Short Communication

Use of Visual and Olfactory Cues for Individual Nest Hole Recognition by the Solitary Bee *Epicharis metatarsalis* (Apidae, Anthophorinae)

Brian D. Inouye[1,2]

INTRODUCTION

Both social and solitary Hymenoptera have a remarkable ability to navigate and to find their way back to their nests (Wehner, 1981). Nest location and recognition by Hymenoptera can involve both visual and olfactory cues (Hölldobler and Michener, 1980). It has been proposed that the use of olfactory cues for individual nest recognition is an important "preadaptation" for the multiple evolutions of sociality among Hymenoptera, since chemical pheromones are frequently used by social Hymenoptera to distinguish nest mates or kin from outsiders (Wcislo, 1990, 1992). Previous studies on the use of visual and olfactory cues for individual nest location and recognition (summarized in Wcislo, 1992) suggest that the use of both categories of cues is widespread among bees (Apiformes), although most hunting wasps (Sphecidae) may use only visual cues. The wide taxonomic distribution of the use of both chemical and visual traits among bees implies that all bees can use both kinds of cues for either nest location or recognition, but there have been few studies of nest location or recognition in the acorbiculate Apidae (*sensu* Roig-Alsina and Michener, 1993). This study

[1]Center for Population Biology, Storer Hall, University of California, Davis, Davis, One Shields Ave., Davis, California 95616.
[2]Correspondence should be sent to Brian D. Inouye, Center for Population Biology, Storer Hall, University of California, Davis, Davis, California 95616; e-mail: bdinouye@ucdavis.edu

investigated the use of visual and olfactory cues for individual nest location and recognition by a Neo-tropical, solitary apid bee.

Epicharis metatarsalis constructs simple nests in the ground. Although females are solitary within their individual nests, nests are found in relatively dense aggregations consisting of up to several hundred females at a site. The nest entrances can be within a few centimeters of each other, with up to about 25 active nests/m^2, yet females returning from foraging reliably return to their own nest entrance without mistakes or lengthy searches. Because the brood cells are approximately a meter under ground (Thiele and Inouye, unpublished data), any visual or olfactory cues associated with the nest entrance at ground level are spatially well separated from olfactory cues associated with the brood cells (i.e., Dufour's gland secretions). Here I describe a series of six experiments that were designed to investigate the role of visual and olfactory cues in nest hole location and recognition by females returning from foraging.

METHODS

Experiments and observations were conducted at La Selva Biological Research Station (McDade *et al.*, 1994), Heredia, Costa Rica, near Puerto Viejo de Sarapiquí, where several large aggregations of *E. metatarsalis* are active from May through July. *Epicharis metatarsalis* are robust bees, approximately 20 mm long, with dense brown-orange pubescence. Each nest has a single entrance, which may have a 2–8-cm mud turret above the ground, leading directly into a vertical tunnel. The nesting aggregations used in this study were in an abandoned guava plantation (described in Folgarait *et al.,* 1995). The understory in this area is kept cleared of vegetation, but trees form a closed canopy. Experiments, conducted during July of 1997 and 1998, manipulated visual cues and olfactory cues associated with the nest entrances, and local landmarks near the nest entrances. I first describe the methods that were common to all of the experiments, and then describe each experiment in turn.

Visual cues were manipulated by covering the area surrounding the nest entrance with a square of thin, opaque blue plastic, 6 cm on a side, with a 14 mm diameter hole in the center (cf. Steinmann, 1985; Shimron *et al.,* 1985). Females readily exited and entered through the hole in the center of the square. When entering and exiting the nest entrances, the bees would usually briefly contact the surface of the cover, or the edges of the hole in the cover. An insect pin through one corner of the square prevented movement during frequent rains.

After leaving their nests, females often circled or hovered over their

nest entrance for several seconds before flying off. When returning, the females would approach their nest entrances rapidly, and then fly more slowly, hovering near their nest entrances for 2–17 seconds (mean 5.4 ± 2.8 SD, $n = 89$). When females landed they immediately entered their tunnels. For all experiments I recorded the seconds elapsed between the time a returning female was first observed and when she landed at a nest entrance. The p values reported below were calculated using nonparametric two-tailed Mann–Whitney U-tests (StatView v. 4.56).

