Natural History Note

How Patrollers Set Foraging Direction in Harvester Ants

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abstract: Recruitment to food or nest sites is well known in ants; the recruiting ants lay a chemical trail that other ants follow to the target site, or they walk with other ants to the target site. Here we report that a different process determines foraging direction in the harvester ant *Pogonomyrmex barbatus*. Each day, the colony chooses from among up to eight distinct foraging trails; colonies use different trails on different days. Here we show that the patrollers regulate the direction taken by foragers each day by depositing Dufour's secretions onto a sector of the nest mound about 20 cm long and leading to the beginning of a foraging trail. The patrollers do not recruit foragers all the way to food sources, which may be up to 20 m away. Fewer foragers traveled along a trail if patrollers had no access to the sector of the nest mound leading to that trail. Adding Dufour's gland extract to patroller-free sectors of the nest mound rescued foraging in that direction, while poison gland extract did not. We also found that in the absence of patrollers, most foragers used the direction they had used on the previous day. Thus, the colony's 30–50 patrollers act as gatekeepers for thousands of foragers and choose a foraging direction, but they do not recruit and lead foragers all the way to a food source.

Keywords: pheromones, regulation of foraging behavior, harvester ant, foraging direction.

Social insect colonies adjust the intensity and location of foraging according to food availability and colony needs (Gordon 1991; Camazine 1993; Beismeijer et al. 1998; Schafer et al. 2006). The direction foragers take in search of food is influenced by interactions with other workers. Returning honeybees recruit other foragers to food resources using waggle dances that provide information about direction and distance to food (von Frisch 1967; Gould and Gould 1988). Ants and termites use chemical trails to recruit foragers and lead them to food resources and nest locations (Reinhardt and Kaib 2001; Andara et al. 2004; Blatrix et al. 2004; Akino and Yamaoka 2005; Jackson et al. 2006). The ant *Temnothorax albipennis* uses tandem running, where one ant leads another from the nest to the food source (Franks and Richardson 2006). Here we report that a different process regulates foraging direction in the red harvester ant (*Pogonomyrmex barbatus*). Patrollers regulate the direction taken by foragers each day by depositing Dufour's secretions on a sector of the nest mound about 20 cm long. The secretions lead to the beginning of a foraging trail but do not recruit foragers all the way to food sources, which may be up to 20 m away.

In a harvester ant colony, patrollers are the first ants to leave the nest each morning, and they scout the nest mound and the surrounding foraging area of about 30 m^2 around the nest (Gordon 1991). Their return to the nest, at a rate of about 1 patroller/10 s (Greene and Gordon 2003, 2007), stimulates the onset of foraging. Foragers recognize returning patrollers during brief antennal contacts by detecting cues in task-specific cuticular hydrocarbons (Greene and Gordon 2003).

Pogonomyrmex barbatus foragers travel from the nest in streams, or trails, and then fan out to individually search for seeds (Gordon 1991) that are scattered by flooding and wind (Gordon 1993). Trails extend radially from the edges of a large nest mound, which has a diameter of up to 1 m, and are often cleared of vegetation by the ants (Gordon 1991). In a foraging trip, which lasts about 20 min (Gordon and Kulig 1996; Adler and Gordon 2003), a forager travels along the trail and then, once it finds food, returns directly to the nest along the trail (Gordon 1995; Gordon and Kulig 1996). Since each forager leaves the trail at a different point to search for seeds, a trail does not lead all

