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Research article

Dispersed central-place foraging in the polydomous odorous house ant, *Tapinoma sessile* as revealed by a protein marker

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Abstract. The odorous house ant, *Tapinoma sessile*, is a native ant species common throughout North America. In urban areas, this ant is classified a pest species and exhibits several attributes characteristic of invasive "tramp" ants (sensu Passera, 1994). These include: extreme polygyny, colony reproduction by budding, reduced internest aggression, generalist diet, and polydomy. Here we explore the organization of foraging and the pathways of food distribution in polydomous colonies of *T. sessile* in the laboratory and field using a novel marking technique (rabbit IgG protein) and enzyme linked immunosorbent assay (ELISA). Laboratory assays revealed patterns of food allocation from foragers to other castes and developmental stages. Foragers distributed the IgG- labelled sucrose to the majority of workers within 24 h, and workers retained significantly more sucrose than either queens or larvae. Approximately 50 % of queens tested positive for the IgG marker and some queens received significantly more sucrose than others, indicating a possible reproductive dominance hierarchy. Larvae received little sucrose demonstrating their minor reliance on carbohydrates. The results of field experiments showed that odorous house ants are dispersed central-place foragers whereby ants from individual nests exhibit high foraging site fidelity, travel along well-established trails, and forage on a local scale. Dispersed central-place foraging most likely allows the odorous house ant to more efficiently secure both clumped and dispersed food sources and possibly increases its competitive ability. As a result, colonies become numerically large and ecologically dominant. The results of our study contribute to our understanding of the social behavior and colony organization in *T. sessile*. In addition, they provide a framework for designing more effective ant control programs based on liquid baits.

Keywords: Tapinoma sessile, foraging range, immunomarking, odorous house ant, polydomy.

Introduction

Most Hymenoptera, including ants, bees, and wasps are highly centralized, occupy a single nest, and engage in central-place foraging. Central-place foragers collect food around the nest and bring it back to the colony where it is fed to the more stationary castes and developmental stages and/or stored for future use. The central-place foraging strategies of many ant species have been thoroughly studied and have been reviewed by Traniello (1989) and Hölldobler and Wilson (1990). Some ant species, however, occupy multiple nests, a condition known as polydomy. Polydomous species often engage in dispersed central-place foraging, whereby colonies redistribute workers, queens, and brood among nests in response to the spatiotemporal heterogeneity of food resources. Such foraging strategy maximizes the net energy yield by reducing food transport costs (Orians and Pearson, 1979; Stephens and Krebs, 1986) and may also help defend food sources form competitors. Moreover, Greenslade (1974) demonstrated that polydomous colonies are more efficient at collecting resources in habitats with low productivity. Holway and Case (2000) investigated dispersed central-place foraging in the highly polydomous (and polygynous) Argentine ant and found that Argentine ants relocated nests in response to the spatial heterogeneity of resources. Instead of bringing the food back to the nest, Argentine ants brought workers, brood, and resources closer to the food. Despite the obvious benefits of dispersed central-place foraging and its potential importance for understanding the competitive dominance of many invasive ant species, the foraging ecology of dispersed central-place foragers is poorly understood and field studies are lacking.

In this study, we examine the organization of dispersed central-place foraging in the odorous house ant, *Tapinoma sessile* (Say). The odorous house ant is a native species widely distributed throughout North America, and is a common house-infesting pest throughout its range (Thompson, 1990). *T. sessile* is very opportunistic and inhabits a variety

of nesting sites, both natural and man-made. The nests are usually shallow, under stones, in mulch or debris, or protected inside structures. Our field observations indicate that the nests are frequently moved, most likely due to changes in abiotic conditions and availability of food. We have also observed that odorous house ants may forage in a wandering pattern or along well established trails whenever the ants are tending a permanent food source, such as a homopteran population. Odorous house ant foraging patterns and foraging distance, however, are not well understood due to the great fluidity of colonies with constant fusion and budding of nests and frequent movement of brood, queens, and workers. In other Dolichoderine ants, specifically the Argentine ant, Markin (1968) found workers labeled with a radioisotope (P^{32}) in citrus orchards up to 45 m from the source; Ripa et al. (1999) found up to 21 % of ants marked at 54 m from the feeding station; and Vega and Rust (2001, 2003) found marked ants up to 61 m from feeding stations.

To investigate the foraging ecology of the odorous house ant in urban areas, we used a combination of laboratory and field tests designed to investigate how the distribution of nests and trails affects the distribution of food within a large and a highly polydomous society. To track the movement of food within colonies we used protein marking and sandwich enzyme-linked immunosorbent assay (ELISA). Protein-based marking techniques for insects have recently been developed (reviewed in Hagler and Jackson, 2001). Immunoglobin G (IgG) proteins derived from various vertebrates can be applied internally or externally to the target insect and later detected by the highly sensitive sandwich ELISA (Hagler, 1997a, b). The protein marking technique is less costly, more sensitive, and safer than using radioactive isotopes, which had been used in previous studies in ants (Markin, 1970; Sorensen et al., 1980). In addition, they are photostable and resist degradation by heat and water (Hagler, 1997a), and therefore are an ideal marker for studying natural populations. For example, DeGrandi-Hoffman and Hagler (2000) used protein marking to investigate the flow of sucrose solution through a honeybee colony. Rabbit IgG protein obtained by the foragers from sucrose feeding stations spiked with the marker was readily distributed throughout the hive. The protein was later detected in foraging and non-foraging bees, brood, and the colony's food reserves.

