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Short Communication

A possible pheromone effect as reflected in food handling by *Messor arenarius* ants

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Abstract This work aimed to explore a pheromone effect during food handling using food choice field experiments with *Messor arenarius* ants. The results showed that the proportion of times ants touched the two food items placed in the artificial food patch (collection point) with their antennae was significantly higher in their first collection attempt than in subsequent attempts. However, when the soil was replaced after each collection attempt, there were no significant differences in the proportions of cases in which ants touched the two food items with their antennae between the first and subsequent food collection attempts. This difference may indicate that ants deposited a pheromone in their first food collection attempt that faded away when the soil in the patch was replaced after each collection attempt.

Keywords antennae; ants; food handling; Formicidae; *Messor*; pheromone

Introduction

Pheromones are used by ants for chemical communication, especially for nest-mate recognition or foraging (Hölldobler and Wilson, 1990; Vander Meer *et al.*, 1998). When a scout ant finds a food item good enough for a collection, it usually deposits a pheromone at the collection point and sometimes marks a pheromone trail from the collection point back to the nest entrance (Hölldobler and Wilson, 1990). Ants, like many insect groups, also use their antennae for smelling, by chemoreceptors, or for tasting, by contact chemoreceptors (Chapman, 1998; Reinberg, 2012). Ants also have contact chemoreceptors on their antennae. When ants reach a food item, they first touch it with their antennae, a behavior defined as antennation (Culver and Beattie, 1978; Gunther and Lanza, 1989).

Messor arenarius (Fabricius) are harvester ants distributed mainly in the deserts of the Middle East and North Africa (summarized in Warburg, 2000), primarily feeding on plant parts, usually seeds (Steinberger *et al.* 1991). Besides collecting seeds from the ground, *M. arenarius* ants also collect seeds directly from plants by cutting them with their mandibles (Delye, 1971). *M. arenarius* ants usually forage solitarily, i.e. individual foraging (Ben-Mordechai, 1981; Warburg, 1996). Some of these ants search for food and collect it in radial sectors, covering an area of 360° around their nest (Warburg, 1996). This research aimed to check whether the handling process of food items by *M. arenarius* ants differs between the first and subsequent collection attempts from the same food patch. The working hypothesis is that ants touch more food items when encountering a new food patch while also depositing a pheromone. Thus, ants arriving at the same patch in subsequent food collection attempts will likely touch fewer food items.

Methods

The experiments were conducted between 1994-1997 in the following areas: Or-Akiva (32.51°N; 34.92°E), Netanya (32.13°N; 34.60°E), Holon (32.00°N; 34.80°E), Ashdod (31.75°N; 34.60°E), Ashkelon (31.60°N; 34.57°E) in the Coastal Plain of Israel, and Revivim (31.04°N; 34.52°E) and Sede-Boqer (30.85°N; 34.75°E) in the Negev Desert of Israel. Most of the experimental nests in the Coastal Plain of Israel were in sand dunes, dominated by *Artemisia monosperma* Delile and by *Retama raetam* (Forsskal) Webb. The seeds of *R. raetam* serve as a primary natural food source for *M. arenarius*. Most of the experimental nests in the Revivim or the Sede-Boqer areas were in dry-river banks or valleys with loess soil, dominated by *Hammada scoparia* (Pomel) Iljin, or on stony slopes, dominated by *Zygophyllum dumosum* Boiss or by *Artemisia sieberei* Besser (*Artemisia herba-alba* Asso).

A 20 × 20 cm artificial food site was located 1 m from the nest entrance. Each food site contained four food patches placed at a distance of about 10cm between each other (Figure 1). A whole wheat seed and a half wheat seed

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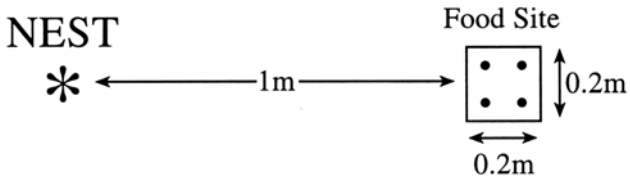

Figure 1. A scheme of an artificial food site used in this research.

