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Patterns of nest-site selection by *Colletes thoracicus* within a forested watershed

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Abstract – Despite the importance of suitable nesting habitat for bee conservation, the nesting requirements of most species remain unknown. We sought to better understand the distribution of *Colletes thoracicus* Smith (Hymenoptera: Colletidae) nesting aggregations within a forested watershed in GA, USA. We found 13 and 17 aggregations within the watershed in 2022 and 2023, respectively, and seven of these occurred at the same locations between years. The aggregations were found exclusively along the banks of stream channels and, compared to randomly selected non-aggregation sites, were associated with sandier and drier soil and with the presence of the herbaceous perennial, *Podophyllum peltatum* L. We suggest that the association with *P. peltatum* may result from favorable disturbances to the litter layer caused by the springtime emergence of the plant. Our results demonstrate that closed-canopy forests, and especially the sandier soils bordering streams, can provide suitable nesting sites for *C. thoracicus* and possibly other ground-nesting bee species.

Liriodendron tulipifera **/ mayapple / nest site / philopatry / pollinators / site fidelity / soil texture**

1. INTRODUCTION

Like the ground-nesting wasps from which they diverged, roughly three-quarters of all bee species today nest below ground in tunnels and chambers specially excavated for this purpose (Antoine and Forrest 2021). Despite this, and although suitable nesting habitat is just as important to the reproductive success of bees as the availability of floral resources, the nesting requirements of most species remain unknown. Decisions ground-nesting bees make about where to nest undoubtedly have important implications for their fitness. Because numerous factors—including distance to floral resources, exposure to pathogens and predators, and microclimatic conditions—interact to affect nesting success (Gathmann and Tscharntke 2002; Harmon-Threatt 2020; Antoine and Forrest 2021), nests can be expected to be patchily distributed. Open habitats with exposed soil are generally thought to provide the most favorable nesting conditions for many ground-nesting bee species (Potts et al. 2005; Ulyshen et al. 2021). However, in landscapes historically dominated by closed-canopy forests, such conditions would have been relatively uncommon. While the exposed soil and gaps created by tree falls may satisfy the nesting requirements for some bees in forests (Campbell et al. 2017), the possibility that some species nest beneath closed canopies remains, except for certain bumblebees (Williams et al. 2019), largely unexplored. The main challenge hindering progress in this area is the difficulty of locating a suitable number of nest

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sites for quantitative study. This challenge is more easily overcome for species that form conspicuous nesting aggregations.

Colletes thoracicus Smith (Hymenoptera: Colletidae) is a univoltine spring-active bee native to eastern North America. Although known to visit a wide range of flowers, flowering trees (especially *Liriodendron tulipifera*) are a major source of pollen for the species (Batra 1980). Active nesting aggregations of *C. thoracicus* are quite conspicuous as large numbers of males patrol for females above nest entrances. Previous work suggested the species may largely avoid nesting within closed-canopy forests, instead preferring open or disturbed areas such as canopy gaps, suburban back yards, or even agricultural fields (Batra 1980). However, beginning in 2019, we began noticing a consistent association between *C. thoracicus* aggregations and riparian forest habitats in northeastern GA. The association appeared so predictable that we decided to undertake an effort to record *C. thoracicus* aggregations within a single-forested watershed to better understand the spatial distribution and nest-site characteristics of the species.

2. METHODS AND MATERIALS

2.1. Study site

This study took place at the Georgia Botanical Gardens in Clarke County, GA, USA. The site consisted of a small watershed within a mature closed-canopy forest (33.901, – 83.379) (Fig. 1). Dominant overstory trees were typical for the region and included *Quercus*, *Carya*, *Liriodendron*, *Liquidambar*, *Fagus*, *Fraxinus*, etc. The watershed drains into a beaver wetland just before it reaches the Middle Oconee River. The entire watershed above the wetland was searched for *C. thoracicus* aggregations in 2022 and 2023. This involved walking on both banks of all obvious channels (as depicted in Fig. 1), including side channels that are dry for most of the year. To facilitate locating aggregations, searches were made only from mid-morning to mid-afternoon on warm clear days in April when the bees were most active. Although we did not systematically search non-riparian areas of the watershed, no aggregations were observed more than 10 m from a stream channel at our study site.

