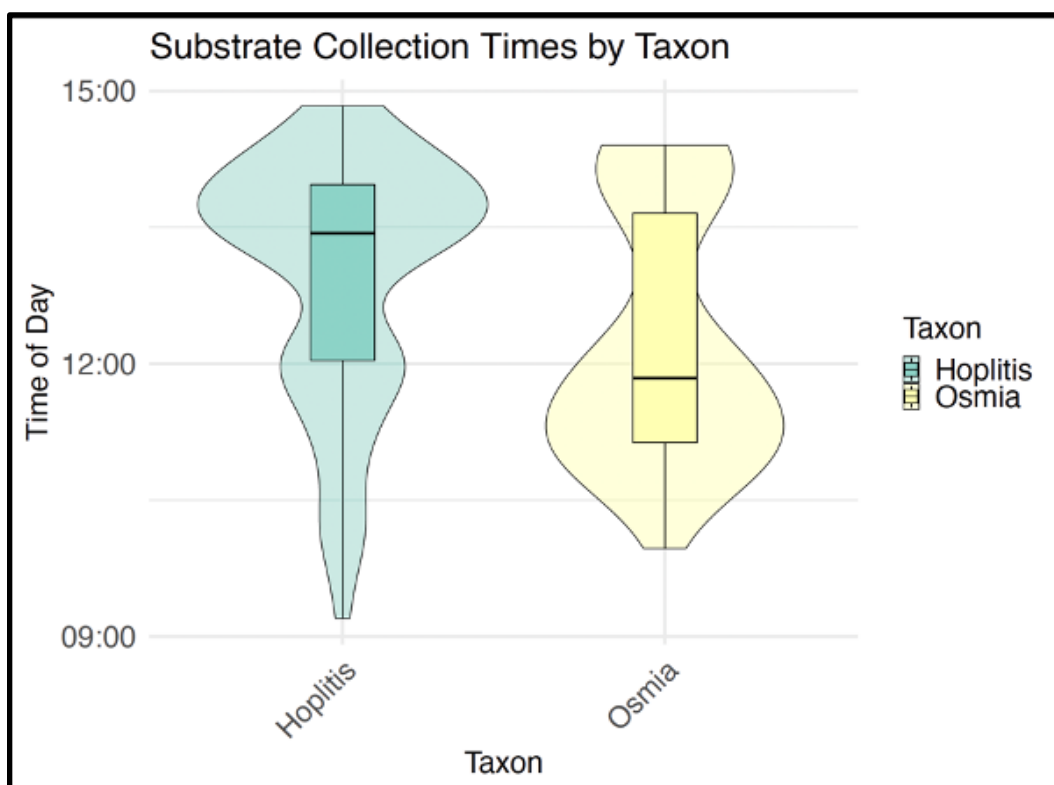
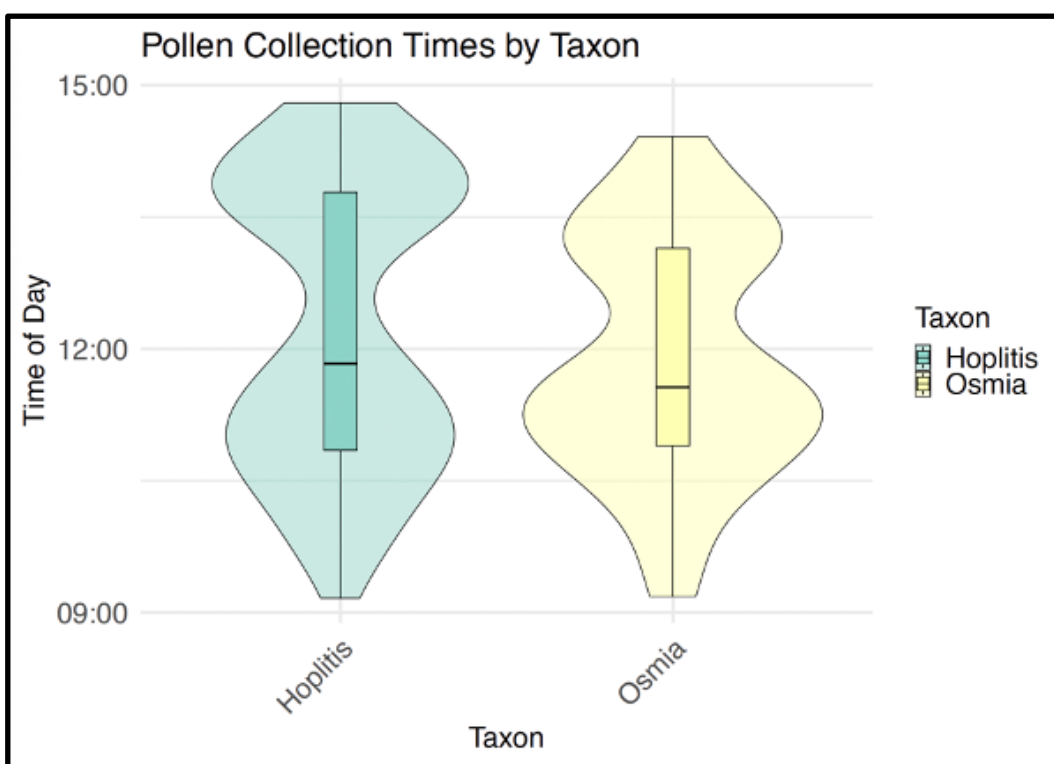


Figure 1 (above): Substrate Collection Times by Taxon. *Hoplitis* collected substrate over a longer period than *Osmia*, but with opposing peak collections in the afternoon and morning, respectively. Data biased by rapid substrate-collection trips demonstrated by *Hoplitis*, and not by *Osmia*. *Megachile* and *Wasp* excluded from figure due to low sample sizes.

Figure 2 (below): Pollen Collection Times by Taxon. Consistent, overlapping pollen collection demonstrated. Consistent with findings that the majority of foraging bouts serve to collect pollen. *Megachile* and *Wasp* excluded due to small sample sizes and lack of characterization on the purpose of foraging trips.