To obtain a clear understanding of the foraging ecology in the odorous house ant we first examined the uptake and retention of marker under laboratory and field conditions. Second, we used immunomarking to investigate the flow of liquid food (sucrose) within laboratory colonies of *T. sessile* to gain a better understanding of social behavior and colony organization in this species. Finally, we used protein marking to investigate the foraging pattern and foraging range in odorous house ants under field conditions. Combined, the results of these objectives provide a framework for future studies utilizing protein marking to study the biology and behavior of ants and other social insects, and may contribute to developing control methods based on the use of baits.

Materials and methods

Colonies

Stock colonies of *T. sessile* were collected on the campus of Purdue University, West Lafayette, IN. Debris (leaves, mulch) containing ant nests was placed in plastic trays provided with moist plaster nests. As the debris dried, the ants moved into plaster nests. Subsequently, colonies were maintained in debris-free, Fluon™-coated trays. Colonies were reared on 30 % sucrose solution *ad libidum* and hard-boiled egg and minced crickets once a week. Colonies were maintained at 25 ± 2 °C, 60 ± 10 % RH, and 14:10 L:D cycle. Ants were held in the laboratory for 2–4 weeks before use in assays.

Uptake and retention of the IgG marker

The uptake and retention of the IgG protein marker were examined under laboratory and field conditions. We hypothesized that the marker might be picked up faster and retained longer in laboratory colonies for several reasons: relatively small colony size allowing faster spread with more marker available per ant, no competing food sources, no dilution effect (i.e. continuous influx and mixing of unexposed ants with ants that fed on the marker). To estimate uptake under laboratory conditions a colony of approximately 500 workers was provided with 0.5 mg technical grade rabbit immunoglobin (IgG) protein (Sigma Chemical Co., St. Louis, MO) in 30 % sucrose:water solution. The 0.5 mg IgG/mL concentration was selected based on the results of preliminary tests, which revealed that the increases in optical density were minimal above 0.5 mg/ml. Thus we elected to use the 0.5 mg/mL concentration in all subsequent lab and field tests. The ants were starved for 24 h and subsequently fed the solution *ad libidum* for 2 h. After 2 h, the nest containing the colony was transferred to a clean, fluoned box and provided with unlabeled food (30 % sucrose water) for the duration of the experiment. To estimate uptake, 10 workers were randomly sampled from the colony 6, 12, and 24 h after feeding. To estimate retention, 10 workers were sampled at 2, 4, 6, 8, 10, and 12 d after the transfer to unlabeled food. To investigate the spread and the persistence of the IgG marker under field conditions, we chose the 3 longest of the identified trails (trails 1, 3, and 4; Fig. 1) and placed a single feeding station next to each trail, 30 cm away from the nests. The feeding station was a 50 mL plastic tube with a slightly smaller plastic floater that rested on top of the liquid. The floater prevented the ants from drowning in the solution, minimized evaporation, and prevented direct contact between the ants and the solution, thus minimizing external contamination with the marker. The feeding station contained 20 mL of 30 % sucrose water and the protein marker (0.5 mg rabbit IgG/ml sucrose water). Subsequently, 15 ants were sampled from the beginning, middle, and end of each trail 3, 12, 48, and 72 h after the stations were put out. The beginning sampling location was 30 cm away from the feeding station where ants visibly started foraging, the middle was halfway between the beginning and end of trail, and the end was where ants visibly stopped foraging. Therefore, the beginning sampling location was constant for all trails (always 30 cm away from the feeding station), while the middle and end distances changed depending on the length of individual trails (e.g. trail #1, middle = 22.5 m from beginning, end = 45 m away from beginning). All individuals were frozen in individual tubes at –20 °C and later analyzed by ELISA (see below). Both lab and field experiments were replicated 3 times.

Trophallactic exchange of food

To estimate uptake and the distribution of the marker to the various castes and developmental stages, a colony of approximately 1,000 workers, 10–15 queens, and numerous brood of various stages was starved for 24 h and subsequently provided with 0.5 mg IgG/mL in 30 % sucrose solution. We randomly sampled 10 workers, 10 queens, and 10 larvae