V1: Entrance Visual Cues Manipulated

I placed a square cover over a nest entrance, obscuring or altering visual cues, but leaving any olfactory cues intact. As a control, I placed a cover over an entrance and then removed it. The covers were placed while the females were out foraging.

V2: Entrance Visual Cues Manipulated

I placed square covers over nest entrances, and left them until the female had adjusted to the presence of the cover and would return to her nest without hesitation. Then I moved the cover to an artificial nest entrance created approximately 10 cm away from the original nest entrance, or moved the plastic cover and then returned it (control). Artificial entrances were created by pushing a plastic rod into the ground to create a hole 10–15 cm deep, and similar in diameter to the nest tunnels. Moving the plastic covers to a fake entrance displaced local landmarks that females might associate with their nests, but any possible chemical cues associated with the soil at or near the nest entrances were not disturbed. Larger local landmarks that bees might use (e.g., trees, branches, and large leaves) were also undisturbed. As a control for olfactory cues possibly associated with the plastic covers rather than the soil at the nest entrances, see Experiment O1.

V3: Local Landmarks Near the Entrance Manipulated

First I placed a blue plastic arch directly above a nest entrance. The arches were half-circles with a 10-cm radius cut from plastic hoops of material about 10 mm in diameter. After 3 days I returned to these nests, and either moved the arch to an artificial nest entrance created about 10 cm away from the original entrance, or else removed the arch and then

replaced it on the original entrance (control). These treatments left the nest entrance itself undisturbed.

O1: Entrance Olfactory Cues Manipulated

This experiment was designed to detect olfactory cues deposited on the square plastic covers used in Experiments V1, V2, and O3. After females had become accustomed to the square covers (at least 24 hr) I switched the covers on pairs of nests. The nest entrances themselves were not manipulated. I compared the results of switching covers to the data for completely unmanipulated nests (no covers present).

O2: Entrance Olfactory Cues Manipulated

To manipulate olfactory cues, I washed the entrances to nests with cotton swabs soaked in hexane. Hexane is a solvent for many of the hydrocarbons that could be potentially used for nest recognition. As a control, I washed the entrances to other nests with cotton swabs soaked in water. Water-soluble chemical cues are unlikely to be used by ground-nesting bees in this habitat, because of the frequent rains at La Selva during June and July.

O3: Entrance Olfactory Cues Manipulated

In this experiment olfactory cues associated with the nest entrances were manipulated by moving intact nest entrances. The high clay content of the soil around these nests meant that a nest entrance could be removed in a block, approximately 6 cm on a side, with the nest entrance and the first 5–10 cm of the nest's tunnel left intact and undisturbed. Presumably, any olfactory cues or pheromones deposited at or near the nest entrance would be transferred along with the block of soil. First, I placed square covers over nest entrances and left them there for at least 24 hr, until the female returned to her nest normally. After females had adjusted to the plastic cover, I changed the block containing the nest entrance, or else returned this block to the original nest (control). Finally, I returned the original plastic cover to the entrance. The plastic covers meant that the external appearance of the entrance was unchanged, but any olfactory cues associated with the nest entrances were switched. As a second control, see O1, which switched only the plastic covers above entrances, and left the

soil blocks around the entrances alone. Experiment O1 would detect an effect of olfactory cues deposited on the plastic covers.

RESULTS

The results are shown in Fig. 1, which compares the median number of seconds required for a female to find her nest entrance under different experimental treatments. The results are consistent with females using only visual cues for nest recognition. When visual cues were manipulated it required significantly longer for a female to find her nest entrance ($p < 0.005$ for V1, V2, and V3). In contrast, for the three experiments that manipulated potential olfactory cues there were no significant effects of the experimental treatments ($p > 0.28$ for O1, O2, and O3). For all six experimental controls, times until females entered their nests were not significantly different from times recorded from unmanipulated nests.