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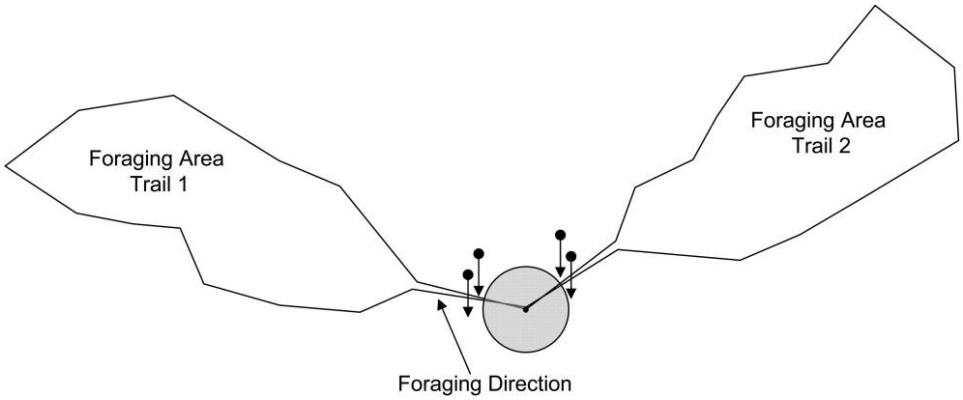

Figure 1: Diagram of a harvester ant nest mound and two foraging trails. The gray circle indicates the nest mound, which can be up to about 1 m in diameter in a mature colony. The nest entrance is usually located at the center of the nest mound, indicated here by a small black circle. Foraging trails extend up to 20 m from the nest mound. The paired arrows indicate the location of flags where forager counts were made along each trail. Maps from observations of foraging activity are from Gordon (1995).

the foragers to a particular food source but instead forms the base of a fan-shaped region in which ants search for seeds (Gordon 1995). The directions of foraging trails used by a harvester ant colony vary from day to day (Gordon 1991)

Each day the colony chooses from among up to eight distinct foraging trails (Gordon 1991). If the same direction is used on successive days, then the same individual foragers tend to use that direction (Gordon 1991). Foraging direction varies in response to overlap with the foragers of neighboring colonies; older, mature colonies avoid foraging areas used by conspecific neighbors, while younger colonies are more persistent in aggressive encounters with neighbors (Gordon 1992; Gordon and Kulig 1996). Previous work showed that foragers tend to travel in the direction from which most patrollers return, indicating that patrollers somehow choose the day's foraging direction (Gordon 1991). Recruitment by pheromone trails leading all the way to a food source occurs very rarely in *P. barbatus*, except in response to experimental baits that provide an extremely large and concentrated source of seeds (Gordon 1983, 2002).

We hypothesized that patrollers influence foraging direction by depositing chemical secretions on the nest mound. We observed that patrollers scout the nest mound with their abdomens tucked under their bodies (Gordon 1991). The secretions from the poison gland and the Dufour's gland exit from the stinger apparatus located in the abdomen. In ants, Dufour's gland secretions have been implicated in a variety of functions, including being territorial markers and propaganda pheromones used by parasitic ants (Hölldobler et al. 2004; Brandt et al. 2006). In the genus *Pogonomyrmex*, Dufour's gland contents are composed mainly of species-specific blends of hydrocarbons (Hölldobler et al. 2004). Poison gland secretions have been shown to act as sex pheromones and trail pheromones in ants (Buschinger 2003; Jackson et al. 2007). Here we show that a harvester ant colony's 30–50 patrollers act as gatekeepers that set the foraging direction for the thousands of foragers that exit the colony in search of food each day (Gordon 2002).

Material and Methods

Experiment 1: Do Patrollers Influence the Direction of Foraging?

In eight colonies, all 5 years old or older, flags were placed on both sides of each foraging trail near the edge of the nest mound (fig. 1). We chose nests that each had two to four habitual foraging directions that had been cleared by the ants through the surrounding grass. To exclude patrollers from a sector of the nest mound leading to a particular foraging direction and trail, a barrier of 15-cm-high aluminum flashing in a flat-bottomed V shape was placed so the bottom of the V was near the nest entrance and the ends straddled the opening to the trail at the edge of the nest mound. Patrollers were allowed access to the remainder of the nest mound. We removed the barrier when patrollers were observed to return to the nest entrance at the rate of 10 ants per 2 min, about 1 per 12 s (known from previous work to stimulate the onset of foraging: Greene and Gordon 2003, 2007), and when at least one ant had returned with a seed. Thirty minutes after removing the barrier, we counted the number of foragers that during 1 min passed an imaginary line between the

flags that marked the opening to each trail. Experiments were performed on two consecutive days for each colony, each time with a different trail.

