Foraging Arena Size and Structural Complexity Affect the Dynamics of Food Distribution in Ant Colonies

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ABSTRACT Food acquisition by ant colonies is a complex process that starts with acquiring food at the source (i.e., foraging) and culminates with food exchange in or around the nest (i.e., feeding). While ant foraging behavior is relatively well understood, the process of food distribution has received little attention, largely because of the lack of methodology that allows for accurate monitoring of food flow. In this study, we used the odorous house ant, *Tapinoma sessile* (Say) to investigate the effect of foraging arena size and structural complexity on the rate and the extent of spread of liquid carbohydrate food (sucrose solution) throughout a colony. To track the movement of food, we used protein marking and double-antibody sandwich enzyme-linked immunosorbent assay, DAS-ELISA. Variation in arena size, in conjunction with different colony sizes, allowed us to test the effect of different worker densities on food distribution. Results demonstrate that both arena size and colony size have a significant effect on the spread of the food and the number of workers receiving food decreased as arena size and colony size increased. When colony size was kept constant and arena size increased, the percentage of workers testing positive for the marker decreased, most likely because of fewer trophallactic interactions resulting from lower worker density. When arena size was kept constant and colony size increased, the percentage of workers testing positive decreased. Nonrandom (clustered) worker dispersion and a limited supply of food may have contributed to this result. Overall, results suggest that food distribution is more complete is smaller colonies regardless of the size of the foraging arena and that colony size, rather than worker density, is the primary factor affecting food distribution. The structural complexity of foraging arenas ranged from simple, two-dimensional space (empty arenas) to complex, three-dimensional space (arenas filled with mulch). The structural complexity of foraging arenas had a significant effect on food distribution and the presence of substrate significantly inhibited the spread of food. Structural complexity of foraging arenas and the resulting worker activity patterns might exert considerable influence on socioecological processes in ants and should be considered in laboratory assays.

KEY WORDS foraging, immunomarking, odorous house ant, protein marking, *Tapinoma sessile*, trophallaxis

Food sharing is a common feature of many animals, both solitary and social, and has been demonstrated in insects (Raveret Richter 2000), birds (Heinrich 1988), and numerous mammals (e.g., Wilkinson 1984, Judd and Sherman 1996), including primates (reviewed by Feistner and McGrew 1989). Sharing food epitomizes the paradox of altruism: a recipient gains fitness benefits at the expense of a donor, either indirectly through kin selection (Hamilton 1964) or through direct mechanisms (Stevens and Gilby 2004). Within highly organized social insect societies, food sharing by trophallaxis is of particular importance (Wilson 1971) and some have postulated that trophallaxis played a key role in the evolution of eusociality (Hunt 1982). In ants, trophallaxis is a highly efficient method for delivering food to the various castes and developmental stages that do not or cannot feed directly (Wilson 1971). Beyond its obvious primary role as a feeding mechanism, trophallaxis also plays a crucial role in the exchange of cuticular hydrocarbons among nestmates (Dahbi et al. 1999, Boulay et al. 2000), nutrient cycling (Machida et al. 2001), exchange of information about available food sources (Farina 1996), as well as transfer of gut symbionts (McMahan 1969), pheromones (Seeley 1995), and caste determination hormones (Moore 1969).

The socioecology of foraging by ant colonies can be analyzed at two levels: the initial process of food acquisition (i.e., foraging) and the subsequent process of food distribution (i.e., feeding). Furthermore, eusociality adds an additional complicating factor: both foraging and feeding can be analyzed at the colony level and the individual level. The process of food acquisition is one of the most thoroughly studied fea-

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tures of ant colonies with ant foraging behavior being relatively well understood, especially in species that use central-place foraging (reviewed in Traniello 1989). In contrast, the process of food distribution has received little attention, largely because of the lack of methodology that allows accurate monitoring of food flow. To date, most work in this area has been done in economically important species such as red imported fire ants, *Solenopsis invicta* (Buren), and Argentine ants, *Linepithema humile* (Mayr) in relation to developing management methods for these invasive pests. For example, the patterns of food transfer by trophallaxis and food allocation from foraging to nonforaging individuals have been examined and the rate of food exchange has been shown to vary with season (Khamala and Buschinger 1971), temperature (Howard and Tschinkel 1981), starvation (Markin 1970, Meudec and Lenoir 1982), and caste (Wilson and Eisner 1957, Sorensen and Vinson 1981). Recently, progress has also been made investigating the quantitative aspects of trophallaxis at the collective and individual levels in other ant species (e.g., Dussutour and Simpson 2008, Dussutour et al. 2009, Buczkowski and Bennett 2009, Buffin et al. 2009).