2.2. Data collection

We recorded the coordinates of all aggregations as well as the approximate size of each aggregation in square meters based on where patrolling males were concentrating their activity. In 2022, several bees were collected from each aggregation to confirm their identity as *C. thoracicus* (using discoverlife.org and an established reference collection), and voucher specimens have been deposited in the Georgia Museum of Natural History, Athens, GA, USA. In 2023, we used a pin flag to mark the center of each aggregation and returned to collect environmental data from ten aggregations that were located across the watershed and that were separated by at least 35 m (Fig. 1). We collected the same information from 24 points without aggregations and these locations were selected randomly within 15 m of the river channel using ArcMap Version 10.4. Random points were more than 10 m from aggregation sites and 20 m from other random points (Fig. 1). Based on our observations or general expectations about nest site suitability for ground-nesting solitary bees, we collected data on fourteen variables we felt might be important predictors of aggregation sites. These were (1) canopy openness (measured using a convex densiometer (Lemmon 1956) in each cardinal direction and then averaged), (2) number of mature (diameter > 20 cm at 1.4 m above ground) *Lirodendron tulipifera* L. (tulip poplar) within a 10-m radius, (3) presence of *Podophyllum peltatum* L. (mayapple) within a 1-m radius, (4) indications of disturbance from flooding during the previous winter (visible as a zone of missing leaf litter); (5) indications of vertebrate digging; (6) distance to nearest stream channel; (7) presence of water within the channel; (8–9) slope and aspect as determined using a digital elevation model (DEM), from the USGS "3D Elevation Program" (https://

