Dorsal landmark navigation in a Neotropical nocturnal bee

Highlights

- The nocturnal bee *Megalopta genalis* has excellent night vision
- *Megalopta* is able to learn dorsal landmarks to find its nest during homing
- *Megalopta* is the first flying insect known with this ability
- This skill may allow bees to use variations in the canopy foliage pattern to navigate

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In brief

The nocturnal bee *Megalopta* visually navigates to and from its nest in a dark rainforest. Chaib et al. show that this bee can learn dorsal landmarks to find its nest, suggesting that spatial variations in local foliage patterns created by the canopy against the brighter night sky might be used as navigational cues during homing.
Dorsal landmark navigation in a Neotropical nocturnal bee

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https://doi.org/10.1016/j.cub.2021.05.029

SUMMARY

Bees, ants, and wasps are well known to visually navigate when traveling between their nests and foraging sites. When leaving their nest, landmarks in the vicinity are memorized and used upon return to locate the nest entrance.1,2 The Neotropical nocturnal sweat bee Megalopta genalis navigates under the forest canopy at light intensities ten times dimmer than starlight.3 Despite these dim conditions, Megalopta is able to memorize visual landmarks around the nest entrance in the frontal visual field.4 Even though frontal landmarks can clearly be discerned by Megalopta, the visual feature of greatest contrast in the rainforest at night is actually the dark dorsal silhouette of the distant canopy against the brighter night sky. Several species of ants,5–10 as well as a subsocial shield bug,11 use bright open gaps in the canopy as dorsal landmarks to navigate home while walking. Here we show that Megalopta is also able to distinguish dorsal landmarks during homing, the first flying insect known with this capacity. Megalopta is able to discriminate between differently oriented dorsal black striped patterns, or an “artificial canopy” of black circles, and to use this information to locate its nest entrance. These results suggest that the local foliage patterns created by the canopy against the brighter sky could potentially provide the bee with reliable landmark information for navigation during foraging and homing at night.

RESULTS AND DISCUSSION

Megalopta genalis (Figure 1A) is a facultatively eusocial tropical bee that nests in hollowed-out wooden sticks entangled in the understory of the forest.1,2 Typically, each bee forages during roughly 70 min windows bordered by astronomical twilight, once during dusk and once during dawn.2 Due to the dense foliage in the rainforest, the light intensity during these twilight periods is typically around that of a starlit night under an open sky.3

Megalopta can use a dorsal landmark to find the entrance of its nest

In all but the last experiment of this study, an occupied nest stick and four sham (empty) nest sticks (wooden sticks with a drilled hole in one end), were arranged side-by-side within a box-like experimental apparatus (STAR Methods) positioned on a stand around 1.5 m above the ground (Figure 1B). This box hid the nests from direct view while also allowing their positions to be swapped easily. The front panel of the box consisted of a transparent Perspex sheet holding a horizontal row of five identical circular Perspex disks (100 mm in diameter), each covered in gray paper. At the center of each disk, a hole (diameter 5 mm) allowed the bee to access the nest connected to the rear side of the disk. The sides of the box were made of gray plastic sheets while the back was opened for easy access to the nesting sticks. A loose Perspex lid was placed on top of the box and then covered with a black cloth. In the first three of our four experiments, we monitored bees returning to this apparatus to determine which of the five nest disks (with their associated landmark) the bees first chose to land on (recorded as a “correct” choice; see below).

First, we tested whether the bees could learn to associate the position of their nest entrance with a single three-dimensional landmark (a 30 × 130 × 5 mm black bar) positioned 100 mm above the center of the disk fronting its nest stick (Experiment 1). Prior to testing, bees were allowed to habituate to the presence of this landmark for a couple of days (STAR Methods). Between foraging bouts during habituation, the nest (together with the landmark) was swapped pseudorandomly with one of the artificial nest sticks to ensure that the relative position of the nest within the five-nest array (one real and four shams) could not be used to guide the bee to its nest.