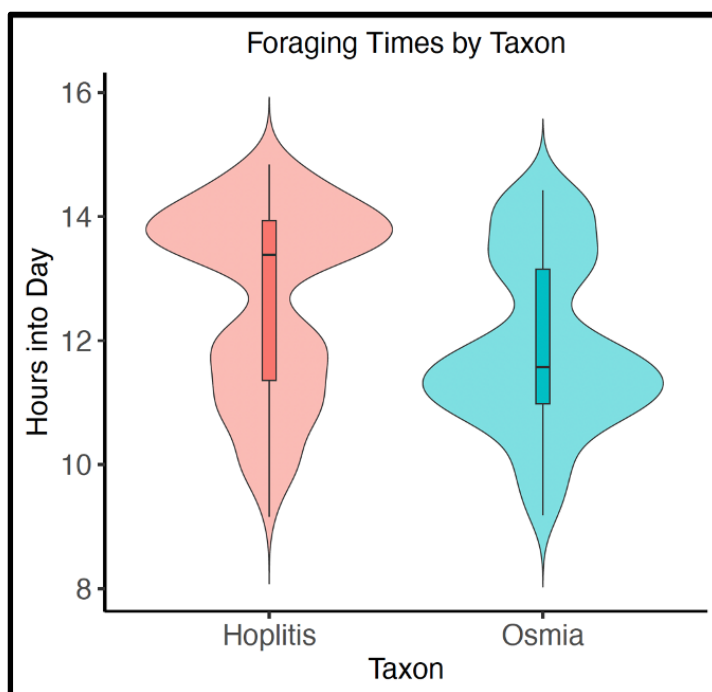
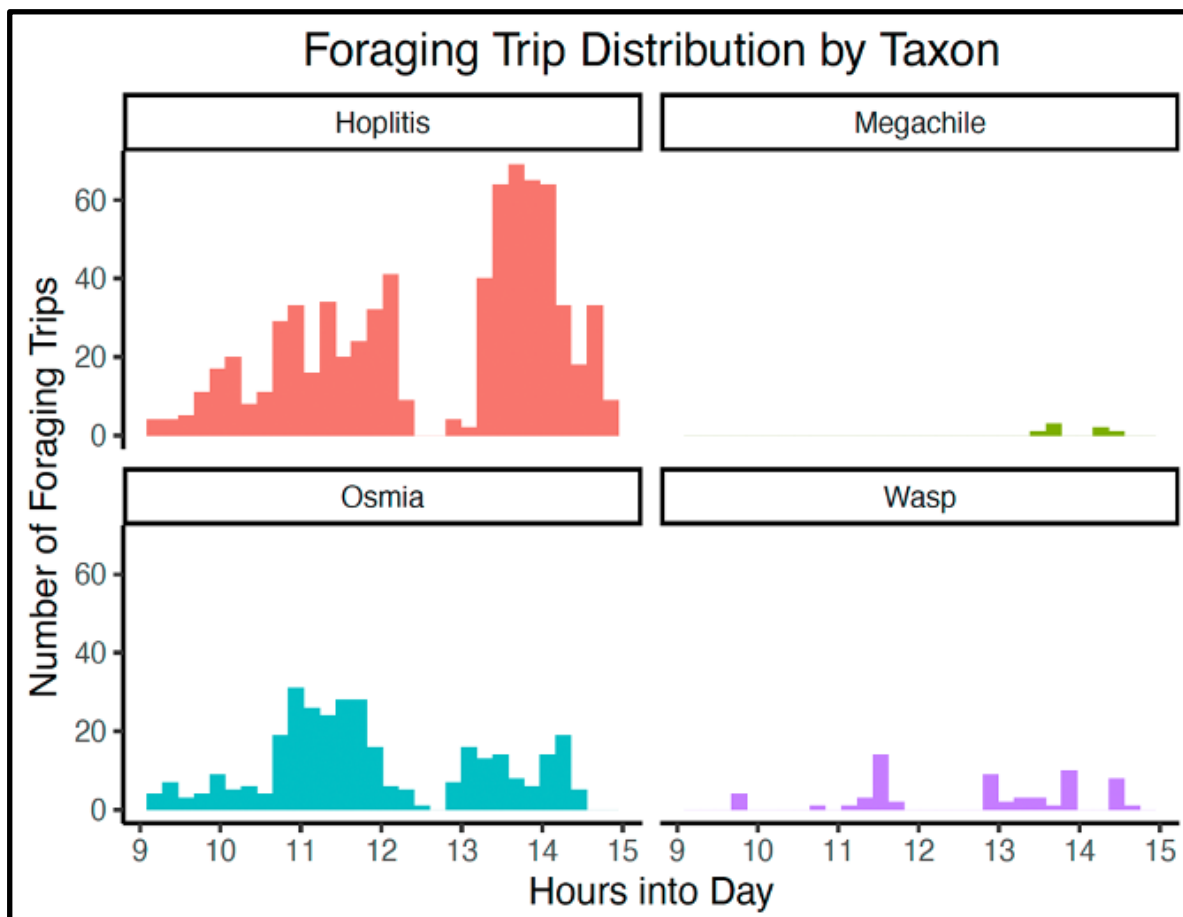


Figure 3 (above): Foraging Times by Taxon (combined box and violin plot). Temporal partitioning between taxa clear, with a late-day density in activity for *Hoplitis* and an early-day density for *Osmia*. *Megachile* and *Wasp* excluded due to small sample sizes.

Figure 4 (below): Foraging Trip Distribution by Taxon (fixed grid histograms). Abundance of foraging trips observed for each taxon visible on the y-axis, with total counts for *Hoplitis* being much higher than any other taxa. Limited sample sizes for *Megachile* and *Wasp* visible. Though consistent activity by *Wasp* was observed. With late-day emergence of *Megachile* being seen.



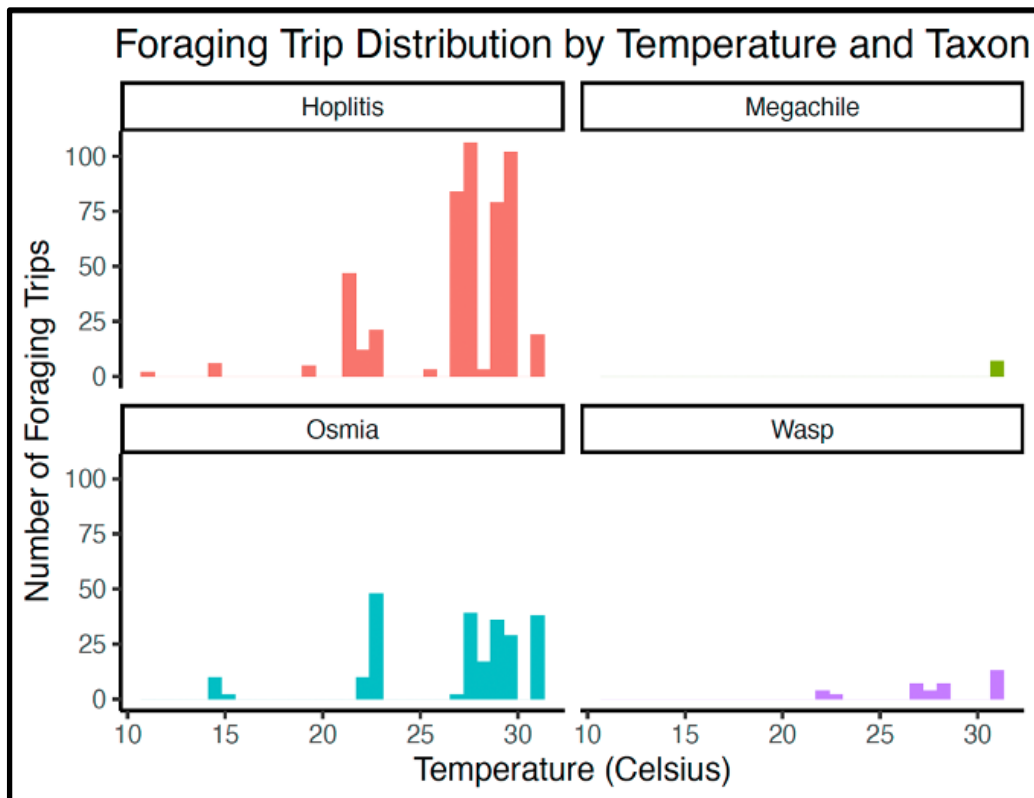
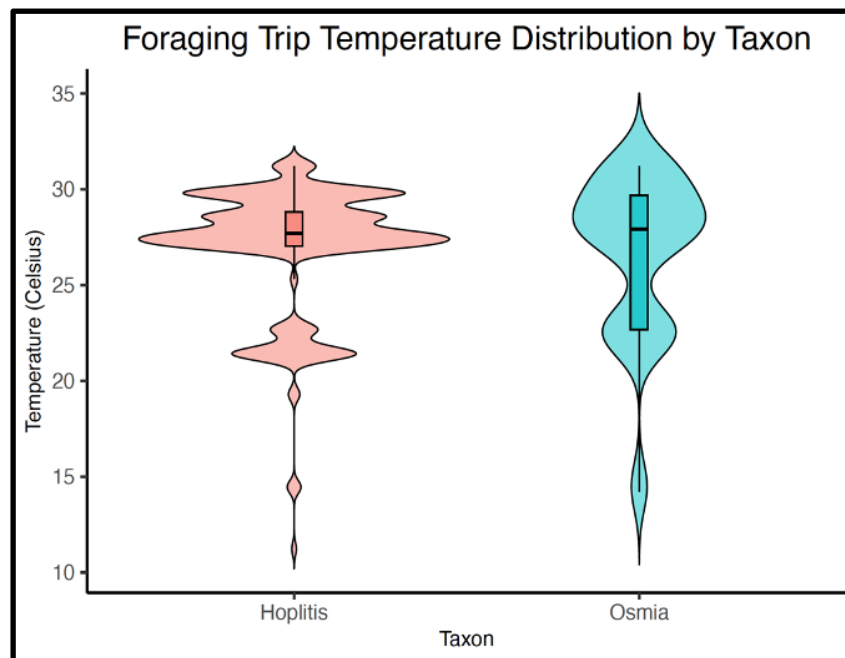


Figure 5 (above): Foraging Trip Distribution by Temperature and Taxon (fixed grid histograms). *Hoplitis* and *Osmia* active around similar temperature hotspots, though *Hoplitis* demonstrates more consistent activity, even emerging at lower temperatures than *Osmia*. Temperature data extrapolated from HOBO loggers, with data perhaps not overlapping consistently for *Megachile* and *Wasps*. Wasps prefer higher temperatures, as do *Megachile*. Though low sample sizes are observed.