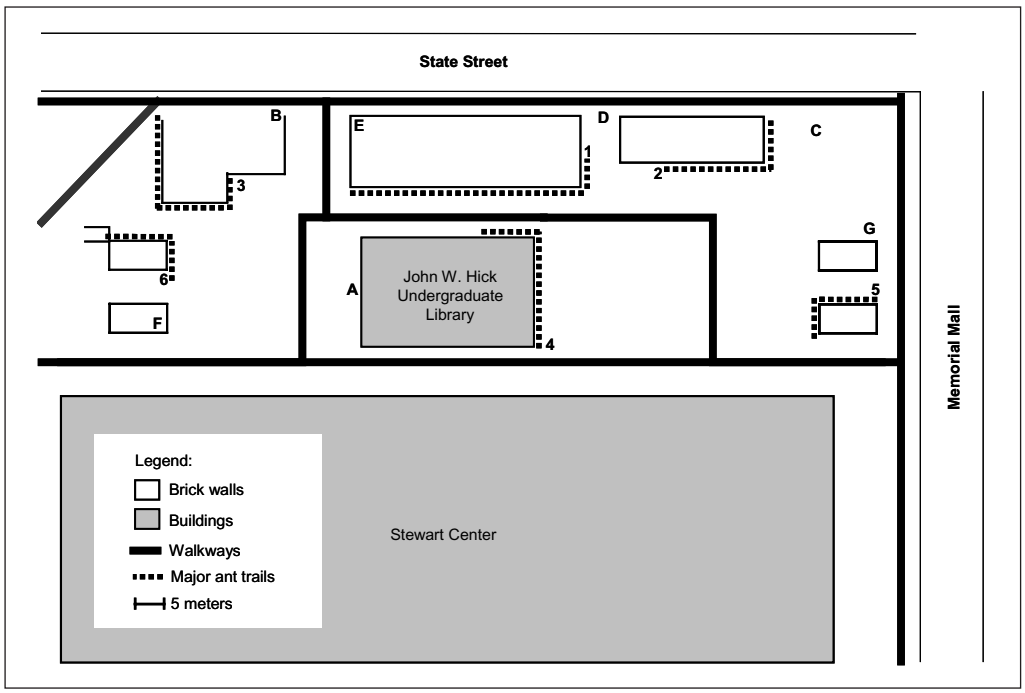

Figure 1. Schematic diagram, drawn to scale, showing the extent of the *T. sessile* colony on the campus of Purdue University. The location of major trails used to investigate the pattern of food flow, the location of bait placement, and ant sampling locations are also indicated.

after 24, 48 and 72 h of *ad libidum* feeding on labeled solution to determine the amount of protein marker acquired by the different developmental stages and castes. All individuals were frozen in individual tubes and later analyzed by ELISA. Three replicates were performed for each time point.

Determination of foraging distance and foraging patterns: field study

The objectives for this experiment were to: (1) estimate the distance that odorous house ants carry liquid carbohydrate food under field conditions, and (2) investigate how the distribution of nests and trails affects the distribution of food within a highly polydomous colony. The test site was a large colony of *T. sessile* on the Purdue University campus (Fig. 1). This colony covers an area of approximately 4,000 m^2 and consists of numerous nests and an extensive three-dimensional network of trails with ants trailing on tree trunks and building walls. All nests appeared to belong to one colony as was evidenced by lack of aggression between even the most distant nests (points F and G, 110 m). To test for intraspecific aggression we used an assay that measured the level of aggression in single worker introductions into a resident colony (Roulston et al., 2003) and aggression was scored on a 0–4 scale described by Suarez et al. (1999). Ants were collected from the distant boundaries of the colony (sites B, C, F, and G, Fig. 1) and one central location (site A). Aggression tests were performed between all possible colony pairs with 10 replications for each pair. We detected no aggression (average score of 0.0 ± 0) between any of the nests. Odorous house ants from other colonies were highly aggressive toward the Purdue campus colony. Ants usually nested in debris (leaves, mulch) and hollow brick walls. We selected ant trails close to those locations (numbers 1–6 in Fig. 1) to place the feeding stations. The visible (aboveground) portion of each trail was measured and ant activity was estimated for each trail by counting the number of ants that crossed an imaginary line in 1 min (Table 3). Ant activity was estimated at 1 PM and three counts were performed for each trail. The location, number, and intensity of foraging trails varied throughout the test period (May–August). However, trails 1–6 remained consistently utilized throughout the season as they were associated with attractive nesting sites (hollow brick walls) and/or permanent feeding sites (aphid-infested trees and shrubs or garbage bins).

To determine the distance sucrose solution was carried, we provided the ants with a single feeding station containing 20 mL of 0.5 mg rabbit IgG/ml of 30 % sucrose solution. The same colony of *T. sessile* was used for replicating the study. At each trial, a single feeding station was placed at one of the trails (numbers 1–6, Fig. 1) and left in place for 24 h. Subsequently, 10 ants were collected from each of 10 locations throughout the site: the beginning, middle, and end of each foraging trail as described above and 7 locations throughout the test site (locations A–G). Locations A–G were active nesting and/or trailing sites and there was no evidence of those sites being connected by trails to trails 1–6. This sampling scheme was repeated 3 times at approximately 4–7 d intervals. All tests were carried out during the peak of colony activity (May–August). Prior to subsequent replications we sampled ants throughout the entire test site to confirm that the IgG marker had been purged (excreted, metabolized, and/or diluted beyond detection) from the colony. Ants were returned to the laboratory and analyzed for the presence of the marker using sandwich ELISA. Each ant was scored positive if it was carrying the protein marker, or negative if not. For each sample point, we calculated the percentage of ants that tested positive.