At the beginning of each test trial the nest occupied by the bee was positioned behind the central disk in the array, and the landmark was placed above it. Because we did not know how many bees were living and foraging from one nest stick, as well as to separate potentially different individuals, we decided early on to treat each nest as one test unit. Directly after the bee(s) flew out to forage, the nest together with its associated landmark (Test 1) was moved to one of the other four possible positions (8 trials) or kept at its original central position (2 trials) on the experimental apparatus (chosen pseudorandomly; Figure 2A).
We then waited until the bee returned, typically up to 30 min later. If the bee directly returned to the “correct” nest disk (i.e., the nest marked with the overhead landmark), without first landing on one of the other four disks, this was considered a correct choice. In order to control if the bees were using any additional cues, for example smell, to identify their nest entrance upon return, we performed a control test on the same bees—instead of moving both the nest and the landmark to a new position after the bee left, only the landmark was moved (Test 2; Figure 2B). The results from all nests and both test conditions (Tests 1 and 2) were pooled. We performed 71 trials in total on bees from 6 different nests (Test 1, 10 trials/nest; Test 2, 1–2 trials/nest). The choice frequencies (binomial) were fitted to a generalized linear mixed model (GLMM) with test condition (Test 1 or 2) as a fixed effect and nest identity as a random effect to avoid pseudoreplication. This model was compared to a reduced model excluding the effect of test condition did not provide a significantly better fit to the data compared to the model that excluded this effect \( \chi^2 = 0.009, df = 1, p = 0.93 \). Additionally, the model excluding the effect of test condition had the lower Akaike Information Criterion (AIC) value (89.03 versus 90.12), suggesting that this model provided a better fit to the data (when the qualities of two models are compared—as judged by a trade-off between the goodness of fit of each model and the number of parameters required to obtain this fit—the model with lowest AIC is considered the better model). Thus, the bees had the same success rate regardless of whether the nest was moved together with the landmark or not, indicating that no additional “nest-associated” cues helped the bees to pinpoint their nests upon return. The bees made a correct choice in 71.8% of their returns \( (N = 71, 95\% CI: [55.3\%–83.9\%]) \). If the bees did not use the dorsal cue as a landmark, we would have expected them to make a correct choice in 20% of the trials, which is well outside the 95% CI of their performance, thus supporting our hypothesis that the bees used the dorsal black bar to pinpoint their nest entrance. (Figure 3, Experiment 1).

**Megalopta can use dorsal stripe patterns to find the entrance of its nest**

Next, we covered the array of five nests with a clear UV-transparent overhanging Perspex roof. Onto this roof, above the entrance to the bee’s nest stick, we placed a pattern of black stripes (separated by identically wide clear stripes) that was oriented perpendicular to the frontal panel, and above each of the sham nests we placed the same pattern of black stripes, but now oriented parallel to the frontal panel (Figure 2C). To prevent bees from seeing the natural canopy pattern above through the clear stripes, we placed a sheet of UV-transparent diffuser paper on the roof above the patterns.

Prior to testing, bees were again allowed to habituate to the presence of these landmarks for a couple of days, with the home nest again swapped pseudorandomly with a sham nest (with their landmarks) to ensure that nest position was an unreliable cue (see above). Again, bees always exited from the central nest position, and following the bee’s departure, either the position of the nest and the landmark were shifted to a new position (or kept at its original position; Test 1; Figure 2C) or only the landmark was moved to the new position, with the nest remaining in the central position (Test 2; Figure 2D). In this experiment (Experiment 2), we used 8 new nests from which we again tested the bees 10 times each by moving the landmark and the nest (Test 1) and 2 times each by only moving the landmark (Test 2). We fitted a GLMM with individual nest as random effect and again we found that a model including test condition as a fixed effect did not provide a significantly better fit to the data compared to a reduced model \( (\chi^2 = 0.009, df = 1, p = 0.93) \), nor did it have a lower AIC value (full model, 138.0 versus reduced model, 136.0), indicating that the bees did not use olfaction (or any other nest-associated cue) to identify the nest. The bees returned to the nest with the correct dorsal cue in 55.2% \( (N = 96, 95\% CI: [44.7\%–65.3\%]) \) of the trials, the chance frequency (20%) again being outside of the CI, indicating that the bees can also rely on differences between dorsal patterns to locate their nest entrance (Figure 3; Experiment 2).