Experiment O1 established that there were no olfactory cues for nest location or recognition associated with the square plastic covers, which were meant to change or hide only visual cues. After females adjusted to
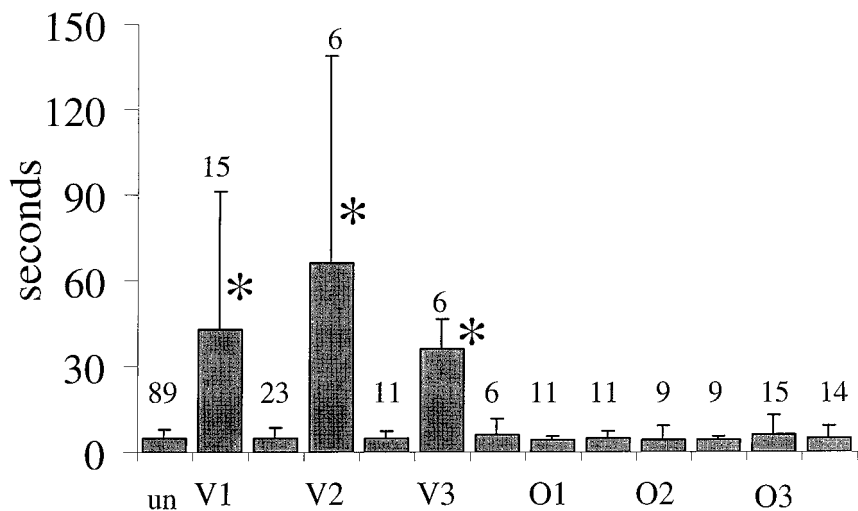

Fig. 1. Median time elapsed between a female's return and entering her nest. The leftmost column presents data from unmanipulated nests. The results for each control are shown in the column to the right of the manipulations. Numbers above columns show sample sizes for each category, and the asterisk shows significant differences ($p < 0.005$) from a Mann–Whitney U-test.

the presence of a cover around their nest entrances, switching covers did not significantly prolong time for nest entering, relative to leaving the familiar cover in place (V2, $p = 0.325$), or females in unmanipulated nests ($p = 0.289$). The result that switching covers did not require females to spend significantly longer searching for their nests means that Experiments V2 and O3 can be interpreted as manipulating only the intended cues.

When local landmarks or the appearance of nest entrances were experimentally manipulated, females returning from foraging would often hesitate before entering a nest. These females would fly in large slow circles above the nesting area, and hover above several entrances before landing at a nest entrance, which was not always the correct one. The observed females never showed these behaviors in olfactory manipulations or in control treatments. In Experiment V2, which moved a familiar cover to a nearby fake entrance, four of the six females in the treatment group landed with little hesitation at the fake entrance and entered the short tunnel (mean of 38 ± 55 sec in the fake nest tunnel), before exiting to make slow circular searching flights. Three of these six females reentered the fake entrance more than twice before finding their correct nest entrance. None of the females in Experiment 6, which moved potential local landmarks to a fake entrance, actually entered the fake entrance, although the females did repeatedly hover over the fake entrance before entering their correct nest entrance.

DISCUSSION

The results of the experiments presented above indicate that *E. metatarsalis* females returning from foraging use only visual cues for location and recognition of their nest entrances. Moving the blocks of soil that contained the nest entrances, or moving the plastic covers among nest entrances, had no effect on how quickly a bee found and entered her nest. This implies that there are no olfactory cues associated with the nest entrances. The strongest visual cues seem to be those surrounding the nest entrance itself, since females trained to enter a hole in a plastic cover can be tricked into entering a fake nest entrance if the covers are moved. Manipulating visual landmarks slightly further from the nest entrance was not sufficient to cause females to enter fake nest entrances; however, it did greatly increase the length of time required for females to locate their nest entrances.