ELISA procedure

Sandwich ELISA was performed on individual ant samples using previously described techniques (Hagler, 1997; Hagler and Jackson, 1998). Frozen samples were individually homogenized in 200 µL phosphate buffered saline (pH = 7.4) and assayed for the presence of the rabbit immunoglobin protein. Each well of a 96-well microplate was coated with 100 µL of anti-rabbit IgG (developed in goat) (Sigma Chemical Co., St. Louis, MO) diluted 1:500 in distilled water and incubated for 2 h at 4 °C. After incubation, the primary antibody was discarded and 310 µL of 1 % non-fat dry milk (Bio-Rad Laboratories, Hercules, CA) in distilled water was added to each well to block any remaining non-specific binding sites. After 30 min. incubation at 26 °C the milk was discarded. Ant samples were vortexed and a 70 µL aliquot of sample was added to each well and incubated for 1 h at 26 °C. The samples were then discarded and

each well was washed 3 times with PBS Tween 20 (0.05 %) and 2 times with PBS. Anti-rabbit IgG conjugated to horseradish peroxidase (50 µL) diluted 1:1,000 in 1 % non-fat milk was added to each well and incubated at 26 °C for 1 h. All wells were washed again as above and 50 µL of TMB HRP substrate (BioFX Laboratories, Owings Mills, MD) was added to each well and incubated for 30 min. Samples were analyzed on a Beckman Coulter AD 340 Absorbance Detector set at 620 nm. The mean (± SE) optical density value and the percentage of samples scoring positive for rabbit protein were determined. Six negative controls (ants never exposed to rabbit IgG) and 6 blanks (PBS buffer only) were run on each plate.

Statistical analysis

The samples were scored positive for the presence of the protein marker if the ELISA optical density value exceeded the mean negative control value by three standard deviations (Hagler, 1997a, b). The percentage of samples testing positive for the IgG protein was tabulated by first calculating the percent of individuals testing positive within a replicate and then averaging across replicates. Percentage data were arcsine transformed to stabilize the variance (Sokal and Rohlf, 1995). ANOVA tests were conducted to determine the significance of developmental stage, time, and bait location on the spread of the marker. This was accomplished by using the PROC GLM procedure in SAS 8.1 (SAS 2002), followed by post-hoc Tukey's HSD tests to separate the means. For all experiments the results are expressed as both: (1) the mean number of individuals testing positive, and (2) the mean optical density (OD).

Results

Uptake and retention of the IgG marker

Ant workers rapidly acquired the IgG protein marker after feeding on sucrose water containing the marker (Fig. 2). Within 6 h, 90 ± 0 % of ants tested positive. At 12 h, 100 ± 0 % of ants tested positive and at 24 h 97 ± 3 % tested positive. This indicates that all workers within the colony either feed directly or receive nutrition from nestmates through trophallaxis. The retention study was designed to determine the length of time after ingestion IgG-labeled sugar water remained detectable in the ants. In laboratory studies rabbit IgG was readily detectable at 4 days with 93 ± 3 % ants testing positive and the mean OD value of 0.59 ± 0.06 (Fig. 2). After 4 d, the amount of IgG retained dropped sharply (Fig. 2). On d 12, 23 ± 3 % of ants still tested positive and the mean OD values decreased to 0.11 ± 0.02. In contrast to laboratory tests conducted on relatively small colony fragments (500 workers) under controlled environmental conditions, field studies revealed that the IgG marker was relatively short lived (Table 2). At 72 h, only 2–7 % of ants still tested positive for the marker and the mean OD values dropped to background levels. The difference in marker retention between lab and field tests can be most likely attributed to the dilution effect, i.e. in the field, the labeled sugar water was quickly distributed by trophallaxis to thousands of individuals and became undetectable by ELISA. In addition, environmental factors such as availability of alternative foods and exposure to heat and solar radiation may have affected the longevity of marker in the field.

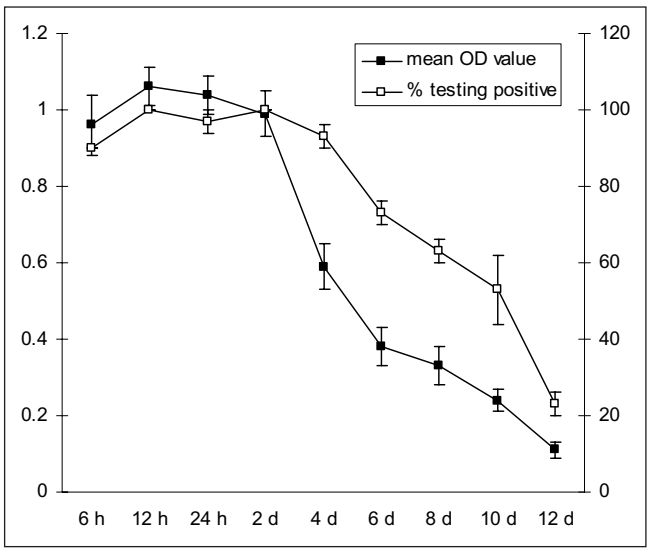

Figure 2. Uptake and retention of the IgG protein marker by odorous house ants under laboratory conditions. Mean optical density (OD) values ± SEM are given (n = 30). Mean ± SEM percent of ants testing positive for the marker is also provided.