Because foraging activity differs between colonies and from day to day (Gordon 1992; Gordon and Kulig 1996), we calculated a foraging score for each trail by dividing the forager count for that trail by the expected number of foragers at each trail if the foragers were evenly distributed on all trails. We used a signs test to determine whether the foraging score for directions for which the nest mound leading to that sector was blocked was less than 1, the ratio that indicates the observed number of foragers equaled the expected number of foragers.

Experiment 2: What Is the Source of the Patroller Signal?

This experiment tested whether the Dufour's gland and the poison gland contain chemicals that influence the number of foragers using trails. In six of the eight colonies used in experiment 1, all with well-defined foraging trails cleared through the grass by the ants, trails were marked by flags as above. At least 2 days before the start of the experiment, patrollers were collected from each colony and killed by freezing. Patrollers were thawed and the stinger apparatus was dissected from ants under water using a dissecting microscope, yielding the stinger, a portion of the hindgut, the Dufour's gland, and the poison gland. The Dufour's gland of a single ant in each colony was removed by pinching the base of the gland with forceps and pulling the gland from the other material. The gland was placed in 2 mL of 100% pentane to extract its contents. The poison gland was dissected from one ant for each colony, and its contents were extracted using 2 mL of 100% pentane. Extractions were performed overnight at room temperature in sealed, screw-top vials that were thereafter stored at −20°C.

To determine whether foraging could be rescued by the deposition of glandular extracts on the nest mound, simulating the action of live patrollers, we blocked one sector of the nest mound leading to a foraging trail, using a V-shaped piece of 15-cm-high aluminum flashing, as in experiment 1. We thus prevented patrolling on an approximately 20-cm-long sector of the nest mound leading to a trail. We allowed patrolling on the rest of the nest mound. When patroller return rate to the nest entrance reached 10 ants in 2 min, the rate previously shown to elicit foraging (Greene and Gordon 2007), and at least one ant had returned with a seed, we used a Pasteur pipette to distribute, as evenly as possible, Dufour's gland extract, poison gland extract, or 100% pentane on the nest mound inside of the barrier. A few minutes later, when the solvent was completely dry, we removed the barrier. The three treatments were applied in a random order on consecutive days, and the same trail was not used on consecutive days. The poison gland and Dufour's gland extracts that were used were taken from patrollers of the focal colony. Thirty minutes after removing the barrier, a count was made of the number of foragers passing an imaginary line between the flags at the opening to each trail during a 1-min interval. This count was made at all trails in each colony. We did not know which stimulus had been added until the end of the trial. Foraging scores were calculated as described for experiment 1. Foraging scores were compared using a Friedman ANOVA; post hoc comparisons were made using Nemyeni's test.

Experiment 3: In the Absence of Patrollers, Do Foragers Use the Direction They Used the Previous Day?

This experiment tested whether patrollers influence the trail fidelity of foragers. On the day before the experiment, we collected 100 foragers from two foraging trails at each of five colonies. We painted the abdomens of foragers of each trail with a unique color (Uni-Paint markers, Sanford Ink, Oak Brook, IL) and released the marked ants the same afternoon. This marking procedure does not affect foraging behavior (Brown and Gordon 1997). We then stimulated foraging in the absence of patrollers. On the morning of the experiment, patrollers exiting the nest entrance were collected and kept in a container, so no patrollers reached the nest mound. After 30 min had passed with no patrollers emerging from the nest entrance, we artificially stimulated foraging by introducing 10 beads coated with patroller hydrocarbons from the same colony; the beads were added to the nest entrance at a rate of 1 bead per 10 s (Greene and Gordon 2003, 2007).