**Megalopta relies on pattern recognition, rather than the magnitude of optic flow, to find the entrance of its nest**

Like all bees, as *Megalopta* leaves its nest it performs a so-called orientation flight, \(^{1,13}\) flying from side to side in front of the nest to learn the arrangements of visual landmarks. This movement should generate a larger magnitude of optic flow when flying beneath a set of stripes oriented perpendicular to the direction of movement (as for the stripes marking the real nest in Experiment 2) than when stripes are oriented parallel to the direction of movement (as for the stripes marking the sham nests). Thus,
Megalopta might discriminate its nest entrance simply by using differences in optic flow provided by the two patterns rather than by recognizing the geometrical pattern differences between the dorsally placed stripes.

To test this (Experiment 3), we repeated Experiment 2 but instead placed a diagonal pattern of stripes (oriented at 45°) above the entrance to the bee's nest stick and an identical but perpendicular pattern of stripes (oriented at 135°) above each of the four sham nest entrances (Figures 1B and 2E). These two patterns provide no systematic differences in the optic flow input for a bee flying beneath. Despite this, the orientation of the stripes was clearly sufficient to help the bees to locate their nest significantly more often than by chance (Figure 3; Experiment 3, 59.4% correct choices, N = 83, 95% CI: [43.7%–73.5%]). As in the two previous experiments, we ruled out the possibility that bees used nest-associated cues, like odor, when locating the nest (Figure 2F) by comparing a GLMM including test condition as a fixed effect to a model excluding this effect (χ² = 0.287, df = 1, p = 0.59; AIC full model, 116.3; AIC reduced model, 114.6).

Megalopta relies on dorsally presented “canopy-like” patterns to define the direction to its nest entrance

We next tested whether Megalopta can make use of large “canopy-like” patterns to find its nest. A rotatable artificial canopy was constructed from two sheets of UV-transparent Perspex (70 x 70 cm, 1.2 m above ground) with three solid black circles of two sizes (20, 20, and 30 cm diameter) placed between the two sheets (Figures 1C and 2G). On top of this artificial canopy, we placed a sheet of UV-transparent diffuser paper to hide the circles when viewed from above, and to conceal the view of the real canopy in the gaps between the circles when viewed from below. An occupied Megalopta nest stick was mounted at one edge of the artificial canopy, approximately 10 cm below it, with the nest entrance directed inward under the canopy (Figure 1C). In an identical fashion, but on the opposite edge, we placed a sham nest stick. We mounted identical white circular discs (with a central 5 mm diameter entrance hole) onto the end of each nest stick. To conceal potential landmarks in the background, black fabric was used to create walls behind the two nest sticks. The two remaining sides were left uncovered to allow the bees to easily travel in and out under the artificial canopy.

Once a bee left its nest to forage, it would fly out under the artificial canopy and perform an orientation flight before leaving the apparatus through one of the open sides. In this experiment (Experiment 4), we would either rotate the canopy and the nests by 180° (4 trials) after the bee had left (Test 1; Figure 2G), or simply leave everything in position (4 trials). The starting position of the nest, the orientation of the artificial canopy, and the

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**Figure 2. The experimental design**

Orange arrowheads denote location of the occupied nest—all other nests were unoccupied.

(A and B) Experiment 1: single 3D bar landmark.
(C and D) Experiment 2: stripes parallel and perpendicular to front panel (pattern-specific differences in optic flow cues present).
(E and F) Experiment 3: stripes 45° and 135° to front panel (pattern-specific differences in optic flow cues absent).
(G and H) Experiment 4: artificial canopy.