Trophallactic exchange of food

The results of the trophallactic flow of food are presented as either mean percentage of ants testing positive for the marker (Table 1) or in histograms displaying the distribution of ELISA results (optical density values) (Fig. 3). The data in Table 1 is largely quantitative and only indicates the number of ants that tested positive, i.e. exceeded a predetermined threshold level. A disadvantage of this approach is that certain ants may have received relatively little marker and only slightly exceeded the threshold level, but still scored positive. Such individuals would not be differentiated from those that ingested substantially larger amounts of the marker. Therefore, in addition to Table 1 we also provide a more qualitative picture of the data, in a form of histogram with the range of OD values broken into 5 arbitrary intervals. Workers readily fed on the sucrose solution (indicating a lack of repellency at the concentration used) and 97 % of the workers tested positive for the marker at 24 h. This indicates that liquid carbohydrate food is rapidly distributed among workers or that all workers feed directly. At subsequent sampling periods, 100 % of workers tested positive for IgG indicating that the exchange of food continues beyond the initial 24 h. In comparison to workers, queens received substantially less sucrose. At 24 h, 50 % of the queens tested positive. The number of queens testing positive increased to 63 % at 48 h, then decreased to 43 % at 72 h, indicating loss of marker possibly due to excretion/metabolism, regurgitation back to the workers, and/or egg-laying. Less than 50 % of the larvae tested positive for the marker at any given sampling period. While some larvae received liquid sugars, others did not suggesting a possible feeding hierarchy that that may depend on larval age and, consequently, nutritional needs.

A histogram of ELISA results (frequency distribution of OD values) for workers, queens, and larvae is presented in Figure 3A–C. The majority of workers had OD values >1.0 indicating ingestion of a relatively large amount of marker and rapid sharing of food with non-foraging workers. The distribution of OD values for queens assumed a parabolic shape whereby some queens received a relatively large amount of marker, while others received little, possibly indicating a reproductive dominance hierarchy. The distribution of sugar water to larvae was delayed and the majority of larvae showed low (0.00–0.25) OD values at 24 h, even though 43 % of larvae tested positive for the marker at 24 h (Table 1). At 48 h, the OD values in larvae increased suggesting that larvae require a longer period of time to communicate hunger to the workers. Overall, the majority of larvae received little sucrose demonstrating that carbohydrates are mainly used by workers.

Table 1. Mean percentage ± SE of ants testing positive for rabbit IgG protein 24, 48, and 72 h after feeding on sucrose solution. Mean optical density (OD) values ± SE are given in parentheses (n = 30). Means followed by the same letter are not significantly different by Tukey's HSD test ($P \leq 0.05$). First letter indicates within row comparisons, second within column comparisons.

Sample type	Mean % positive samples		
	24 h	48 h	72 h
workers	96.7 ± 3.3 % a,a (0.97 ± 0.05)	100 ± 0 % a,a (1.10 ± 0.04)	100 ± 0 % a,a (1.12 ± 0.03)
queens	50.0 ± 10 % a,b (0.47 ± 0.09)	63.3 ± 3.3 % a,b (0.47 ± 0.09)	43.3 ± 3.3 % a,b (0.42 ± 0.09)
larvae	43.3 ± 14.5 % a,b (0.26 ± 0.05)	46.7 ± 8.8 % a,b (0.50 ± 0.10)	43.3 ± 6.7 % a,b (0.32 ± 0.07)

Determination of foraging distance and foraging patterns: field study

The results of the field experiment are summarized in Table 3 and provide a useful description of foraging pattern and foraging distance in the highly polydomous *T. sessile*. Our results indicate that foraging in the odorous house ant is highly localized. Ants testing positive for the marker were found only along the trail that was being provisioned with food and not at locations A–G, even though locations A–G were oftentimes much closer to the feeding station than the

Table 2. Mean percentage ± SE of ants testing positive for rabbit IgG protein 3, 12, 48, and 72 h after sucrose solution was placed next to foraging trails. Mean optical density (OD) values ± SE are given in parentheses (n = 30). Means followed by the same letter are not significantly different by Tukey's HSD test ($P \leq 0.05$). First letter indicates within row comparisons, second within column comparisons.

