


SHORT COMMUNICATION

Dynamics of nest occupation and homing of solitary bees in painted trap nests

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Abstract. 1. The oil-collecting bee *Centris analis* (Fabricius, 1804) is an important pollinator for the Neotropical region. The species can be attracted to nest in human-made cavities. Such trap nests or insect hotels offer the opportunity to study the behaviour of populations in semifield conditions.

2. We studied a newly established trap nest aggregation of *C. analis* in Mato Grosso do Sul, Brazil and tested the effect that differentially painted nesting options have on the rate of nest foundation, and on the ability of relocating the nest when returning from a foraging trip (homing behaviour). Moreover, we tested if the duration of foraging trips decreased with time.

3. We found that females preferred to nest in painted nests compared to unpainted nests, with blue nests being the most occupied ones, followed by purple, yellow, white, and green. Furthermore, bees improved their homing behaviour with time, however, nest colour did not seem to have an effect on this process. Moreover, we found that bees reduce the duration of their foraging trips with time. This could be an indicator of improved foraging efficiency through learning.

4. These findings could inform a new and fruitful line of research on the behaviour and ecology of trap nesting solitary bees.

Key words. *Centris analis*, foraging activities, nesting ecology, oil bees, painted nest preference.

Introduction

Trap nests are a commonly used tool for the study of above-ground nesting bee and wasp communities and have provided means to study the optimisation of trap-nest sizes in pollination systems (Oliveira & Schlindwein, 2009), nesting dynamics (Moure-Oliveira *et al.*, 2017), rate of nest colonization (Martínez-Núñez *et al.*, 2019), and foraging ranges (Gathmann & Tschardt, 2002). These behavioural aspects can help to understand mechanisms of nest occupation and homing behaviour of solitary bees.

Centris analis (Fabricius, 1804) (Hymenoptera: Apidae: Centridini) is a multivoltine neotropical oil bee species with a broad geographic distribution (Moure *et al.*, 2012). This species is associated with several plant species (Santos *et al.*, 2013)

and is used in crop pollination (Magalhães & Freitas, 2013). The females of these solitary bees build nests in cavities and human-made trap nests.

In this study, we addressed questions regarding nesting dynamics of *C. analis* in a newly established trap nest aggregation. We tested if the rate of nest foundation was influenced by differentially painted nesting options and if painted trap nests helped females to locate their nests. We specifically tested the following hypothesis: i) the number of occupied nests differs between painted and unpainted blocks, ii) bees learn the location of nests with time and painting helps with the recognition process, and iii) the duration of foraging trips decreases with time.

Material and methods

The bee hotel (BH) was built in August 2016 on the campus of the Federal University of Grande Dourados, in Dourados,

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Mato Grosso do Sul, Brazil (22°11'41''S 54°55'50''W). It was composed of 95 wooden blocks, with a total of 1549 drilled cavities of 0.6 and 0.8 cm diameter and 12 cm length. Thirty-three blocks (35%) with 395 cavities were painted with different water based colours (green_{blocks} = 7, white_{blocks} n = 6, purple_{blocks} n = 8, yellow_{blocks} n = 8 and blue_{blocks} n = 4, Metalatex Eco, Sherwin Williams, Brazil), 62 blocks (65%) with 1154 cavities were not painted (wood). Observations started in June 2017, in the beginning of the breeding season of *C. analis*, when females were starting to establish new nests.

Number of occupied nests differs between painted and unpainted blocks

We monitored the nesting dynamics of the aggregation weekly from June to November 2017, by counting the number of established nests in each block. To test if the number of occupied nests was different between painted (human perception) and unpainted blocks, we used a generalised linear model with the ratio of occupation in a block as response variable (using the `cbind` function) and colour as predictor variable. Blocks were randomly assigned to treatment. We took the number of cavities per block as proxy for cavity density into account by including it as offset variable into our model. The nest cavities in our study had two different diameters (termed 'small' 0.6 and 'big' 0.8 cm), we took the different sizes into account by including the proportion of small nesting cavities as random factor into our model (`glmer(cbind(number of occupied nests, number of empty nests) ~ colour + (1|proportion of small nesting cavities), offset = log (total number of nests), family = binomial)`).