In this study, we used the odorous house ant, *Tapinoma sessile* (Say) to investigate the effect of two abiotic factors, foraging arena size and foraging arena structural complexity, on the rate of spread of liquid carbohydrate food (sucrose solution) throughout a colony. To track the movement of food we used protein marking and double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA; Buczkowski and Bennett 2006). Protein marking (a.k.a. immunomarking) has proven highly effective to study the foraging ecology of various social insects including ants (Buczkowski and Bennett 2006, Buczkowski and Bennett 2007), honey bees (DeGrandi-Hoffman and Hagler 2000), and termites (Buczkowski et al. 2007, Hagler et al. 2009) in field and laboratory settings. The goals of this study were two-fold. The main objective was to investigate the effect of foraging worker density on the rate and the extent of food distribution. Worker density was determined by an interaction of two factors: foraging arena size and colony size. As the size of the foraging arena decreased and colony size increased, worker density increased and vice versa. In ants, trophallaxis is an open-ended system whereby each worker will share food with one or more nestmates (Wilson and Eisner 1957). These primary recipients then become secondary donors and each share food with several other nestmates in a process called trophallactic cascade (Suárez and Thorne 2000). Such pattern of food distribution requires that workers encounter each other and suggests that food flow within the colony should be most effective when worker density is high. The basic spatial relation between group size and the interaction pattern predicts that the number of encounters and the interval that elapses between encounters will depend on the number or density of ants present (Gordon 1999). Despite theoretical predictions and the potential importance of worker density on food distribution empirical data are lacking. The second objective was to investigate the effect of foraging arena structural complexity on the rate and the extent of food distribution. The majority of laboratory studies on various ant behaviors are carried out in artificial foraging arenas, most often plastic trays that do not contain any substrate. We hypothesized that the presence of substrate might have an effect on the rate of encounters among individuals and ultimately an effect on the rate of food distribution. This hypothesis was tested by comparing the rate and the extent of food distribution in colonies foraging in experimental arenas with and without nesting material.

Materials and Methods

Biological Model and Test Colonies. The odorous house ant, *T. sessile* is a widespread species native to North America (Fisher and Cover 2007) and occurs in a variety of habitats ranging from forests to urban areas. Colonies vary greatly in size and social structure and range from small, single-nest, monogyne colonies in natural areas to large, multi-nest, polygyne supercolonies in urban areas (Buczkowski 2010). *T. sessile* have an opportunistic diet and exploit various liquid carbohydrate resources including Hemipteran honeydew, flower nectar, and tree sap. Test colonies were collected on the campus of Purdue University, West Lafayette, IN, from a large, polydomous supercolony (Buczkowski and Bennett 2008). Given the supercolonial nature of *T. sessile* at the collection site, ants were collected from several nests, but later raised as a single colony. Debris containing the ants was placed in plastic, Fluon-coated trays provided with moist plaster nests. As the debris dried, the ants colonized plaster nests and were subsequently maintained in debris-free trays. Colonies were reared on 30% sucrose solution and vitamin-enriched artificial diet (Bhatkar and Whitcomb 1970) ad libitum and crickets twice a week. Colonies were maintained and all experiments were conducted at 25 ± 2°C, 60 ± 10% RH, and 14:10 L:D cycle.