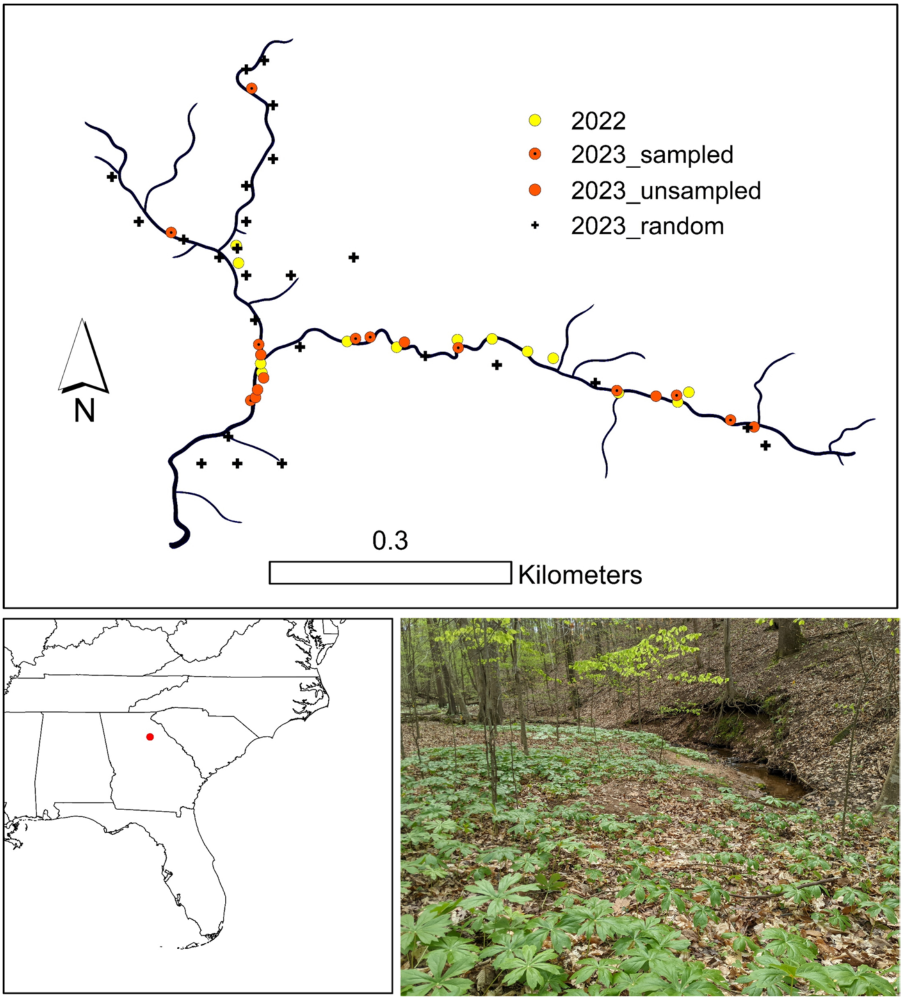

Figure 1 Map showing the watershed in northeastern Georgia that was searched for *Colletes thoracicus* aggregations in 2022 and 2023 (yellow and red dots, respectively). Dots from 2023 with black centers and black plus signs indicate aggregations and random locations, respectively, at which environmental data were collected in 2023. The photograph shows a typical aggregation site in the foreground (note the presence of *Podophyllum peltatum*) and the stream in the background

www.usgs.gov/3d-elevation-program), with a resolution of 1 m in ArcMap; (10) leaf litter weight (described below); (11) soil water content (described below); and (12–14) the sand, silt, and clay contents of soil (described below). For leaf litter weight, we collected all litter within a 25 × 25-cm quadrat, using a serrated knife to cut around the edges. These litter samples were dried for 4 days at 60 °C and weighed after removing any sticks, nuts, stones, or other non-leaf material. After collecting the litter samples and scraping away any other organic matter, we sampled the mineral soil to a depth of 10 cm. We determined the water content of each soil sample by comparing weights before and after drying for 4 days at 60 °C. Soil texture analysis was performed by the Cornell Soil Health Laboratory. This consisted of using sieves and suspensions to separate soil samples into sand, silt, and clay components and calculating the percentage

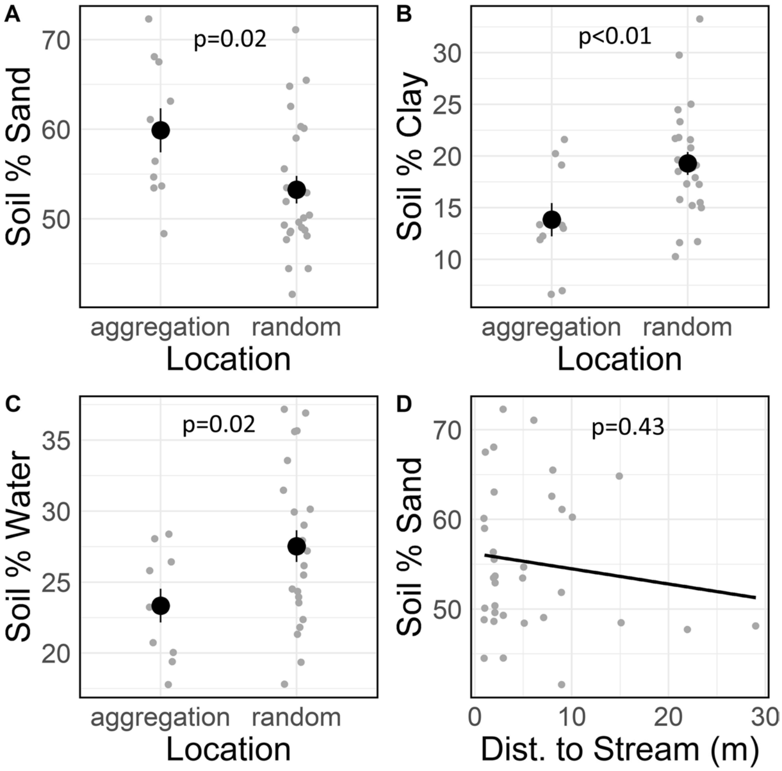

Figure 2 Mean ± SE of percent sand (**A**), clay (**B**), and water (**C**) contents of soil collected at the centers of *Colletes thoracicus* aggregations ($n = 10$) and at randomly selected locations ($n = 24$). The relationship between soil sand content and distance from stream is shown in panel **D**

of each by dry weight (for full methodological details, see https://soilhealth.cals.cornell.edu/).