Bait placement	Trail length	Time since bait placement	Ant sampling location			Mean of all 3 sampling locations
			beginning of trail	middle of trail	end of trail	
Trail 1	45 m	3 h	83 ± 32 % a,a (0.86 ± 0.08)	60 ± 6 % a,a (0.67 ± 0.10)	77 ± 3 % a,a (0.85 ± 0.09)	73 ± 4 % (0.79 ± 0.1)
		12 h	77 ± 7 % a,a (0.86 ± 0.09)	87 ± 3 % a,a (0.94 ± 0.07)	77 ± 3 % a,a (0.80 ± 0.09)	80 ± 3 % (0.86 ± 0.05)
		48 h	40 ± 6 % a,b (0.22 ± 0.04)	23 ± 7 % a,b (0.14 ± 0.03)	47 ± 3 % a,b (0.26 ± 0.05)	37 ± 4 % (0.21 ± 0.02)
		72 h	13 ± 7 % a,c (0.09 ± 0.01)	7 ± 3 % a,c (0.07 ± 0.01)	0 ± 0 % a,c (0.05 ± 0.00)	7 ± 3 % (0.07 ± 0.01)
Trail 3	34 m	3 h	83 ± 3 % a,a (0.91 ± 0.08)	77 ± 3 % a,a (0.77 ± 0.09)	80 ± 6 % a,a (0.80 ± 0.08)	80 ± 2 % (0.83 ± 0.05)
		12 h	67 ± 3 % a,a (0.69 ± 0.09)	67 ± 3 % a,a (0.73 ± 0.10)	60 ± 6 % a,a (0.60 ± 0.10)	64 ± 2 % (0.67 ± 0.06)
		48 h	27 ± 9 % a,b (0.18 ± 0.05)	17 ± 3 % a,b (0.10 ± 0.02)	3 ± 3 % a,b (0.06 ± 0.01)	16 ± 4 % (0.12 ± 0.02)
		72 h	17 ± 3 % a,c (0.11 ± 0.03)	0 ± 0 % a,c (0.05 ± 0.00)	0 ± 0 % a,b (0.05 ± 0.00)	6 ± 3 % (0.07 ± 0.01)
Trail 4	30 m	3 h	80 ± 6 % a,a (0.89 ± 0.09)	63 ± 3 % a,a (0.60 ± 0.09)	67 ± 9 % a,a (0.66 ± 0.09)	70 ± 4 % (0.72 ± 0.05)
		12 h	70 ± 0 % a,a (0.68 ± 0.09)	83 ± 3 % a,a (0.81 ± 0.08)	73 ± 3 % a,a (0.74 ± 0.09)	76 ± 2 % (0.74 ± 0.05)
		48 h	40 ± 6 % a,b (0.22 ± 0.04)	33 ± 3 % a,b (0.22 ± 0.05)	13 ± 3 % a,b (0.09 ± 0.02)	29 ± 5 % (0.17 ± 0.02)
		72 h	7 ± 3 % a,c (0.06 ± 0.01)	0 ± 0 % a,c (0.05 ± 0.00)	0 ± 0 % a,c (0.05 ± 0.00)	2 ± 2 % (0.06 ± 0.0)

Table 3. Mean percentage ± SE of ants testing positive for rabbit IgG protein 24 h after sucrose solution was placed next to foraging trails. Mean optical density (OD) values ± SE are given in parentheses ($n = 30$). Means followed by the same letter within rows are not significantly different by Tukey's HSD test ($P \leq 0.05$).

Bait placement	Trail length (m)	Ant count per minute	Ant sampling location								
			beginning of trail	middle of trail	end of trail	A	B	C	D	E	F	G
Trail 1	45	166 ± 12	73 ± 9 % a (0.60 ± 0.08)	70 ± 0 % a (0.60 ± 0.08)	57 ± 9 % ab (0.53 ± 0.09)	0 ± 0 % c (0.05 ± 0.0)	0 ± 0 % c (0.05 ± 0.0)	0 ± 0 % c (0.05 ± 0.0)	0 ± 0 % c (0.05 ± 0.0)	37 ± 9 % b (0.20 ± 0.04)	0 ± 0 % c (0.05 ± 0.0)	0 ± 0 % c (0.05 ± 0.0)
Trail 2	26	125 ± 15	70 ± 10 % a (0.74 ± 0.09)	67 ± 3 % a (0.62 ± 0.09)	63 ± 3 % a (0.53 ± 0.08)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)
Trail 3	34	81 ± 9	80 ± 6 % a (0.67 ± 0.09)	70 ± 6 % a (0.68 ± 0.09)	57 ± 3 % a (0.46 ± 0.08)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)
Trail 4	30	149 ± 14	90 ± 6 % a (0.93 ± 0.07)	83 ± 7 % a (0.85 ± 0.08)	77 ± 3 % a (0.67 ± 0.09)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)
Trail 5	18	64 ± 10	83 ± 3 % a (0.74 ± 0.08)	70 ± 0 % a (0.68 ± 0.09)	73 ± 9 % a (0.53 ± 0.08)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)	0 ± 0 % b (0.05 ± 0.0)
Trail 6	18	45 ± 5	60 ± 6 % ab (0.62 ± 0.09)	77 ± 9 % a (0.71 ± 0.09)	57 ± 3 % b (0.54 ± 0.09)	0 ± 0 % c (0.05 ± 0.0)	0 ± 0 % c (0.05 ± 0.0)	0 ± 0 % c (0.05 ± 0.0)	0 ± 0 % c (0.05 ± 0.0)	0 ± 0 % c (0.05 ± 0.0)	20 ± 6 % c (0.13 ± 0.0)	0 ± 0 % c (0.05 ± 0.0)

end of the trail. Two exceptions are location E where 37 % of sampled individuals tested positive when the bait was placed at trail 1 and location F where 20 % of sampled individuals tested positive when the bait was placed at trail 6. We did not observe any direct mixing of workers from trail 1 and location E or from trail 6 and location F. It is possible, however that the trails continued underground or mixing of workers occurred inside of the wall.