Effect of Foraging Arena Size and Colony Size on Food Distribution. The effect of worker density on the rate and the extent of sucrose distribution were examined. Worker density was determined by an interaction of two factors: foraging arena size and colony size. Colony fragments consisting of 250, 500, or 1,000 workers and 20 queens were placed inside shallow, debris-free, Fluon-coated plastic arenas of varying size: small (25 × 25 cm; 625 cm^2), medium (50 × 50 cm; 2,500 cm^2), and large (100 × 100 cm; 10,000 cm^2). Thus, colony size doubled each time, but foraging arena (patch size) quadrupled. Each colony size was tested in all three arena sizes for a total of nine tests. Testing all the possible combinations allowed us to examine the effect of various worker densities on the rate and the extent of food flow. Overall, seven different worker densities were tested which ranged from 0.025 workers/cm^2 (250 workers in a large arena) to 1.6 workers/cm^2 (1,000 workers in a small arena). The ants were allowed to colonize a moist plaster nest

(8 cm Ø) placed in the center of each arena. They were provided with drinking water and allowed to acclimate to the nest for 48 h without food. After the acclimation period, a group of 10 replete donors was introduced into the recipient colonies. The donors were introduced into the area outside the nest and allowed to freely interact with foragers present throughout the arena. To prepare the donors, a group of ≈200 workers was starved for 24 h and subsequently allowed to feed on a droplet of 30% sucrose solution containing technical grade rabbit immunoglobin (IgG) protein (Sigma, St. Louis, MO) at a concentration of 0.5 mg IgG/ml sucrose. This concentration was selected based on the results of previous studies that revealed that the increases in optical density (OD) were minimal above 0.5 mg/ml (Buczkowski and Bennett 2006). Ants that fed to repletion were gently removed from the feeding box by allowing them to walk onto a toothpick and immediately transferred to the recipient colony.

To estimate the amount of protein marker acquired by the recipients we randomly sampled 5% of the workers (i.e., 12, 25, or 50 individuals) from each recipient colony at 1 h and 8 h after introducing the donors. Random sampling was accomplished by collecting workers from all areas of the arena including those in close proximity to the nest. The number of workers sampled at each time point was equal to 5% of the original colony size, not the number of workers remaining after prior sampling events. Queens were not sampled because previous work in *T. sessile* demonstrated that feeding in the queens is delayed and that workers retain the majority of liquid carbohydrates (Buczkowski and Bennett 2006). All individuals were frozen in individual tubes at −20°C and later analyzed by DAS-ELISA using previously described methodology (Buczkowski and Bennett 2006, 2007). Three replicates were performed for each colony size/box size combination using independent colony fragments. The mean (±SE) OD value and the percentage of samples scoring positive for rabbit immunoglobin protein were determined.

Effect of Foraging Arena Structural Complexity on Food Distribution. The structural complexity of the foraging arena might have an effect on ant foraging behavior and ultimately the rate of food distribution. Specifically, the presence of substrate might decrease the rate of encounters and increase the interval that elapses between encounters. Consequently, the rate of food distribution throughout the colony might also decrease. This hypothesis was tested by comparing the results from the above-described test in which 500 workers foraged in substrate-free arenas to an identical test with the substrate present. Briefly, colony fragments consisting of 500 workers and 20 queens were placed within small, medium, and large experimental arenas. The ants were allowed to colonize a moist plaster nest and were acclimated as above. Each arena was filled with a 2 cm layer of shredded hardwood mulch consisting of the original nesting material collected in the field. After the acclimation period, a group of 10 replete donors was introduced into the recipient colonies. The rate and the extent of food distribution were again examined as above by randomly sampling 5% of the workers from each recipient colony at one and 8 h. Three replicates were performed for each arena size using independent colony fragments.

Statistical Analysis. The samples were scored positive for the presence of the protein marker if the ELISA OD value exceeded the mean negative control value by three standard deviations (Sutula et al. 1986, Buczkowski and Bennett 2006). The percentage of samples testing positive for the protein was tabulated by first calculating the percent of individuals testing positive within a replicate and then averaging across the three replicates, Analysis of variance (ANOVA) tests were conducted to determine the significance of colony size, arena size, and time on the spread of the marker. This was accomplished by using the PROC ANOVA (repeated measures ANOVA) procedure in SAS 9.2 (SAS Institute 2008), followed by post hoc Tukey's honestly significant difference 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 OD.