Figure 6 (below): Foraging Trip Temperature Distribution by Taxon (combined box and violin plot). Similar median foraging trip temperatures shown between *Hoplitis* and *Osmia*. Fluctuating preferences in temperature demonstrated by *Hoplitis*, could be attributed to extraneous weather events. *Megachile* and *Wasp* excluded due to low sample sizes.



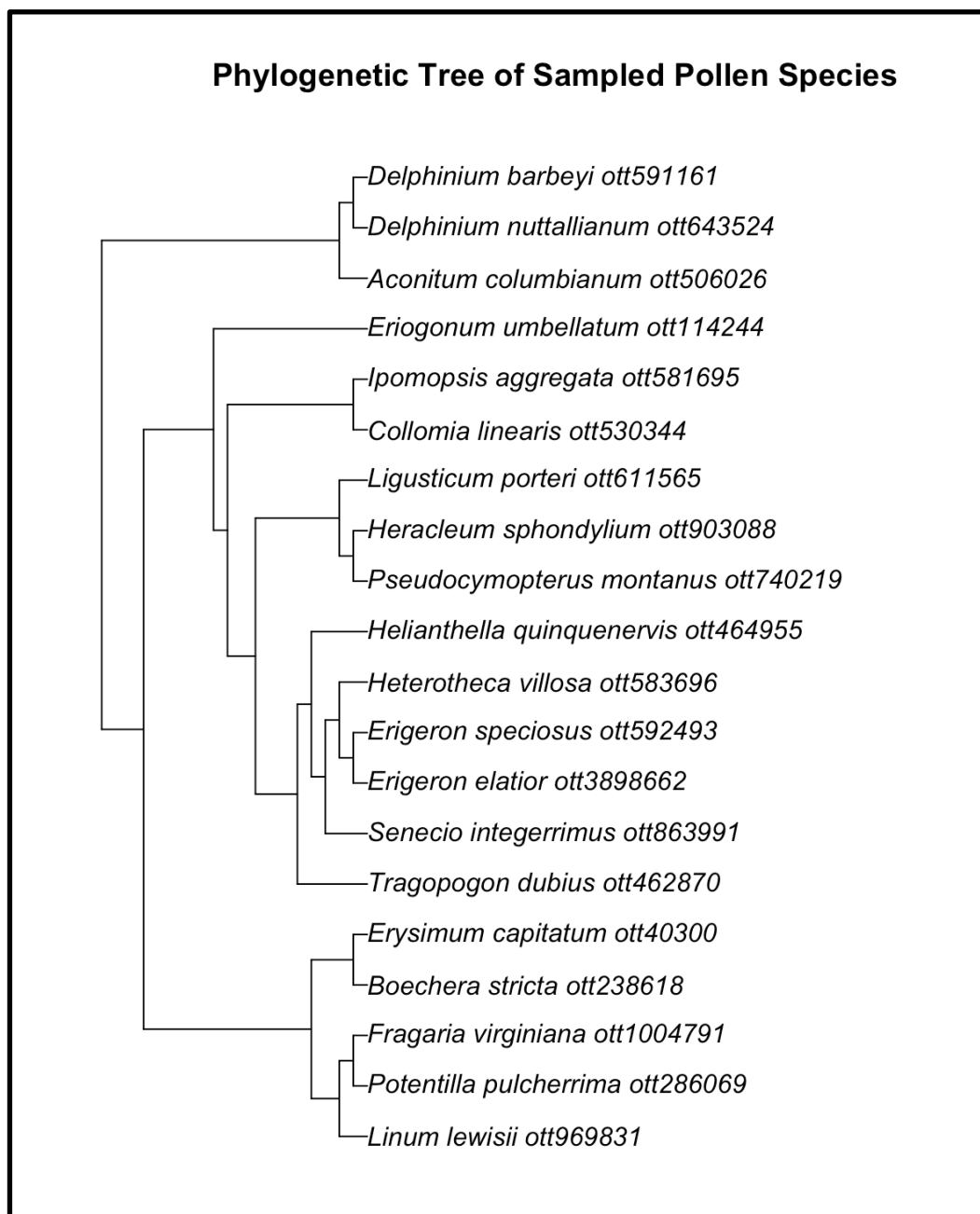


Figure 7 (above): Phylogenetic Tree of Sampled Pollen Species. Diversity of flowering species sampled from *Hoplitis* nests shown. Species shown come from 18 genera, 8 families and 8 orders. Abundance of families sampled clarifies the polylectic behavior of *Hoplitis*. Select sampled species not shown due to inability of database to correlate species names.

Phylogenetic Tree of Surveyed Flower Species

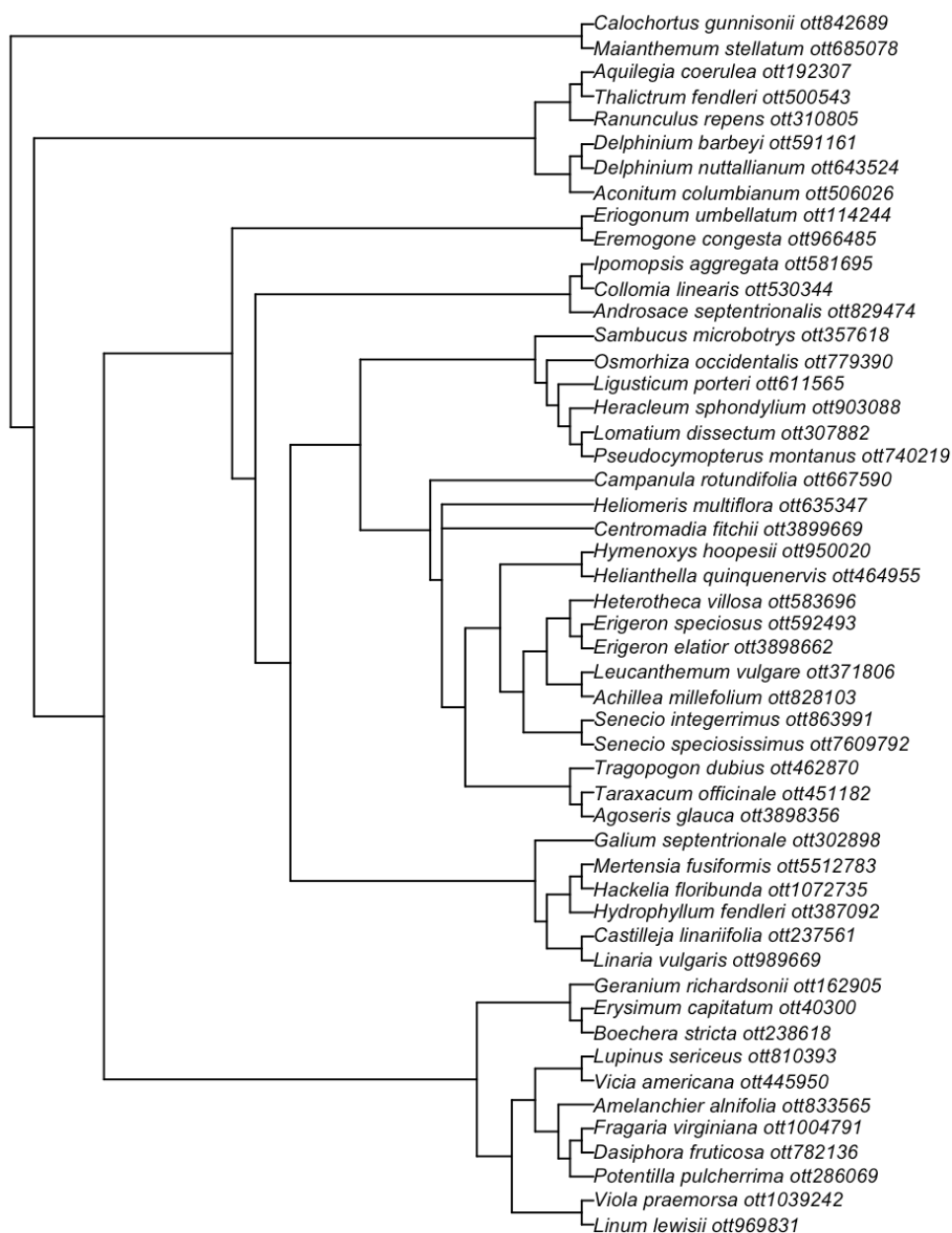


Figure 8 (above): Phylogenetic Tree of Surveyed Flower Species. Diversity of surveyed flowering species across sites shown. The 50 species portrayed come from 42 genera, 19 families and 14 orders. Select surveyed species not shown due to inability of database to correlate /confirm species names.