Although there are no olfactory cues used for nest entrance location, this does not necessarily demonstrate that olfactory cues are not used for individual nest recognition. For example, bees may use information about

individual nest architecture, or olfactory cues that are associated with the brood cells. In this species, brood cells are lined with chemical secretions (Thiele and Inouye, unpublished data) probably derived at least in part from Dufour's gland compounds (Hefetz, 1987), as is true for many bees. Shimron *et al.* (1985), working on *Eucera palestinae* (Apidae, Eucerini), found that extracts from the Dufour's gland of other females placed on a nest entrance delayed nest recognition. However, the behavioral observations on *E. palestinae* are consistent with the hypothesis that only visual cues are normally used for nest location, and that in natural circumstances individuals' glandular secretions are only associated with the brood cells. Steinmann (1985) was able to trick returning female *Anthophora plagiata* (Apidae, Anthophorini) into entering the wrong nest entrance by switching only visual cues, using covers over entrances similar to those described here. Females that entered the wrong entrance were able to recognize their mistakes, presumably after encountering foreign nest architecture or olfactory cues further inside a neighbors' nest.

The reliance of *E. metatarsalis* on visual landmarks for individual nest entrance location is somewhat surprising, given their habitat. The rate of leaf and litter fall in the forests where these bees nest is high, and it was not uncommon for nest entrances to be partially to completely obscured by newly fallen leaves. When this happened, returning females would often have difficulty finding their nest entrance, though they were usually able to push the leaves aside while walking on the ground and searching (personal observation). Females that were inside their nests when a leaf fell over the entrance would either push the leaf aside or chew a hole through the leaf. These females would then hover above their entrance or fly slowly around it before leaving again for foraging, presumably to learn the new visual cues associated with their nest entrance.

ACKNOWLEDGMENTS

REFERENCES

Folgarait, P. J., Marquis, R. J., Ingvarsson, P., Braker, H. E., and Arguedas, M. (1995). Patterns of attack by insect herbivores and a fungus on saplings in a tropical tree plantation. *Environ. Entomol.* **24**(6): 1487–1494.

Hefetz, A. (1987). The role of Dufour's gland secretions in bees. *Physiol. Entomol.* **12:** 243–253.

Hölldobler, B., and Michener, C. D. (1980). Mechanisms of identification and discrimination in social Hymenoptera. In Markl, H. (ed.), *Evolution of Social Behavior: Hypotheses and Empirical Tests,* Verlag-Chemie, Weinheim, pp. 35–58.

McDade, L. A., Bawa, K. S., Hespenheide, H. A., and Hartshorn, G. S. (eds.). (1994). *La Selva: Ecology and Natural History of a Neotropical Rain Forest,* University of Chicago Press, Chicago.

Roig-Alsina, A., and Michener, C. D. (1993). Studies of the phylogeny and classification of long-tongued bees (Hymenoptera: Apoidea). *Univ. Kansas Sci. Bull.* **55:** 123–173.

Shimron, O., Hefetz, A., and Tengo, J. (1985). Structural and communicative functions of Dufour's gland secretions in *Eucera palestinae* (Hymenoptera; Anthophoridae). *Insect Biochem.* **15**(5): 635–638.

Steinmann, E. (1985). Die Wand-Pelzbiene *Anthophora plagiata* (Illiger) (Hymenoptera: Apoidea). *Jber. Natf. Ges. Graubünden* **102:** 137–142.

Wcislo, W. T. (1990). Olfactory cues in nest recognition by solitary bees (*Lasioglossum figueresi;* Halictidae) as a preadaptation for the evolution of kin associations. In *Social Insects and the Environment,* Veeresh, G. K., Mallik, B., and Viraktamath, C. A. (eds.), New Delhi, Oxford & IBH Publishing Co. PV.T., pp. 412–413.

Wcislo, W. T. (1992). Nest localization and recognition in a solitary bee, *Lasioglossum (Dialictus) figueresi* Wcislo (Hymenoptera: Halictidae), in relation to sociality. *Ethology* **92:** 108–123.

Wehner, R. (1981). Spatial vision in arthropods. In Autrum, H. (ed.), *Handbook of Sensory Physiology, Vol. VII, 6 C. Vision in Invertebrates,* Springer-Verlag, New York, pp. 287–616.