2.3. Analysis

Our analysis, conducted in R 4.3.0 (R Core Team 2022), was mostly limited to data collected in 2023. However, we also included slope and aspect data, which were collected remotely from DEM layers, from 2022 aggregations. Because proportional data are rarely normal or homoscedastic, we used the betareg function of the betareg package (Zeileis, Cribari-Neto et al. 2016) to compare the sand, silt, clay, and water contents of soil between aggregations and randomly selected plots. We also fitted betareg models to examine the relationships between sand or clay proportions and the distance to the water. We then compared all non-proportional continuous responses (i.e., number of *L. tulipifera*, slope, aspect, and litter weight) using the Mann–Whitney *U* test after finding they violated the assumptions of *t*-tests. Finally, categorical responses (i.e., water in the channel, number of *P. peltatum*, recently flooded, and vertebrate digging) were compared using Chi-square tests.

3. RESULTS

We recorded 13 and 17 nesting aggregations of *C. thoracicus* within the watershed in 2022 and 2023, respectively. The total (mean ± SE) area occupied by the aggregations in 2022 and 2023 were 271 (20.85 ± 3.70) and 238

(14.00 ± 4.01) m^2, respectively. All aggregations occurred under closed-canopy conditions, with canopy openness above aggregations in 2023 ranging from just 1–7% (Table 1). Seven of the observed aggregations occurred at about the same locations in 2022 and 2023. By contrast, six of the aggregations observed in 2022 were no longer present at those locations in 2023, and ten of the aggregations in 2023 were in different locations compared to the previous year.

The soil at nine out of ten aggregations was classified as sandy loam while the remaining site was classified as sandy clay loam (Fig. 3). Compared to the random sites, the soil at aggregations was characterized by a significantly higher proportion of sand, lower proportion of clay, and lower water content (Table 1 and Fig. 2). Although we observed a negative trend between the sand content and the distance from

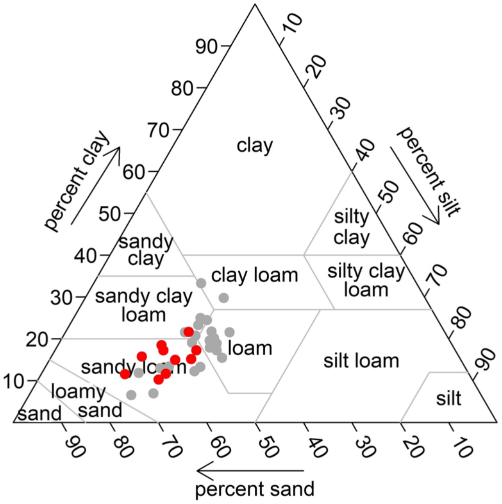

Figure 3 Soil-texture triangle showing soil properties at aggregation (red) and random (grey) sites

Table I Summary statistics of variables collected at each *Colletes thoracicus* aggregation (n = 10 except for slope and aspect for which n = 30) and random location (n = 24). For continuous variables, mean ± SE are provided, while percentages are given for categorical variables. The last column provides test statistics with p-values based on Beta Regression, Mann–Whitney U tests, or Chi-square tests