Taxonomic Summary - Sampled Pollen Species	
Rank	Unique Count
class	1
division	1
family	8
genus	18
infrakingdom	1
kingdom	1
order	8
species	21

Table 9 (above): Taxonomic Summary of Sampled Pollen Species. 21 species matched with a wide array of diversity portrayed. Species sampled came from 18 unique genera, 8 families and 8 orders. Select sampled species excluded due to inability of database to match species names.

Table 10 (below): Taxonomic Summary of Surveyed Flowering Species. 50 species matched with a wide array of diversity portrayed. Species surveyed same from 42 unique genera, 19 families and 14 orders. Selected surveyed species excluded due to inability of database to match species names.

Taxonomic Summary - Surveyed Flowers	
Rank	Unique Count
class	1
division	1
family	19
genus	42
infrakingdom	1
kingdom	1
order	14
species	50

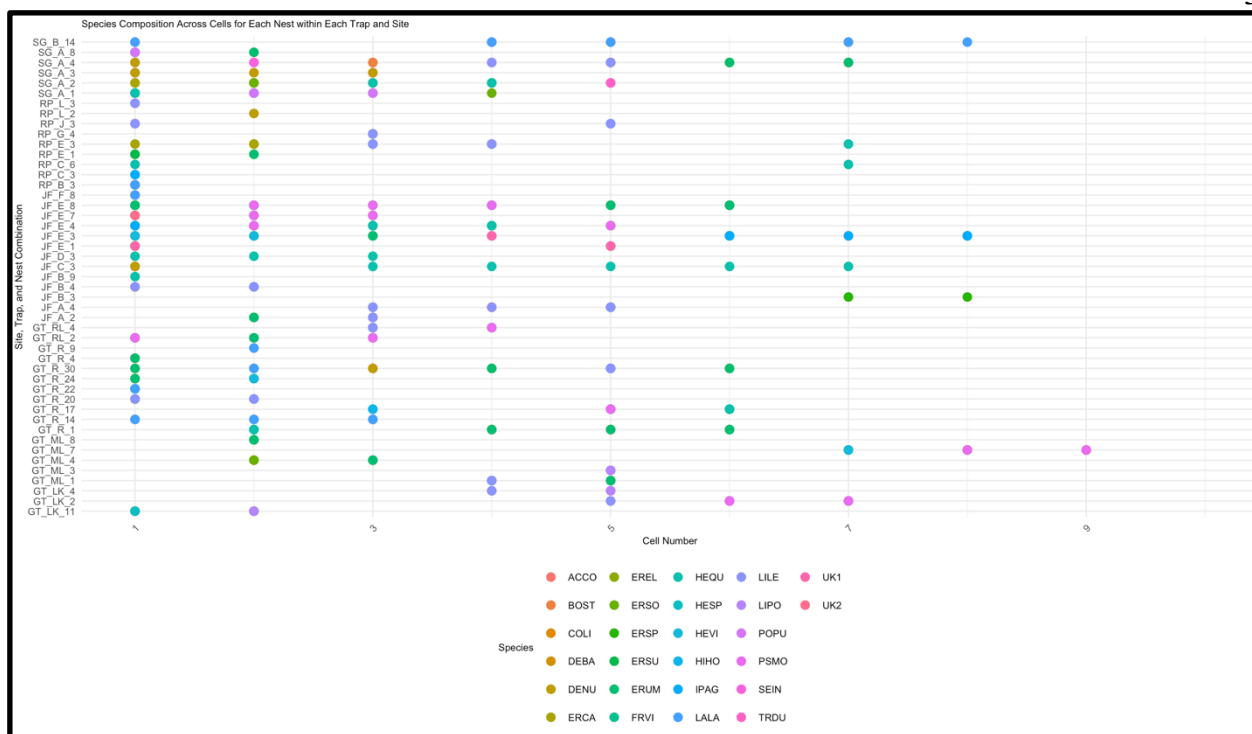
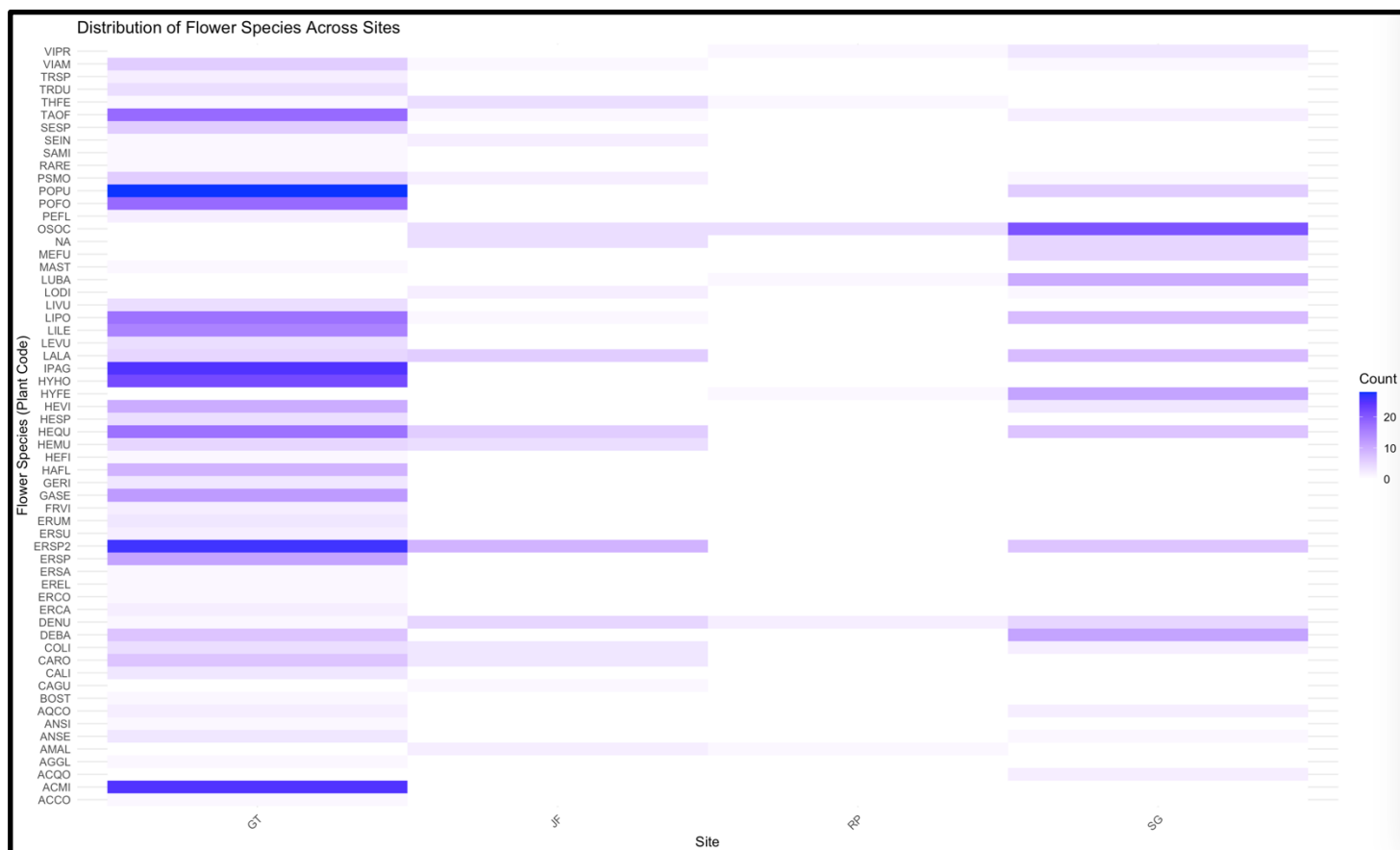


Figure 9 (above): Species Composition Across Cells for Each Unique Nest. Primary pollen composition for each cell is shown, with the y-axis showing unique site-block-nest combinations that cells belong to. Primary pollen composition is seen to be consistent in some nests and vary across the construction of others.