(A, C, E, and G) Landmark and nest were moved after the bee exited the nest.
(B, D, F, and H) Only the landmark was moved after bees exited (controls for nest-associated sensory cues, e.g., odors).

Total number of bees tested (N) and correct choices made (c) (basis for data shown in Figure 3): (A) N = 60, c = 41; (B) N = 11, c = 9; (C) N = 80, c = 44; (D) N = 16, c = 9; (E) N = 70, c = 42; (F) N = 13, c = 7; (G) N = 64, c = 52; (H) N = 14, c = 12.
The bee’s nest was marked by a single bar (Experiment 1), stripes perpendicular to the front panel (Experiment 2), stripes at 45° to the front panel (Experiment 3), or by orientation of an artificial canopy (Experiment 4). Horizontal dashed lines: random choice (20% Experiments 1–3, 50% Experiment 4). Error bars: 95% CI. See also Figure S1.

Figure 3. Nocturnal sweat bees use dorsal landmarks to correctly choose their nest during homing
The bee’s nest was marked by a single bar (Experiment 1), stripes perpendicular to the front panel (Experiment 2), stripes at 45° to the front panel (Experiment 3), or by orientation of an artificial canopy (Experiment 4). Horizontal dashed lines: random choice (20% Experiments 1–3, 50% Experiment 4). Error bars: 95% CI. See also Figure S1.

The bees made the correct choice on 82.1% of their returns (N = 78, 95% CI: [71.9%–89.1%]), when 50% of the choices would have been expected to be correct just by chance (Figure 3; Experiment 4), again ruling out the possibility that the bees used odor (or any other nest-associated cue) as a navigational cue. The bees made the correct choice on 82.1% of their returns (N = 78, 95% CI: [71.9%–89.1%]), when 50% of the choices would have been expected to be correct just by chance (Figure 3; Experiment 4), again ruling out the possibility that the bees used odor (or any other nest-associated cue) as a navigational cue.

Can Megalopta navigate using the dorsal pattern of gaps in the rainforest canopy?
Our results show that while flying toward its nest upon return from a foraging trip, Megalopta can use patterns presented in the dorsal visual field—stripe patterns as well as a large artificial “canopy” of circular shapes—to locate its nest. These abilities in Megalopta set them apart from the honeybee Apis mellifera, which use optic flow in their dorsal visual field for flight-speed control and geometrical cues for frontal pattern recognition, but are unable to discriminate between two dorsally positioned striped patterns oriented at 45° and 135° to their flight path. This indicates that Megalopta, unlike honeybees, have evolved the ability to discriminate patterns in the dorsal visual field (using the frontal-dorsal regions of its eyes; Figure S1), which may be of great advantage for navigation in a nocturnal rainforest environment.

For a human observer standing in a rainforest at night, the most obvious visible cues are the bright patches of starry night sky seen through gaps in the dark silhouetted forest canopy above. Humans see this high-contrast spatial pattern as complex because of the myriad arrangements of branches and leaves that penetrate these gaps, but seen through the significantly coarser acuity of a bee’s compound eye, the canopy pattern is likely to be a lot less complex. Moreover, this pattern may be reinforced by the celestial pattern of polarized light, which at the dusk/dawn times of Megalopta’s foraging trips is extremely uniform, with the direction of polarized light in all parts of the sky—and thus within every gap in the canopy—aligned roughly north-south, providing a powerful directional cue overlaid on the pattern cue. Megalopta would easily see this directional cue—the dorsal rim areas of its compound eyes contain ommatidia whose very large rhabdoms are exquisitely sensitive to linearly polarized light.

Could Megalopta make use of these canopy cues during homing? Diurnal bees, wasps, and ants can recall a sequence of memorized “visual snapshots” of the landscape along a foraging route and use them to retrace this route back to the nest. It is not impossible that Megalopta does the same, memorizing (among other landmarks) changes in canopy cues from one location in the rainforest to another. Indeed, several species of forest-dwelling ants, some with nocturnal activity in the wild, as well as day-active shield bugs, clearly make use of the canopy pattern for short-range orientation around the nest (at least in the bright conditions under which these species were tested). Larger walking animals with distinctly greater homing ranges—such as the remarkable three-striped poison frog, which appears to employ a map-like navigational mechanism during rainforest homing—might also make use of the forest canopy pattern.