At most trails, the highest proportion of ants testing positive was detected at the beginning of each trail (average for trails 1–6: 76 %, range: 60–90 %), closer to where the feeding station was placed. Fewer ants tested positive when sam-

Figure 3. Histogram of ELISA results (optical density values) for (A) workers, (B) queens, and (C) larvae 24, 48, and 72 h after continuous feeding on sucrose solution containing rabbit IgG protein.

pled downstream from the feeding station: 72 % in the middle of the trail (range: 67–83 %; not significantly different from the beginning of the trail, $P = 0.086$) and 64 % at the end of the trail (range: 57–73 %; not significantly different from the beginning of the trail, $P = 0.086$). Workers rapidly spread sucrose over relatively long distances. This distance is at least 45 m (our longest trail, Table 2 and 3) and can perhaps be greater depending on the length of the trail. *T. sessile* workers appear to exhibit high nest and foraging site fidelity and forage mainly along well-established trails without crisscrossing into the territory of nearby nests (even though internest aggression is absent). For example, trail 1 was situated in close proximity to sampling location D. Location D was a large tree with an active, yet independent foraging trail and it appeared that most ants visiting that tree nested in the mulch at the base of the tree. Despite the close proximity of trail 1 to site D (ca. 5 m), no marked ants were detected on the tree.

Discussion

The results of our study provide unique experimental data on the organization of foraging in the odorous house ant, *T. sessile*. We show that polydomous colonies of odorous house ants are dispersed central-place foragers whereby members of individual nests utilize local food sources with little or no exchange of individuals or food among neighboring nests. In the odorous house ant, dispersed central-place foraging may be a response to the patchy distribution of nests/food as to more efficiently utilize randomly distributed resources.

Experiments on the trophallactic flow of sucrose from foragers to other castes and developmental stages revealed that workers retained the majority of sucrose. Markin (1970) investigated the distribution of food in the Argentine ant, a Dolichoderinae with colony attributes similar to the odorous house ant, and discovered that workers were the primary consumers of liquid carbohydrate food. In other ants too, workers utilized that majority of carbohydrates entering the colony (e.g. Wilson and Eisner, 1957; Sorensen et al., 1985). The distribution of sucrose to queens varied greatly, with some queens receiving substantially more food than others. This finding suggests a possible reproductive dominance hierarchy, often observed in polygynous ant species (Fournier and Keller, 2001; Vargo, 1990). The larvae received relatively small amounts of sucrose with some individuals receiving substantially more food than others. This suggests a possible feeding hierarchy that that may depend on larval age and consequently nutritional needs. In the Argentine ant, the smallest larvae experienced a delay in feeding and received the least amount of either protein or carbohydrate food, while the largest larvae were fed within seconds and received the most food (Markin, 1970). In addition, prepupal larvae were not fed. Our results shed light on the social behavior and colony organization in the odorous house ants and suggest that odorous house ants utilize liquid sugar food in a manner similar to other polygynous ant species (Markin, 1970; Sorensen et al., 1985). This finding may have implications in designing control programs based on liquid baits where the toxicant is taken back to the nest and shared through trophallaxis with other colony members (Knight and Rust, 1991; Hooper-Bui and Rust, 2000).