Figure 10 (below): Distribution of Flower Species Across Sites. Saturation demonstrates abundance, while the number of bars present demonstrates richness. Gothic Townsite is seen to have the highest species richness and abundances amongst those species. Followed sequentially by South Gothic, Judd Fall and Rosy Point. Data likely biased by the number of observations that occurred at each site.



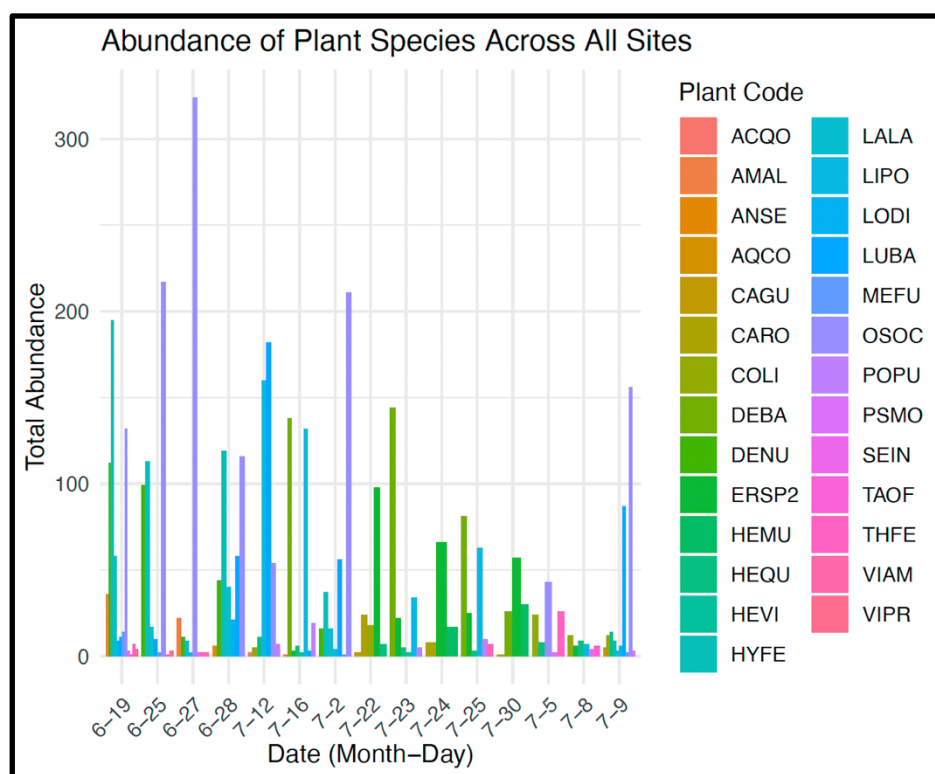
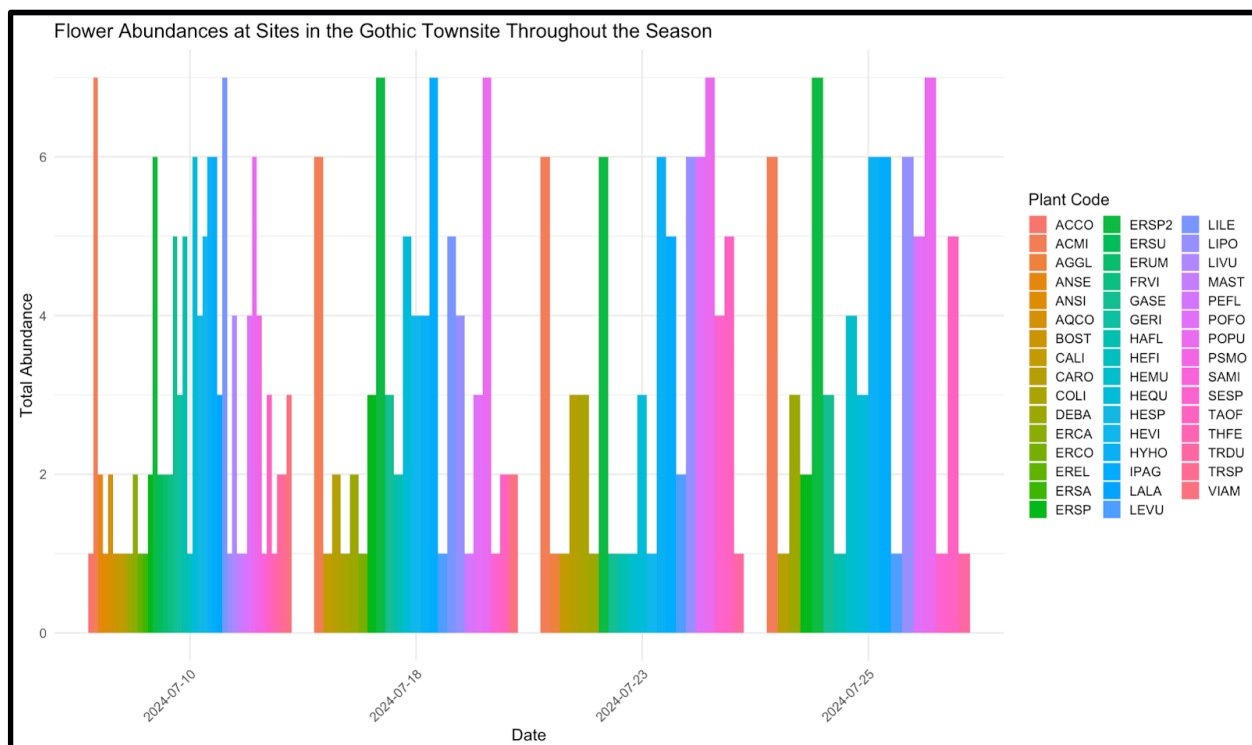


Figure 11 (above): Abundance of Plant Species Across All Sites. Excluding the Gothic Townsite, where species counts were not performed and only the names of species present were recorded. Species counts seem to rise steadily until mid-July, when counts begin to decrease. This may be accurate but may also reflect the decreasing number of surveys that were performed at sites with surveyed counts, as Judd Falls and South Gothic were visited less due to decreasing bee populations.

Figure 12 (below): Flower Abundances at Sites in the Gothic Townsite Throughout the Season. The number of times that species were present at sites within the Gothic Townsite were totaled within each of the four blocks that Townsite surveys were performed. Consistent species presences are reflected, with increasing diversity throughout the season.