Results

Both colony size (ANOVA; $F_{2,65} = 46.84$; $P < 0.0001$) and foraging arena size (ANOVA; $F_{2,65} = 20.20$; $P < 0.0001$) had a significant effect on the spread of the marker and the number of workers testing positive decreased as arena size and colony size increased (Table 1). The importance of arena size on food distribution was especially evident when the size increased from 625 cm^2 (small box) to 2,500 cm^2 (medium box) and the percentage of workers testing positive dropped off considerably. Time had no significant effect on the distribution of the marker (ANOVA; $F_{1,65} = 1.61$; $P = 0.21$), indicating that food is distributed rather quickly and reaches the maximum level within the first hour. Worker density, which was dependent on arena size and colony size, did not seem to play a clear role in the distribution of food among the workers. This result is evident in Table 2 where different arena size and colony size combinations resulted in identical worker densities which can be compared. Results suggest that colony size is the dominant factor affecting food distribution within colonies. Among the nine different arena size/colony size combinations, two comparisons stand out. The first one is between 250 workers in a small arena (625 cm^2) versus 1,000 workers in a medium arena (2,500 cm^2). Worker density in both tests was identical and equal to 0.4 workers/cm^2. However, the percentage of workers testing positive for the marker was vastly different with 64 ± 6% of workers testing positive in a colony of 250 and only 7 ± 4% testing positive in a colony of 1,000 after 1 h of trophallactic interactions with the donors. A second comparison is between 250 workers in a medium arena (2,500 cm^2) versus 1,000 workers in a large arena (10,000 cm^2). Worker density in both tests was equal to 0.1 workers per cm^2, but the percentage

Table 1. Mean percentage ±SE of workers testing positive for rabbit IgG protein 1 and 8 h after introducing 10 sucrose-fed donors

Donor: recipient ratio	Substrate	Mean % positive samples					
		1 h			8 h		
		Small	Medium	Large	Small	Medium	Large
10:1000	Absent	64 ± 6 a,a (0.69 ± 0.03)	47 ± 10 ab,a (0.50 ± 0.03)	19 ± 7 b,ab (0.21 ± 0.02)	89 ± 6 a,a (0.98 ± 0.02)	58 ± 13 ab,a (0.51 ± 0.03)	36 ± 12 b,a (0.29 ± 0.02)
		59 ± 3 a,a (0.48 ± 0.02)	39 ± 11 b,a (0.30 ± 0.02)	33 ± 8 b,a (0.26 ± 0.02)	52 ± 5 a,b (0.24 ± 0.02)	44 ± 14 a,b (0.15 ± 0.01)	41 ± 16 a,a (0.16 ± 0.01)
		14 ± 3 a,b (0.07 ± 0.00)	7 ± 4 ab,c (0.06 ± 0.01)	3 ± 1 b,c (0.05 ± 0.01)	13 ± 3 a,c (0.07 ± 0.00)	1 ± 1 b,d (0.04 ± 0.00)	3 ± 1 b,c (0.04 ± 0.00)
10:500	Present	47 ± 4 a,ab (0.53 ± 0.03)	23 ± 4 b,b (0.25 ± 0.02)	8 ± 4 c,b (0.15 ± 0.02)	45 ± 7 a,b (0.20 ± 0.02)	36 ± 12 a,c (0.19 ± 0.01)	16 ± 14 b,b (0.10 ± 0.01)

Mean optical density (OD) values ±SE are given in parentheses. Means followed by the same letter are not significantly different by Tukey's honestly significant difference test ($P \leq 0.05$). First letter indicates within row comparisons, second within column comparisons.

Table 2. The effect of worker density on the distribution of the protein marker throughout the colony

Colony size	Arena size (cm^2)	Worker density	1 h	8 h
250	625	0.4	64 ± 6 b,ab	89 ± 6 a,a
500	625	0.8	59 ± 3 a,a	52 ± 5 b,b
1,000	625	1.6	14 ± 3 a,ef	13 ± 3 a,cd
250	2500	0.1	47 ± 10 a,bc	58 ± 13 a,b
500	2500	0.2	39 ± 11 a,cd	44 ± 14 ab
1,000	2500	0.4	7 ± 4 a,f	1 ± 1 a,d
250	10,000	0.025	19 ± 7 a,def	36 ± 12 b,bc
500	10,000	0.05	33 ± 8 a,cde	41 ± 16 a,b
1,000	10,000	0.1	3 ± 1 a,f	3 ± 1 a,d

Mean percentage (±SE) of workers testing positive for rabbit IgG at 1 and 8 h is given. Means followed by the same letter are not significantly different by Tukey's honestly significant difference test ($P \leq 0.05$). First letter indicates within row comparisons, second within column comparisons.

of workers testing positive was again significantly different with 47 ± 10% of workers testing positive in a colony of 250 and only 3 ± 1% of workers testing positive in a colony of 1,000. From these two comparisons, it is clear that colony size, rather than arena size, is the primary factor affecting food distribution. Food distribution is more complete in smaller colonies regardless of the size of the foraging arena.