With the extra potential foraging range afforded by flight, it is not unreasonable to suspect that Megalopta and other flying nocturnal central-place foragers, like the tropical hunting wasp Apoica, might take advantage of bright canopy cues to find their way home through a tangled and complicated rainforest at night, an environment whose dim features seemingly vary little in other directions of view. Certainly, the excellent abilities shown by Megalopta to identify its nest using dorsal visual patterns argue in favor of this notion. It remains of course for future research to show whether this is indeed the case.

STAR*METHODS

Detailed methods are provided in the online version of this paper and include the following:

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ACKNOWLEDGMENTS

The authors are grateful for the financial support of the Swedish Research Council (VR grant 2016-04014) and also wish to thank the staff of the Smithsonian Tropical Research Institute in Panama for logistical support during these studies. We also thank Dr. Ajay Narendra (Macquarie University, Australia) for the photo of Megalopta shown in Figure 1, Dr. Lina Herbertsson and Dr. Jessica Abbott (University of Lund, Sweden) for expert statistical advice, and Professor Jochen Zeil (Australian National University) and the three anonymous referees for helpful suggestions that improved the manuscript.

AUTHOR CONTRIBUTIONS

S.C. and E.W. conceived the experiments; S.C., E.W., and M.D. discussed and analyzed the results; E.W. provided supervision and financed the project; W.W. provided logistical support in the field; and all authors were involved in writing and editing the manuscript.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Deposited data      | This paper and online data repository | https://doi.org/10.5281/zenodo.4751660 |
| Raw and analyzed data files | | |
| Experimental models: Organisms/strains | Wild caught | N/A |
| Central American sweat bee (Megalopta genalis) | | |
| Software and algorithms | | |
| lme4 | 22 | https://CRAN.R-project.org/package=lme4 |
| RStudio Statistical software | RStudio, Boston MA, USA | https://www.rstudio.com |

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Eric Warrant (Eric.Warrant@biol.lu.se).

Materials availability
The data generated in this study were behavioral observations scored in notebooks and transferred to Excel spread sheets. While video sequences were collected as a safety backup for human observation failures, these sequences are unfortunately no longer available. Custom-made software or new reagents were not used in the study. Statistical analyses were made using commercially available software packages. Drawings showing the designs of apparatus manufactured in the University of Lund workshops are available upon request.

Data and code availability
The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files. No specific computer code was generated for this study. Individual data files used to generate the figures in the paper are available from an online data depository: https://doi.org/10.5281/zenodo.4751660

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals
Female nocturnal bees (Megalopta genalis, Hymenoptera: Halictidae) were used for all experiments. Experiments were carried out at the Smithsonian Tropical Research Institute field station on Barro Colorado Island (79.8°W, 9.2°N) in the Republic of Panama between January and June in 2014 and 2015. Megalopta leaves the nest to forage on only two occasions each day: shortly after sunset and shortly before dawn.3,4 Typically foraging just once for between 1 and 30 min on each occasion. Light intensities encountered by bees in the vicinity of the nest vary depending on the exact time that foraging takes place and nearby vegetation, but bees have been recorded flying at lights levels up to 10 times dimmer than starlight.3,4 All experiments reported here were performed during these morning and evening foraging bouts at a selected site in the rainforest with typical canopy cover and vegetation density. Nest sticks containing one or several female bees were collected from all over the island and brought to this experimental site.