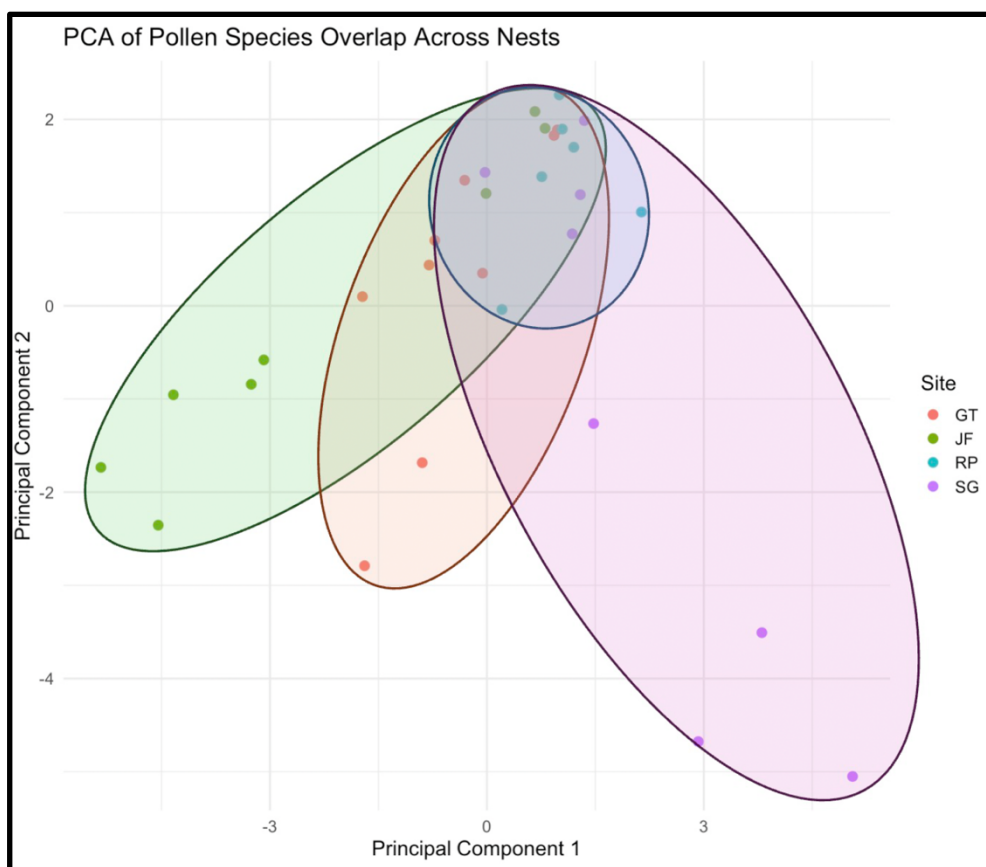
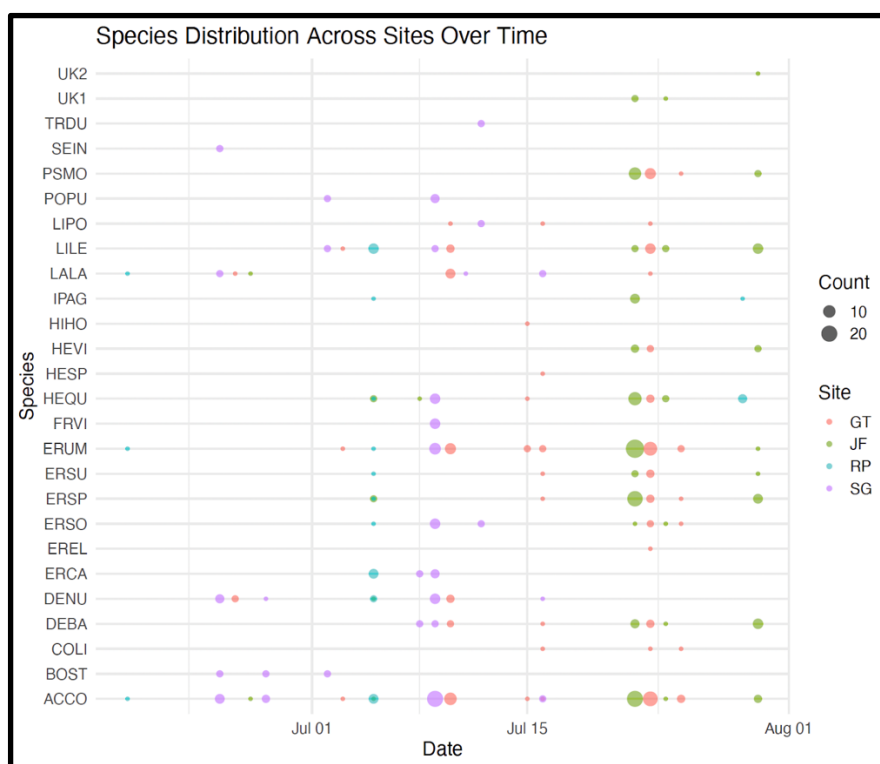


Figure 13 (above): Principal-component-analysis of Pollen Species Overlap Across Nests. Color-coded by the site that each nest belonged to, the pollen composition of nests was reduced and graphed on nondimensional axes to visualize composition overlap. Through a PERMANOVA analysis, the pollen composition across sites was found to differ significantly.

Figure 14 (below): Species Distribution Across Sites Over Time. Bubbles are color coded to distinguish between sites. The pollen of species found in nests varies considerably over the course of the season.



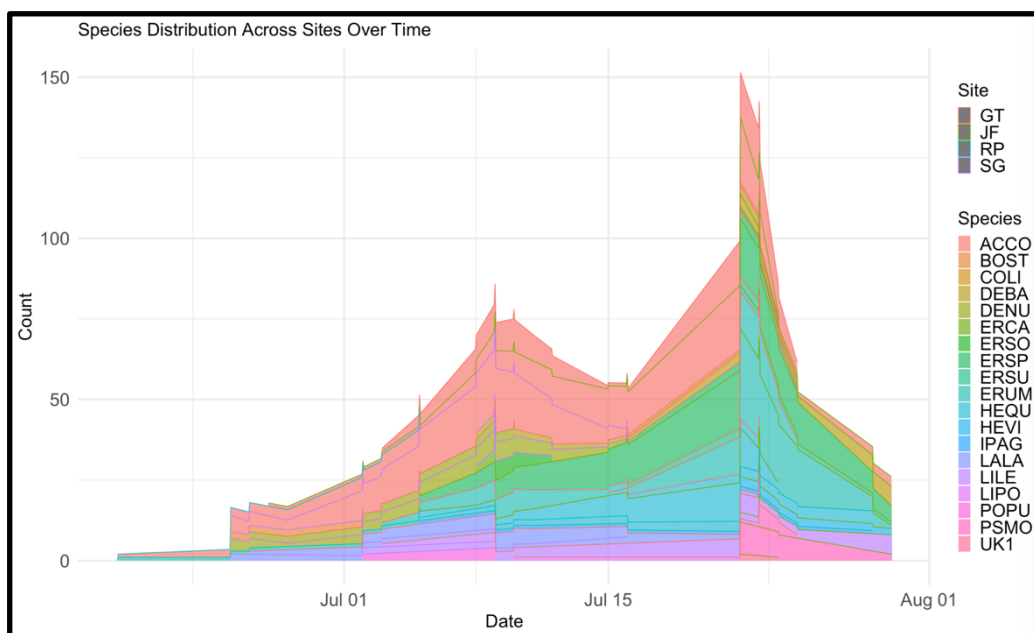
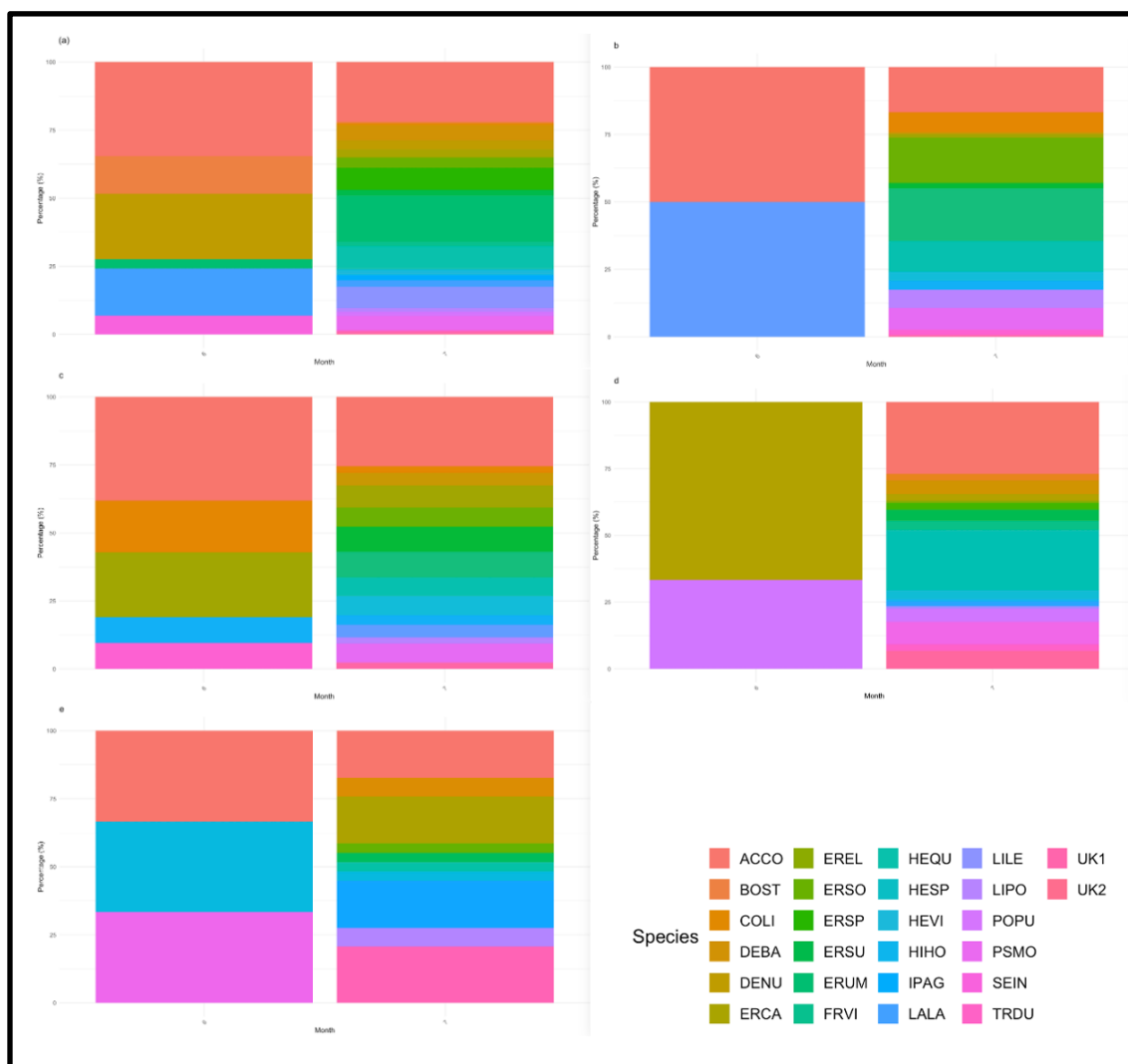