The structural complexity of the foraging arenas had a significant effect on food distribution (ANOVA; $F_{1,65} = 6.15$; $P = 0.015$) and was especially pronounced as arena size increased (Table 1; Fig. 1). The presence of substrate significantly reduced the percentage of workers testing positive for the marker and this effect was especially pronounced in large arenas. In medium arenas, the percentage of workers testing positive was significantly lower in arenas with the substrate present at 1 h, but not different at 8 h. In small arenas the presence of substrate did not affect food distribution at either time point.

Discussion

Social insects are faced with a nutritional challenge whereby the food entering the colony is brought by a small number of foragers, but must be efficiently shared among all members of the colony. To accomplish this task, workers continually assess the nutritional needs of the colony and respond by adjusting foraging effort to changing conditions. The encounter rate between food donors and food recipients is critical for efficient transfer and depends on the number or density of ants present (Gordon 1999). In the current study, the density of food donors was kept constant at 10 workers to isolate the effects of recipient worker density on the rate and the extent of food distribution. Results show that both arena size and colony size have a significant effect on the spread of the food and the number of workers receiving food decreased as arena size and colony size increased. When colony size was kept constant and arena size increased, the percentage of workers testing positive for the marker decreased. This is expected as the

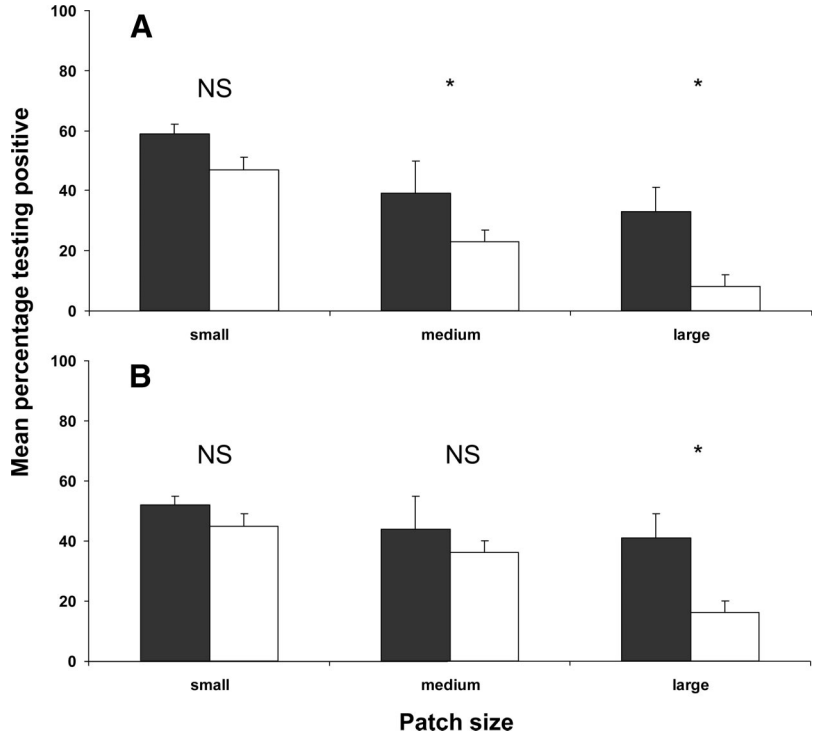

Fig. 1. Mean (± SEM) percentage of 500 *T. sessile* workers testing positive for the protein marker at (A) 1 hour and (B) 8 hours after interacting with 10 donors placed into foraging arenas of increasing size. Filled bars represent arenas with the substrate absent, open bars arenas with the substrate present. NS, not significant; *, $P < 0.001$; $\alpha = 0.05$.