METHOD DETAILS

Objectives of the study
The objective of the study was to determine whether dorsal landmark cues – such as gaps in the rainforest canopy that create an obvious spatial pattern against the brighter night sky – can be used by the nocturnal bee Megalopta during navigation and homing. To achieve this objective, foraging bees were given the opportunity to learn various landmarks and patterns placed above the nest entrance in the dorsal visual field, and then tested to see whether they recognize these (and thus their nest entrance) upon return from a foraging trip.
Experiments 1, 2 and 3

Experimental Apparatus

The experimental apparatus was a box-like structure placed on a stand around 1.5 m from the ground, which is in the height range where most Megalopta nests are found in the wild. The front panel of the apparatus comprised a 580 × 140 mm transparent acrylic plastic sheet with five round gray disks (10 cm diameter) attached side-by-side and about 2 cm apart (Figure 1B). In the middle of each disk was a 5 mm diameter hole extending all the way through the front panel. Behind each of the five disks it was possible to attach a nest stick with its entrance just in line with the hole in the front panel, which thus became an extension of the nest entrance. In this way, the gray disk concealed the visual features of the actual nest as well as served as a landing target for the bee. On the rear side of each circular disk, a plastic collar was used to connect the nest. Small magnets on the collar and on the rear side of the circular disk made it easy to attach and remove the nest sticks from their positions in the apparatus. The sides of the apparatus were covered with gray acrylic plastic sheets, while the back was open for easy access to the nest sticks. To further prevent the bees from recognizing the visual appearance of their nest, all sticks were covered with a black cloth. The emitter of an infrared motion detector (with inbuilt alarm) was mounted at the left-hand edge of the front panel and its receiver at the right-hand edge. The emitter’s unbroken IR light beam was then manipulated to cross the 5 entrance holes on the front panel – when a bee entered or departed a nest, the beam was broken and an alarm sounded to alert us to this event. The experiment was monitored using night vision monoculars (Bushnell 2.5 × 42 mm monocular) and bee flights were recorded with an infrared-sensitive Sony HDR-CX730 video camera (in “Night Shot” mode) mounted on a tripod around three meters in front of the experimental apparatus. Extra infrared illumination was provided by a Sony HVL-HIRL Video IR Light fitted to the hot-shoe of the video camera.

Dorsal landmarks/patterns were placed above the entrances of nests by adding either (1) a bar of thick black cardboard (30 × 130 × 5 mm) that protruded out over the nest, centered 100 mm directly above it (Experiment 1), or (2) black striped patterns held between 2 square sheets of transparent Perspex (UV-transmissive Solacryl SUVT, PolyOne USA, 5.6 mm thick), that protruded 150 mm out over the entire front panel of the apparatus (Experiments 2 and 3). Directly above the entrance of each of the five nests, we placed a 150 × 150 mm pattern of three parallel black stripes (using black electrical tape of 19 mm width). The tape stripes were placed such that they were alternated with three transparent stripes of clear Perspex (of 19 mm width). A UV-transparent diffuser paper (Lee Filter 251, Quarter White Diffuser), placed on top of the upper sheet of Perspex, diffused the view of the forest canopy seen dorsally from below through the clear stipes and also prevented the black stripes from being seen from above. Depending on the experiment, these stripes were either oriented parallel or perpendicular to the front panel of the apparatus (Experiment 2) or at 45° or 135° to it (Experiment 3).

Habituation and training

The nest of the bee(s) to be tested was placed behind one of the five gray disks. The other four possible positions were filled with sham nest sticks (similar sticks found in the forest but with a 5 mm diameter hole drilled into one end).

Experiments relied on the fact that Megalopta, like all bees, learns visual landmarks around the nest entrance before departing on a foraging trip and later uses these landmarks to locate the position of the nest upon return. When a bee leaves its nest, before taking off to forage, it performs an “orientation flight” to memorise the landmarks around the nest site – presumably including the dorsal landmarks we supplied. Before being tested, the bee(s) from one nest went through a habituation period to get used to the experimental apparatus and to learn to find its way back to the nest after foraging. Between foraging bouts, the nest (together with the landmark) was swapped pseudorandomly with one of the artificial nest sticks (and its associated landmark) to make the position of the nest an unreliable cue (the full complement of nests and landmarks – as presented during testing – was also present during habituation). This habituation went on for a maximum of three days or until the bee(s) made three successful returns in a row (without landing on any of the other “incorrect” entrances).