Figure 15 (above): Species Distribution Across Sites Over Time. Counts of sampled pollen identities across the season. Demonstrates bimodal peaks in pollen-collection and nest-construction in early July and late July.

Figure 16 (below): Diversity of Pollen Species Monthly. Stacked histogram visualizes the massive increase in diversity of cell-pollen composition between the two months where pollen was sampled. Graph does not accurately represent the number of sampling periods that were performed in each month.



Pollen Distribution by Species and Month

Month	Species	Count	Percentage
6	ACCO	10	34.500
6	BOST	4	13.800
6	DENU	7	24.100
6	ERUM	1	3.450
6	LALA	5	17.200
6	SEIN	2	6.900
7	ACCO	84	21.900
7	BOST	2	0.522
7	COLI	3	0.783
7	DEBA	21	5.480
7	DENU	13	3.390
7	ERCA	11	2.870
7	EREL	1	0.261
7	ERSO	14	3.660
7	ERSP	31	8.090
7	ERSU	8	2.090
7	ERUM	65	17.000
7	FRVI	6	1.570
7	HEQU	32	8.360
7	HESP	1	0.261
7	HEVI	7	1.830
7	HIHO	1	0.261
7	IPAG	7	1.830
7	LALA	9	2.350
7	LILE	30	7.830
7	LIPO	5	1.310
7	POPU	6	1.570
7	PSMO	20	5.220
7	TRDU	2	0.522
7	UK1	3	0.783
7	UK2	1	0.261

Chi-Squared Test Results

Statistic	Value
X-squared	237.47
df	75
P-value	< 2.2e-16

PERMANOVA Results

Term	Df	SumOfSqs	R2	F	Pr(>F)
site	3	172.18	0.22075	2.5495	0.001
Residual	27	607.82	0.77925	NA	NA
Total	30	780.00	1.00000	NA	NA

Pollen Distribution by Species and Month for Site SG

Month	Species	Count	Percentage
6	ACCO	8	38.095238
6	BOST	4	19.047619
6	DENU	5	23.809524
6	LALA	2	9.523810
6	SEIN	2	9.523810
7	ACCO	22	25.581395
7	BOST	2	2.325581
7	DEBA	4	4.651163
7	DENU	7	8.139535
7	ERCA	6	6.976744
7	ERSO	8	9.302326
7	ERUM	8	9.302326
7	FRVI	6	6.976744
7	HEQU	6	6.976744
7	LALA	3	3.488372
7	LILE	4	4.651163
7	LIPO	2	2.325581
7	POPU	6	6.976744
7	TRDU	2	2.325581

Pollen Distribution by Species and Month for Site GT

Month	Species	Count	Percentage
6	DENU	2	66.666667
6	LALA	1	33.333333
7	ACCO	32	26.8907563
7	COLI	3	2.5210084
7	DEBA	6	5.0420168
7	DENU	3	2.5210084
7	EREL	1	0.8403361
7	ERSO	3	2.5210084
7	ERSP	5	4.2016807
7	ERSU	4	3.3613445
7	ERUM	27	22.6890756
7	HEQU	4	3.3613445
7	HESP	1	0.8403361
7	HEVI	2	1.6806723
7	HIHO	1	0.8403361
7	LALA	6	5.0420168
7	LILE	10	8.4033613
7	LIPO	3	2.5210084
7	PSMO	8	6.7226891

Pollen Distribution by Species and Month for Judd Falls

Month	Species	Count	Percentage
6	ACCO	1	50.000
6	LALA	1	50.000
7	ACCO	25	16.800
7	DEBA	11	7.380
7	DENU	1	0.671
7	ERSO	2	1.340
7	ERSP	25	16.800
7	ERSU	3	2.010
7	ERUM	29	19.500
7	HEQU	17	11.400
7	HEVI	5	3.360
7	IPAG	5	3.360
7	LILE	10	6.710
7	PSMO	12	8.050
7	UK1	3	2.010
7	UK2	1	0.671

Pollen Distribution by Species and Month for Site RP

Month	Species	Count	Percentage
6	ACCO	1	33.333333
6	ERUM	1	33.333333
6	LALA	1	33.333333
7	ACCO	5	17.241379
7	DENU	2	6.896552
7	ERCA	5	17.241379
7	ERSO	1	3.448276
7	ERSP	1	3.448276
7	ERSU	1	3.448276
7	ERUM	1	3.448276
7	HEQU	5	17.241379
7	IPAG	2	6.896552
7	LILE	6	20.689655

Table 11 (starting left): Percent composition of pollen found in *Hoplitis* nests across sites and month. From left to right: all sites → South Gothic → Gothic Townsite → Judd Falls → Rosy Point.

Table 12 (middle): Chi-Squared Analysis on the pollen-composition of nests across sites. The small p-value indicates that pollen composition varies significantly across sites. However, analysis was performed on a contingency table, which may have been limited by a small sample size and lower than expected frequencies.

Table 13 (top right): PERMANOVA Results of Analysis of Principal-Component-Analysis. Multivariate analysis of the reduced dataset reduced by the principal-component-analysis of pollen composition across sites. Significant value indicates that the pollen composition of nests across sites is not due to random chance.