Bees learn the location of nests with time and painting helps with the recognition process

Additionally, we studied the learning curve of females to locate their own nest after returning from foraging trips. For this, we individually marked 25 foundress females that were provisioning nests (observed carrying nesting material/pollen to the nest) with non-toxic markers (Edding®). In the afternoon when foraging activity ceased, the nest entrances of marked bees were blocked with a 1.5 µl Eppendorf tube, that was perforated to allow air circulation. At 6 am the next day, before foraging activity started again, the tube was removed, and females were allowed to forage normally. Foraging activity started around 8 am.

For the first five foraging trips of the day, we measured the duration (in minutes) of the foraging trips and the 'homing behaviour' which is the time (in seconds) a female spent hovering in front of the BH when returning from a foraging trip before it located and entered its nest. We used a linear mixed model to test if the interaction between the number of return event and block colour had an effect on the time a bee spent hovering in front of the nest entrance (`lmer: time hovering ~ return event + painted vs. unpainted + (1|bee)`).

Duration of foraging trips decreases with time

In a subset of 12 marked females, we tested whether the bees reduced the duration of their foraging trips during the first five foraging events of the day using a generalised linear mixed model with negative binomial distribution and foraging time (in minutes) as response variable, number of foraging event (1–5) as fixed factor, and bee ID as random factor.

All statistical analyses were performed in R (R Core Team, 2019) with the following packages loaded, `lme4` (Bates *et al.*, 2015), `ggplot2` (Wickham, 2016).

Results and discussion

Number of occupied nests differs between painted and unpainted blocks

Centris analis females built a total of 104 nests (ca. 7% of the total number of available cavities) during our observations. In absolute numbers, the majority of nests were built in non-painted blocks, (n = 25) followed by nests built in yellow (n = 21) and purple (n = 21), blue (n = 16), white (n = 12) and green (n = 9). Yet, when taking into account the proportion of available and occupied nests per colour, our first model showed that the rate of nest occupation differed between painted and unpainted blocks ($\chi^2 = 34.344$, $df = 5$, $P < 0.001$). Bees nested significantly more often in painted blocks compared to the unpainted blocks ($P < 0.05$) and proportionally more in blue painted nests (Fig. 1).

Bees learn the location of nests with time and painting helps with the recognition process

Our model showed a significant effect of number of returns from foraging events on the time bees spent hovering in front of their nest (main effect foraging event: $\chi^2 = 30.653$, $df = 4$, $P < 0.01$). Painting had no significant effect (main effect block colour: $\chi^2 = 0.133$, $df = 1$, $P = 0.714$) on hovering time.

Our results indicate that females of the oil bee *Centris analis* improve the relocation of their nests with time, suggesting a learning process (Lehrer, 1993). Upon leaving the nest for the first time of the day, all studied females displayed a recognition flight. The bees were observed hovering in front of the nest, probably performing memory retention before disappearing from the BH. When returning from foraging, the females were hovering again in front of the BH before entering the nest but reduced this hovering time during the 5 first return events ($\chi^2 = 19.767$, $df = 4$, $P < 0.001$, Fig. 2) of the day by on average 13 s between the first return ($R1_{\text{average}} = 17.15 \pm 11$ s) and the fifth return ($R5_{\text{average}} = 4.29 \pm 11.69$ s). The time that the females needed to find their nests was higher after the first foraging trip, which also was found to be the longest foraging trip of the day (see below). It is known that bees might at least partially forget their nest location overnight (Dukas & Real, 1991; Keasar *et al.*, 1996) and need to re-learn on the following day, resulting in an increased homing time after the first foraging trip (Dukas & Real, 1991; Keasar