Variable type	Response	Aggregation	Random	Statistic
Continuous	soil % sand	59.9 ± 2.45 (range: 48.4–72.3)	53.2 ± 1.54 (range: 41.6–71.1)	Estimate: – 0.27, $\chi2 = 5.26$, $p = 0.02$
	Soil % silt	26.3 ± 1.34 (range: 19.9–32)	27.5 ± 0.97 (range: 17.2–35.3)	Estimate: 0.05, $\chi2 = 0.39$, $p = 0.53$
	Soil % clay	13.8 ± 1.62 (range: 6.6–21.6)	19.3 ± 1.11 (range: 10.3–33.3)	Estimate: 0.41, $\chi2 = 8.10$, $p < 0.01$
	Soil % water	23.3 ± 1.19 (range: 17.8–28.4)	27.5 ± 1.12 (range: 17.8–37.2)	Estimate: 0.21, $\chi2 = 4.63$, $p = 0.03$
	% Canopy openness	3.1 ± 0.67 (range: 1–7)	3.39 ± 0.5 (range: 1–10)	$U = 109$, $p = 0.82$
	Distance to water (m)	3.3 ± 0.76 (range: 1–9)	6.83 ± 1.47 (range: 1–29)	$U = 94$, $p = 0.33$
	No. *L. tulipifera*	2.5 ± 0.5 (range: 1–6)	1.88 ± 0.33 (range: 0–6)	$U = 149$, $p = 0.27$
	Aspect	204 ± 11.4 (range: 85–311)	204 ± 18.8 (range: 22.7–322)	$U = 330$, $p = 0.61$
	Slope	13.9 ± 1.3 (range: 2.76–31.2)	13.5 ± 1.25 (range: 3.77–25.4)	$U = 356$, $p = 0.95$
	Litter dry weight	17.9 ± 5.05 (range: 0–48.2)	17.9 ± 2.34 (range: 0–46.3)	$U = 121$, $p = 0.98$
Categorical	Water in stream	100.00%	91.67%	$\chi2 = 0.02$, $p = 0.89$
	Mayapples within 1 m	70.00%	16.67%	$\chi2 = 6.90$, $p = 0.01$
	Recently flooded	30.00%	12.50%	$\chi2 = 0.53$, $p = 0.47$
	Vertebrate digging	40.00%	8.33%	$\chi2 = 2.94$, $p = 0.09$

Figure 4 Bank scouring caused by winter flooding, January 2023

the nearest stream channel, the relationship was not significant (Estimate: – 0.01, pseudo-R^2: 0.02, $\chi 2 = 0.61$, $p = 0.44$) (Fig. 2D). We also found that *Podophyllum peltatum* was more commonly associated with aggregation sites than with random sites (Table 1).

4. DISCUSSION

Contrary to the reliance on open habitats proposed by Batra (1980), our findings show that *C. thoracicus* nests readily within closed-canopy forests. All aggregations observed in the current study occurred within a few meters of stream channels. A preference for sandy soil is the most likely explanation for this riparian association. Many ground-nesting bees prefer to nest in sandy soil (Harmon-Threatt 2020) and such a preference was suggested for *Colletes* in the previous studies (Batra 1980; Cane 1991). Indeed, the proportion of sand reported at *C. thoracicus* aggregations in the current study closely matched those reported for *C. inaequalis* aggregations in NY (López-Uribe et al. 2015). The reasons for selecting sandy sites for nesting are not well-known, but it may simply be that sandier soil is softer and easier to excavate (Antoine and Forrest 2021). Although we did not detect a significant negative relationship between sand content and distance from the stream, it is worth noting that most of our sampling took place within 10 m of the stream channel. We suspect that we would have detected a stronger negative relationship with distance had we sampled farther away from the stream channel. It should be noted that Neff (2004) observed *C. bumeliae* Neff nesting in sandy alluvial deposits along a river in Texas, suggesting riparian associations may be common among members of the genus.