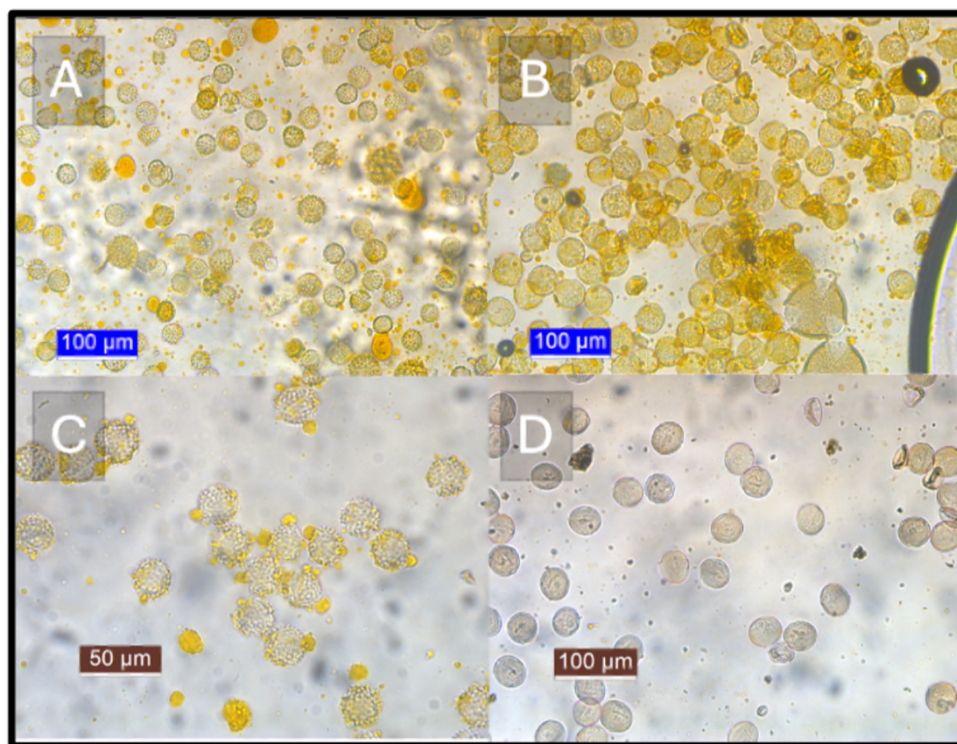
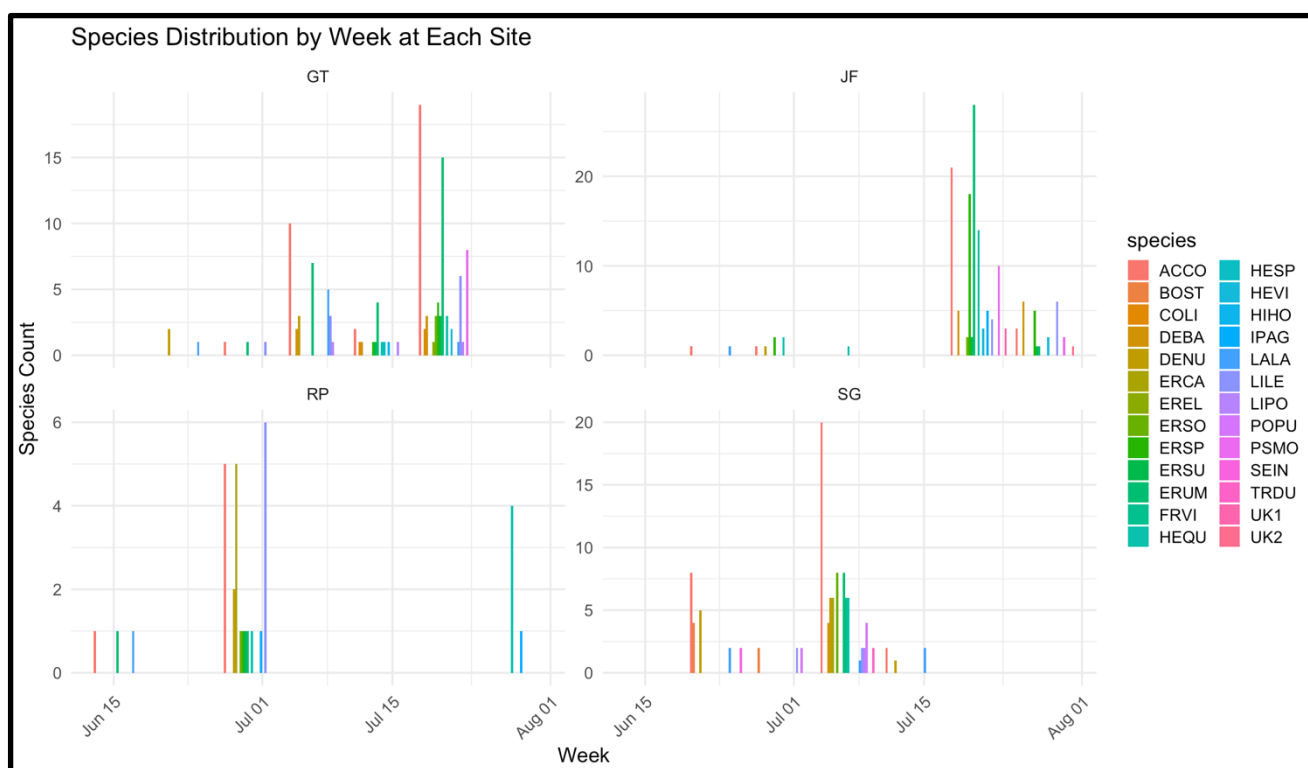


Figure 17 (above): Pollen Sampled from Flowers & *Hoplitis* nests. (A) Pollen sampled from a *Hoplitis* nest in Judd Falls [B3, 7/5/2024]. (B) Pollen sampled from a *Hoplitis* nest in the Gothic Townsite [R20, 7/3/2024]. (C) Pollen sampled from *Erigeron Speciosus*, and (D) Pollen sampled from *Lathyrus lanszwertii* var. *leucanthus*.

Figure 18 (below): Species Distribution by Week at Each Site. Species identity of pollen in *Hoplitis* nests over the season. Spread of distribution reflects the sampling periods that were performed at each site. Therefore, Rosy Point's distribution may not accurately reflect the true seasonally dependent pollen composition of *Hoplitis* nests, as the site was not visited as frequently as the others. Of the remaining sites, Judd Falls and South Gothic exhibit stagnant periods or drop-offs due to decreased use of the site's nests by *Hoplitis* individuals. While the Gothic Townsite demonstrates an increased preference by *Hoplitis* individuals over the course of the summer.



Plant Codes and Scientific Names

Plant Code	Scientific Name
ACCO	Aconitum columbianum
ACMI	Achillea millefolium
AGGL	Agoseris glauca
AMAL	Amelanchier alnifolia
ANSE	Androsace septentrionalis
ANSE	Androsace septentrionalis
AQCO	Aquilegia coerulea
BOST	Boechera stricta
CAGU	Calochortus gunnisonii
CALI	Castilleja linariifolia
CARO	Campanula rotundifolia
COLI	Collomia linearis
DEBA	Delphinium barbeyi
DENU	Delphinium nuttallianum
ERCA	Erysimum capitatum
ERCO	Eremogone congesta
EREL	Erigeron elatior
ERSA	
ERSP	Erigeron speciosus
ERSP2	Erigeron speciosus
ERSU	Eriogonum subalpinum
ERUM	Eriogonum umbellatum
FRVI	Fragaria virginiana
GASE	Galium septentrionale
GERI	Geranium richardsonii
HAFL	Hackelia floribunda
HEFI	Hemizonia fitchii
HEMU	Heliomeris multiflora
HEQU	Helianthella quinquenervis
HESP	Heracleum sphondylium
HEVI	Heterotheca villosa

HYFE	Hydrophyllum fendleri
HYHO	Hymenoxys hoopesii
IPAG	Ipomopsis aggregata
LALA	Lathyrus lanszwertii var. leucanthus
LEVU	Leucanthemum vulgare
LILE	Linum lewisii
LIPO	Ligusticum porteri
LIVU	Linaria vulgaris
LODI	Lomatium dissectum
LUBA	Lupinus barbiger
MAST	Maianthemum stellatum
MEFU	Mertensia fusiformis
OSOC	Osmorhiza occidentalis
PEFL	Pentaphylloides floribunda
POFO	Potentilla forsteriana
POPU	Potentilla pulcherrima
PSMO	Pseudocymopterus montanus
RARE	Ranunculus repens
SAMI	Sambucus microbotrys
SEIN	Senecio integerrimus
SESP	Senecio speciosissimus
TAOF	Taraxacum officinale
THFE	Thalictrum fendleri
TRDU	Tragopogon dubius
TRSP	
VIAM	Vicia americana
VIPR	Viola praemorsa