To coat the beads, surface lipids were extracted by soaking thawed ants in 100% pentane for 10 min (Greene and Gordon 2003, 2007). Cuticular hydrocarbons were isolated by running extracted surface lipids through a silica gel column (60–200 mesh, Sigma Aldrich, Milwaukee, WI) using 100% pentane as an eluent. Each bead contained an amount of hydrocarbon equal to the amount extracted from one ant. One ant-equivalent of hydrocarbon was added to each bead. As in previous work (Greene and Gordon 2003, 2007), the beads usually elicited foraging behavior. In two cases, when foraging activity did not begin within 15 min of introducing the beads, we also returned one to five live patrollers directly to the nest entrance without allowing them to patrol the nest mound.

After foragers emerged from the nest, every 10 min for 60 min, we counted the number of painted foragers of each color observed in 1 min along 2 m of trail extending from the nest entrance. For each trail, we used a *G*-test to compare the observed number of ants painted each color counted along the trail with the expected value. The

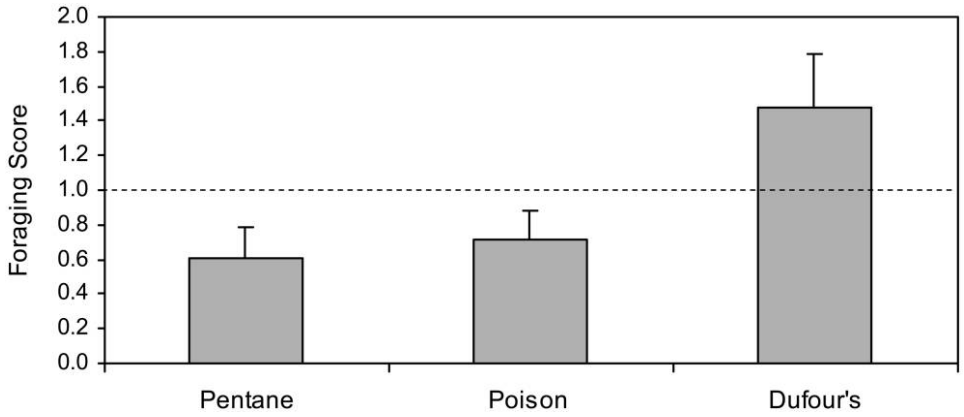

Figure 2: Pentane extracts of Dufour's gland secretions, but not extracts of poison glands, rescued foraging on trails when patrollers were blocked from the sector of the nest mound leading to that trail. Data are reported as means ± SE. The dashed line indicates the expected number of foragers on the trail.

expected value was calculated assuming an equal number of ants of each color on each trail if ants chose trails independently of their choice of trail the previous day.

Results

Experiment 1: Do Patrollers Influence the Direction of Foraging?

The colony's daily choice of foraging direction depends on a cue placed on the ground by patrollers before foragers emerge from the nest. Preventing patrolling, but not foraging, on a sector of the nest mound leading to a foraging trail reduced foraging activity on that trail. Trails leading from nest mound sectors from which patrollers had been blocked had lower foraging scores on both day 1 of the experiment (signs test: $n+ = 0$, $n- = 8$, $P < .01$) and day 2 of the experiment (signs test: $n+ = 0$, $n- = 8$, $P < .01$). We repeated the experiment at all replicate colonies by blocking patrolling on the two nest mound sectors leading to two different trails, each on a different day. Trails leading from nest mound sectors from which patrollers had been blocked on day 1 had significantly lower foraging scores (mean foraging score: 0.45 ± 0.08 SE) than the same trails on day 2 (mean foraging score: 1.00 ± 0.17 SE), when patrollers were not blocked from the nest mound sectors in that direction (Wilcoxon signed-rank test, $P < .025$). Similarly, trails leading from nest mound sectors from which patrollers had been blocked on day 2 (mean foraging score: 0.45 ± 0.09 SE) had a lower foraging score than the same trails on day 1 (mean foraging score: 1.27 ± 0.14 SE), when patrollers were not blocked from those sectors (Wilcoxon signed-rank test, $P < .012$).