The results of field experiments revealed that foraging in the odorous house ant is highly localized. *T. sessile* workers exhibit high foraging site fidelity, travel along well-established trails, forage on a local scale, and consistently deliver harvested resources to specific nests. Indeed, spatial fidelity to nests, trails, or foraging localities is a common attribute of many ant species (Rosengren, 1977; Wehner, 1987; Fernandez and Rust, 2003). The odorous house ant is highly polydomous and nests are interspersed within a complex, three-dimensional network of trails. The distribution of nests in a polydomous colony may be affected by many factors, such as the patchiness of food resources, distance between patches, nest site availability, and pressure from competing species. In our study, odorous house ants occupied a relatively large area with abundant nesting sites and a large number of aphid-infested trees and shrubs which provided stable, local food resources. *T. sessile* was clearly ecologically dominant at the study site, as no other ant species were observed at feeding stations or throughout the study area. Other factors such as grounds irrigation, mowing, periodic debris removal, and insecticide treatments may have also affected the distribution of nests at our study site. We observed that *T. sessile* workers frequently moved nests depending on the environmentral conditions. The ants incubated brood in exposed areas during periods of optimal conditions. As conditions deteriorated (i.e. the nesting substrate became too dry or too wet), workers relocated nests to more protected areas inside of brick walls. While numerous environmental factors may affect the distribution of nests in *T. sessile*, it remains unknown whether the spatial distribution of nests or food sources (or a combination of both factors) ultimately determined the observed foraging pattern. On the one hand, the distribution of nesting sites may have determined the foraging pattern, i.e. the ants colonized the most attractive nesting sites and then utilized the closest stable food source. Alternatively, the distribution of feeding sites may have determined the foraging pattern, whereby the ants relocated nests closer to food and then foraged in close proximity to the nest. Holway and Case (2000) investigated dispersed central-place foraging in the polydomous Argentine ant and discovered that Argentine ants established new nests at sites located near food. Thus, in the Argentine ant, the location of food determines the location of nests, which in turn determines the foraging pattern. The study by Holway and Case (2000) was conducted in a natural area with abundant potential nesting sites. Argentine ants are indiscriminate nesters and invest little time and resources in the construction of nests (Newell and Barber, 1913; Markin, 1970). In contrast, odorous house ants have stricter nesting requirements and various barriers, such as concrete sidewalks, lawns, and buildings, limited nesting at our study site. Therefore, the location of nesting sites and not feeding sites may play a primary role in determining the foraging pattern in *T. sessile*.

Foraging theory predicts that foraging costs should be minimized in order to optimize the yield and thus the net energy gain (Oster and Wilson, 1978). Ant colonies utilize a variety of adaptations to optimize efficiency of foraging, such as group retrieval (e.g. Moffett, 1987; Roulston and Silverman, 2002), worker polymorphism (e.g. Bailey and Polis, 1987; Wetterer, 1994), extreme worker specialization (e.g. Fowler, 1985), and polydomy (e.g. Hölldobler and Lumsden, 1980; McIver, 1991; Davidson, 1997). In *T. sessile*, polydomy may be an effort to improve the foraging efficiency in response to patch quality variation within the territory. The ants mainly utilize food sources that are clumped and relatively stable through time (aggregations of honeydew-producing Homoptera), but may also forage for more ephemeral foods such as insect cadavers and refuse provided by people. Dispersed central-place foraging most likely allows the odorous house ant to more efficiently secure both clumped and dispersed food sources. Relatively stable food sources can be more efficiently exploited by reducing travel distances and travel time (Hölldobler and Lumsden, 1980; McIver, 1991; Davidson, 1997). Other benefits of dispersed central-place foraging may include reduced exposure to natural enemies, decreased likelihood for workers to become disoriented, and increased competitive ability where workers can be quickly dispatched to monopolize food sources by interference competition (Holway, 1998). Dispersed central-place may also allow *T. sessile* to utilize many temporary and patchily distributed food sources. In other polydomous ant species, wide distribution of workers increased the retrieval rate for transient and dispersed food items (Traniello and Levings, 1986; Pfeiffer and Linsenmair, 1998).

Our field observations indicate that the social structure of *T. sessile* varies widely, and may perhaps depend on certain environmental factors such as the availability of nesting sites and presence of competing species. In other ant species availability of nesting sites and food altered the social structure of colonies (Herbers and Banschbach, 1999). Odorous house ant colonies range in size from small (ca. 15–30 workers), isolated, and strictly monogynous colonies found predominantly in undisturbed old-growth forests to large (ca. 500,000 workers), highly polygynous and polydomous supercolonies found in urban areas. Currently, factors responsible for such wide range in colony size and queen number remain unknown, but may depend on factors such as intra- and interspecific competition and availability of food resources and nesting sites. Monogyne colonies of *T. sessile* in natural areas nest in cavities such as hollow acorns and are likely to face intense competition for nesting sites from other cavity-nesting arthropods, especially other ants. Under such circumstances, colony size may be limited by interspecific competition for nest and food. In urban areas, where *T. sessile* is closely associated with human-built structures, protected nesting sites are much more abundant. Released from the constraints of interspecific competition, *T. sessile* flourish and adopt an alternative life-history strategy.

The results of our study may have implications in the areas of basic and applied myrmecology. First, they contribute to our understanding of the social behavior and colony organization in *T. sessile*. Second, our results may have implications in the areas of urban pest management and biological conservation. Specifically, they might help us to design better ant control programs based on liquid insecticide baits to target native and introduced pest ant species. Baits are a popular method to control pest ants and have been used to control numerous invasive ants including the red imported fire ant, *Solenopsis invicta* and the Argentine ant, *Linepithema humile* (Lofgren, 1986; Rust and Knight, 1990; Hooper-Bui and Rust, 2000). Invasive ant species are often highly polygynous and polydomous (Passera, 1994; Moller, 1996) and field studies show that baits frequently fail to eradicate colonies and resurgences are common (Knight and Rust, 1991; Rust and Knight, 1990; Vega and Rust, 2003). To obtain better results with toxic baits, the optimal density and distribution of bait stations must first be determined. To accomplish this, we require a comprehensive understanding of first, nest and trail distribution and second, the foraging pattern and foraging range.

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