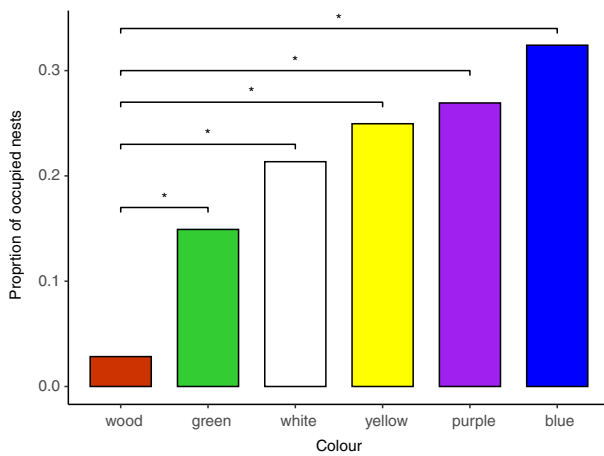


Fig. 1. Proportion of nests occupied by females of *Centris analis* in differently painted nest blocks. Females built their nests preferentially in painted trap nests compared to unpainted blocks; * $P < 0.01$. [Colour figure can be viewed at wileyonlinelibrary.com].

et al., 1996). Furthermore, it is known that bees use visual (Brünnert *et al.*, 1994) and chemical (Guédot *et al.*, 2013) cues for nest recognition and can remember the position of their nests by spatial memory learning (Collett *et al.*, 2013) which can optimise homing behaviour (Ostwald *et al.*, 2019). Differentially painted nesting blocks might therefore facilitate nest site recognition in a similar way. Cognitive processes related to colour have been found to be an important mechanism for bees in different situations such as in bee-flower interactions (Lehrer, 1993; Dyer & Garcia, 2014). This might indicate that the bees choose blocks of distinctive colours as a salient cue, in order to recognise their nest more accurately, which likely helps to decrease the possibility of drifting behaviour; a common feature in gregarious nesting bee species (Field, 1992). The use of painted nests has been found to significantly decrease drifting behaviour in honey bees (Dynes *et al.*, 2019). However, if this is also the case in *C. analis* remains to be tested.

Duration of foraging trips decreases with time

Our model showed that the number of the foraging trip had an effect on the duration of foraging time ($\chi^2 = 13.684$, $df = 4$, $P < 0.01$). The first foraging trip of the day was the longest, and the duration of foraging trips decreased with subsequent foraging events. However, this effect was mainly driven by the first two foraging events of the day. The first foraging trip of the day might have included an initial recognition and/or defecation flight (Cane *et al.*, 2017) and foraging subsequently was optimised along the day.

To our best knowledge, this study with *Centris analis* is the first showing that painted trap nests play a role on nesting occupation of solitary bees. Even though our study is limited to one population of *C. analis*, we believe that our findings open to a new and fruitful line of research on the behaviour and ecology of trap nesting solitary bees.

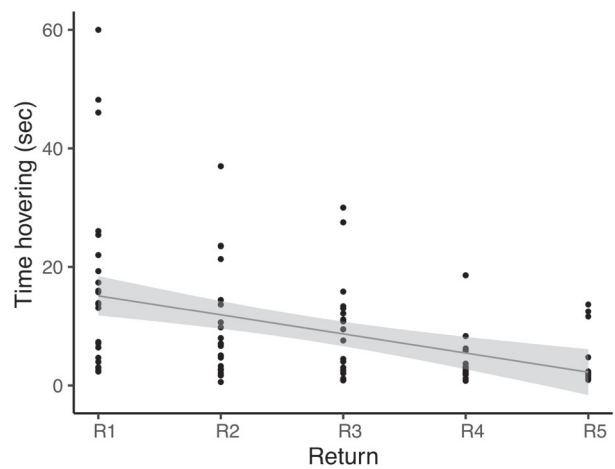


Fig. 2. Hovering time (seconds) of females in relation to the number of return events (R1 = first return of the day, R2 = second return of the day ... , R5 = fifth return of the day). The time females of *Centris analis* spent hovering and searching for their nests in front of the bee hotel decreased with progressive return events.

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Contribution of authors

The authors have equally contributed to all aspects related to this study.

Data availability statement

Data available on request from the authors. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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