In terms of choice behavior, bee returns fell broadly into two categories: either the bees fly quite confidently to the landmarked nest and enter it directly (often the situation for returns at the brighter end of their activity range when the landmarks are easier to distinguish), or they fly slowly from side to side in front of the five nest disks before making a choice.

Testing

Once a bee had habituated to the experimental situation testing began. The initial position of the nest was always in the middle position. However, when the bee left the nest to forage, the position of the nest and the landmark was either kept in its original position or quickly swapped with one of the artificial nests and its landmark (with the position of the nest chosen pseudorandomly from trial to trial). If the bee found its way directly back to the nest possessing the learned landmark this was considered a successful return and a correct choice. The bee’s choice was defined as the gray target disk upon which it first lands. A correct choice was defined as a first landing on the gray disk below the learned landmark. An incorrect choice was defined as a first landing on one of the other four gray disks.

Each bee was tested in 10 trials in which the nest was swapped pseudorandomly with one of the artificial nests or left in its central position. In order to control for the possibility that the bee might be using olfactory cues, or any other cue directly linked to the nest, we performed two extra trials (or one extra trial, in the case of four bees that went missing before the second control trial) in which only the position of the landmark was shifted and the nest was left in its original position.

Exact details of the experimental procedures followed can be found in the main text.
Experiment 4

Experimental Apparatus

Our artificial canopy consisted of two large (700 × 700 mm) sheets of UV-transparent Perspex (Solacryl SUVT, PolyOne USA, 5.6 mm thick) placed on top of each other 1.2 m above the ground (Figure 1C). Between the sheets we distributed three solid-black cardboard circles (20 cm, 20 cm and 30 cm) on the vertices of an equilateral triangle, which constituted the “canopy.” A UV-transparent diffuser paper (Lee Filter 251, Quarter White Diffuser) placed on top of the circles served to cover the “canopy” from being distinguished from above, and the real forest canopy from being distinguished from below. Two of the vertical sides of the setup – opposite to each other – were open, while the other two sides each had a device to attach a nest about 10 cm below the roof with the two nest entrances facing in under the artificial canopy (and facing each other). One nest was occupied by a bee, while the other nest was an empty sham nest. Mounted at the edges of each of these two nest-holding devices, infrared motion-detectors of the same type as used in Experiments 1-3 announced the entrance or exit of a bee. As previously, this experiment was monitored using night vision monoculars (Bushnell 2.5 × 42 mm monocular) and bee flights were recorded with an infrared-sensitive Sony HDR-CX730 video camera (in “Night Shot” mode), although in this case the camera was placed on the ground on a small tripod under the artificial canopy and pointed upward. Extra infrared illumination was provided by a Sony HVL-HIRL Video IR Light fitted to the hot-shoe of the video camera.

Habituation, training and testing

Habituation, training and testing were performed in a manner similar to that described for Experiments 1-3, but with the obvious difference being the use of an artificial canopy as a stimulus. Exact details of the experimental procedures followed can be found in the main text.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data analysis

To test if the bees were using smell as a cue when finding their way home to the nest, we compared the binomial success rate of the returns using generalized linear mixed models (GLMMs). A model including test condition (Test 1 involving displacement of landmark and nest, or Test 2 involving displacement of landmark alone) was compared to a reduced model without a fixed effect using a likelihood ratio test and the Akaike Information Criterion (AIC) value (when the quality of two models are compared – as judged by a trade-off between the goodness of fit of each model and the number of parameters required to obtain this fit – the model with lowest AIC is considered the better model). Individual nest was included as a random effect to account for pseudoreplication. To assess whether or not the bees were using the landmark to locate the nest in the experiments, we compared the 95% confidence interval (CI) of the proportion of successful returns to the proportion expected by chance. GLMMs were fitted using the lme4 package22 in RStudio.23 The data from the four experiments were analyzed separately.