Many of the aggregations occurred at about the same locations in 2022 and 2023, consistent with the multi-year *C. thoracicus* aggregations (e.g., lasting 5 years or more) described previously (Batra 1980). However, the locations of other aggregations changed between the years. It is not clear why some locations were used in both years while others were not. One possibility is that winter floods, which caused considerable scouring along sections of the bank (including the removal of leaf litter, see Fig. 4), reduced the survivorship of some aggregations, as reported previously for other bee taxa (Fellendorf et al. 2004). Although we did not detect a difference in flooding history between 2023 aggregation and the random sites (Table 1), it is noteworthy that none of the aggregations that persisted from 2022 to 2023 experienced flooding. Moreover, we were unable to locate any aggregations farther downstream, bordering the middle Oconee River, where prolonged winter flooding (and considerable sediment deposition) occurs nearly every year. However, because the waterproof cells typical of *Colletes*, like those of some other bee genera (Roubik and Michener 1980), are thought to provide considerable protection from inundation (Albans et al. 1980), flooding may not be a good explanation for the changing aggregation locations observed in this study. In fact, flooded sites without leaf litter may encourage nesting by the species. For example, all three of the 2023 aggregations observed at recently flooded sites were new aggregations, suggesting that the bees may have selected those areas of exposed soil as favorable nesting sites.

One of the most unexpected differences observed between aggregation and random sites concerns *Podophyllum peltatum,* a perennial colony-forming herb native to eastern North America. This species occurred significantly more often at aggregations than at random sites (Table 1). This association is unlikely to reflect a shared preference for sandier soil given the documented tolerance of *P. peltatum* for a wide range of soil conditions (Zheljazkov, Jones et al. 2009). A more probable explanation is that *P. peltatum* creates numerous openings through the litter layer as the plants emerge in the spring, and that these openings provide access points to the soil used by *C. thoracicus*. Another source of litter disturbance observed in this study was digging by vertebrates. We found evidence of such digging at many of our aggregations, although there was only a marginally significant difference in digging between aggregations and random sites (Table 1). However, we suspect that much of this digging was in response to the aggregations rather than a driver of nest-site selection. The high density of nests typical of *C. thoracicus* aggregations may present a significant resource to certain vertebrate species. Indeed, we noticed fresh digging activity centered on the disturbance we caused when taking a soil sample from the center of an aggregation several days before. Our data provide no support for the possibility that podophyllotoxins present in the rhizomes and leaves of *P. peltatum* (Eyberger et al. 2006) discourage vertebrate digging given that the digging was observed at over half (4/7) of the aggregations with *P. peltatum* and at none of the three aggregations without *P. peltatum*. However, it is possible that podophyllotoxins provide *C. thoracicus* with some protection from other natural enemies such as the parasitic meloid beetle, *Tricrania sanguinipennis* (Say) (Parker and Böving 1924), which was commonly associated with *C. thoracicus* aggregations in this study (MDU and CRT, personal observation).

Liriodendron tulipifera, one of *C. thoracicus*' preferred pollen sources (Batra 1980), was common throughout the watershed and may explain the large number of aggregations observed in this study. Although we did not detect a difference in the number of mature trees within a 10 m radius of aggregations and random sites, the availability of this tree nearby may still influence nest-site selection as well as reproductive success. *Colletes thoracicus* may respond to the availability of this tree at larger scales than we considered in this study. For example, Batra (1980) wrote of *C. thoracicus* flying about 200 m from their nests to visit a large flowering *L. tulipifera* tree. Within a foraging radius of that size, *L. tulipifera* was abundant throughout the entire watershed.

In conclusion, our findings show that, contrary to general expectations, some bee species do nest beneath leaf litter in closed-canopy interior forests. This is perhaps not surprising given the close association between *C. thoracicus* and flowering trees (specifically *L. tulipifera*) and should be considered for other similarly forest-dependent bee species. In particular, streamside areas characterized by sandy soil may provide a particularly favorable habitat for many ground-nesting bees within forests. More research in this area would help clarify the role forested habitats play in maintaining diverse bee communities.

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AUTHOR CONTRIBUTION

Michael Ulyshen conceived the study and Clayton Traylor and Bryan Danforth contributed to the design. Michael Ulyshen and Clayton Traylor collected the data. Michael Ulyshen analyzed the data and wrote the first draft of the manuscript. All authors commented on the manuscript and approved the final version.

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DATA AVAILABILITY

Data collected in this study are available from the corresponding author upon request.

CODE AVAILABILITY

The code used in the analysis is available from the corresponding author upon request.

DECLARATIONS

Ethics approval and consent to participate This work did not involve human subjects and did not require ethics approval.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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