density of workers in the smaller arenas was higher, which may have resulted in more trophallactic interactions. Previous studies show that crowding increases foraging efficiency in leaf-cutting ants (Dussutour et al. 2007) and encounter rate is an important factor regulating task allocation in ants (Gordon and Mehdiabadi 1999). When arena size was kept constant and colony size increased, the percentage of workers testing positive for the marker decreased. Generally, food flow within the colony should be the most effective when worker density is high. This is because trophallaxis is an open-ended system whereby each worker will share food with one or more nestmates (Wilson and Eisner 1957). These primary recipients then become secondary donors and each share food with several other nestmates in a process called trophallactic cascade (Suárez and Thorne 2000). However, other factors such as worker dispersion pattern and the amount of food entering the colony may also affect the rate and the extent of food distribution. In the current study, the ants did not disperse uniformly, but tended to aggregate around the nest and water vials and in the corners of the arenas, most likely because of thigmotaxis. Clumped worker distribution might have facilitated food transfer by increasing local worker density and promoting food sharing. However, a clumped distribution may have created isolated pockets of workers and prevented them from interacting with the rest of the colony. The amount of food entering the colony may have also affected the dispersion pattern and previous results demonstrate that increasing colony size while keeping the number of donor workers constant significantly limited the spread of food within colonies of *T. sessile* (Buczkowski and Bennett 2009). Overall, worker density, which is regulated by both arena size and colony size, did not seem to play a clear role in the distribution of food. Results suggest that food distribution is more complete in smaller colonies regardless of the size of the foraging arena and that colony size, rather than arena size, is the primary factor affecting food distribution. This is evident by comparing food distribution in colonies having identical worker densities resulting from different combinations of arena and colony sizes. For example, the density of 250 workers placed in a small arena (625 cm^2) was equal to the density of 1,000 workers placed in a medium arena (2,500 cm^2) and equivalent to 0.4 workers/cm^2. However, the percentage of workers testing positive was significantly higher in the smaller colony. Previous results with *T. sessile* show that in colonies provisioned with a set number of donor workers, the percentage of workers receiving food decreases with increasing colony size (Buczkowski and Bennett 2009). However, the number of workers actually receiving food increases suggesting that per capita food consumption decreases as colony size increases.

The structural complexity of foraging arenas, as mediated by the presence or absence of substrate, had a significant effect on food distribution and the pres-

ence of substrate significantly inhibited the spread of food. This effect was especially pronounced in larger arenas. Observations indicate that the substrate had a significant effect on ant dispersion and encounter patterns and ultimately affected food diffusion throughout the colony. In assays with the substrate absent the ants were restricted to foraging in two dimensional space. This resulted in a clumped worker distribution as the ants tended to aggregate around objects placed in the arena (e.g., nest, water tubes) because of thigmotaxis and formed distinct trails between the objects. Furthermore, excluding the substrate may have increased encounter rates by concentrating and speeding up worker movement along unobstructed paths. Interestingly, however, the presence of substrate did not have an effect on food distribution in the smallest arenas where encounter rates most likely remained high. In contrast, assays with the substrate present more closely approximated natural field conditions where the ants interact in three dimensional space and are free to explore the various topographical features of the environment. Including the substrate forced the ants to travel over the uneven surface of the mulch and the mulch may have inhibited encounter rates as some of the donor workers may have retreated into the mulch. Other studies demonstrate that the physical structure of the environment affects the dispersion pattern in ants (Fewell 1988a, b,; Fourcassié et al. 2003; Pie et al. 2004). Furthermore, the presence of substrate may affect interactions with nestmates and previous studies show that interactions with nestmates greatly affect the dispersion movement of individuals (Robinson 1992, Gordon et al. 1993). Interactions may also affect the internal state of the worker and subsequently have an effect on movement patterns (Pacala et al. 1996, Bonabeau et al. 1998, Gordon and Mehdiabadi 1999). The great majority of laboratory studies using ants exclude the substrate which allows for the ants to be more easily observed and counted. Excluding the substrate, however, may affect the activity patterns and ultimately the behavior that is being observed. The structural complexity of foraging arenas and the resulting worker activity patterns might exert considerable influence on socioecological processes in ants and should be considered in laboratory assays.

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