Experiment 2: What Is the Source of the Patroller Signal?

Patrollers mark foraging direction using Dufour's gland secretions. Pentane extracts of Dufour's gland secretions, but not extracts of poison glands, were sufficient to rescue foraging on trails leading from blocked nest mound sectors (Friedman ANOVA, $P < .01$; fig. 2). Foraging scores observed on a trail when the nest mound sector leading to it had no patrollers but was treated with Dufour's gland extracts were significantly higher than scores on trails associated with nest mound sectors that had no patrollers and were treated with poison gland extracts or with pentane (Nemenyi post hoc test, $P < .05$). There was no difference in the foraging scores on trails leading from blocked nest mound sectors treated with the pentane control and the poison gland extract (Nemenyi post hoc test, $P > .05$).

Experiment 3: In the Absence of Patrollers, Do Foragers Use the Direction They Used the Previous Day?

Foragers were likely to use the same trail they used the previous day when patrollers were prevented from marking the nest mound before the start of foraging. Data from one trail for each colony were analyzed. Significantly more marked ants were found on the trail used the previous day compared with the number of ants marked for the other trail (G-test: $G = 58.22$, $P < .001$). On average, 82.4% of painted ants used the same trail as on the previous day.

Discussion

In a harvester ant colony, patrollers ordinarily do not recruit foragers all the way to food (Gordon 1983). In-

stead, patrollers regulate the direction foragers take to search for food each day by depositing secretions from the Dufour's gland onto the sector of the nest mound, about 20 cm long, that leads to a foraging direction in which ants may travel up to 20 m. Previous work suggested that in harvester ants, Dufour's gland secretions might act as long-lived colony-specific markers along the foraging trail (Hölldobler et al. 2004). Our results demonstrate that Dufour's gland secretions placed on the nest mound act in the day-to-day regulation of foraging direction.

Foragers are more likely to travel on a trail if patrollers have deposited Dufour's gland secretions on the sector of the nest mound leading to that trail. The amount of Dufour's gland extract that one patroller provides was sufficient to set the direction of many foragers along a trail that led away from the nest mound. Each of the 20–50 patrollers that scout the nest mound in a large colony each morning may deposit smaller amounts, and further work is needed to determine whether the quantity of secretion deposited influences forager direction. Even a single day without patrollers depositing secretions on a nest sector leading to a trail greatly reduces the numbers of foragers on that trail. This suggests that the relevant information in Dufour's gland secretions is short-lived.

The patrollers' choice of direction each day is apparently influenced more by encounters with the patrollers of neighboring colonies than by current food availability. Colonies adjust to the foraging direction of neighbors (Gordon 1992; Gordon and Kulig 1996). However, once patrollers trigger the onset of foraging and set its directions, a different process regulates the numbers of ants actively foraging (Schafer et al. 2006; Gordon 2007). Once foraging begins, whether a forager leaves the nest on successive trips depends on the rate at which foragers return to the nest with food (Schafer et al. 2006). This links foraging intensity to food availability, because the more that food is available, the more quickly foragers will find it and return to the nest.

Forager decisions about which trail to take apparently occur almost immediately after exiting the nest. Dufour's gland secretion placed in a sector of the nest mound leading about 20 cm from the nest entrance was sufficient to determine foraging direction. Unlike ants that use chemical recruitment trails, in which a pheromone trail is laid all the way to a food source (Akino and Yamaoka 2005; Jackson et al. 2006), harvester ants use centralized information at the nest mound to regulate foraging direction.

Our results show that foragers remember the direction they took the day before, without information from the patrollers; we do not know what cues the foragers use to do this. When foraging was artificially stimulated in the absence of patrollers, foragers tended to use the direction they had taken the previous day. Thus, both forager memory and patroller signals can influence the direction foragers take. However, since a colony's foraging directions change from day to day, patroller signals must override forager memory. A forager will take a direction different from the one it took the previous day if the patrollers open the gate to another trail.

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