cut longitudinally (with its cut side facing upward) were placed in each patch. Wheat seeds were used because of the following reasons: wheat does not grow naturally in those locations; when *M. arenarius* ants encounter wheat particles, they usually collect them (Warburg, 2000). Those two food items touched or were very close to each other in order to ensure that the collecting ants would not ignore any food item while reaching the artificial patch. A single experiment was conducted on each nest, during which several collection rounds were recorded. During each round, direct observations were used to monitor the number of times ants touched the food items with their antennae or with their forelegs. After each collection round, the ants that had collected a food item were usually marked on one of their legs with correction fluid. This marking was done to identify ants that had already collected a food item from the food site in the same experiment. *M. arenarius* ants usually remove this correction fluid from their legs, thorax, or abdomen within a couple of days after the marking. Therefore, it is assumed that it did not cause any damage to the ants. Marked ants were prevented from collecting food items from the food site again. Hence, each collection round was done by a different ant. After each collection round, a food item like the one removed was added to the patch, ensuring food choice in subsequent rounds. Two experimental series were done. In experimental series 1, the sand or soil in each collection site remained unchanged during all collection rounds. In experimental series 2, the sand or soil in each collection point was swept or replaced with sand or soil from outside the food site after each collection round. This replacement of sand or soil was done to weaken the possible effect of pheromones deposited by the collecting ants.

Observations related to the first or the subsequent food collection attempts were distinguished. Three levels of food examination/handling were considered: T_0 – no touching of the food items; T_1 – touching one food item; T_2 – touching both food items. The proportion of times ants touched the two food items placed in each artificial patch was calculated as $T_2/(T_0 + T_1 + T_2)$. A one-sided Chi-square test was used to examine differences in the proportion of times ants touched the two food items placed in each patch between the first and subsequent collection attempts. A separate test was done for antennae or forelegs touching food items.

Results

The data of antennae or forelegs touching food items are presented in Table 1. In experimental series 1, 26 nests were examined, with 65 first collection attempts and 28 subsequent collection attempts registered. In experimental series 2, 10 nests were examined, with 19 first collection attempts and nine subsequent collection attempts registered. In series 1, the proportion of antennae touching both food items was significantly higher in the first collection attempts than in subsequent collection attempts (0.600 vs 0.393, respectively; Chi-square = 3.378; DF = 1; P = 0.033). A similar but not significant pattern was observed in forelegs touching both food items (0.369 vs 0.250 for the first and subsequent collection attempts, respectively; Chi-square = 1.132; DF = 1; P = 0.287). In experimental series 2, the proportion of antennae touching both food items in the first collection attempt did not differ significantly from that observed in subsequent attempts (0.789 vs 0.667, respectively; Chi-square = 0.491; DF = 1; P = 0.483). A similar pattern was found in forelegs touching both food items (0.368 vs 0.111 for the first and subsequent collection attempts, respectively; Chi-square = 1.981; DF = 1; P = 0.159).

Discussion

When the sand/soil in the artificial food patch remained unchanged, ants touched the two food items placed there with their antennae more frequently in the first than in

Table 1. Distribution of cases where ants touched the seeds or half seeds with their antennae or with their forelegs when the sand/soil in the artificial food patch remained unchanged (Experimental series 1) or when it was replaced after each food collection round (Experimental series 2).[1]

Examination degree	Data group							
	Experimental series 1 (n = 26)				Experimental series 2 (n = 10)			
	First collections		Subsequent collections		First collections		Subsequent collections	
	A	F	A	F	A	F	A	F
T_0	0	1	0	1	0	0	0	0
T_1	26	40	17	20	4	12	3	8
T_2	39	24	11	7	15	7	6	1
$T_0+T_1+T_2$	65	65	28	28	19	19	9	9
$T_2/(T_0+T_1+T_2)$	0.600	0.369	0.393	0.250	0.789	0.368	0.667	0.111

[1] n – number of examined nests; A – number of cases where food items were touched by the antennae; F – number of cases where food items were touched by the forelegs; T_0 – number of cases where food items were not touched; T_1 – number of cases where one food item was touched; T_2 – number of cases where both food items were touched.

subsequent collection attempts. This pattern, however, became no longer significant when the sand/soil in the food patch was replaced after each collection attempt. This finding can indicate pheromone effect. Specifically, ants that encountered a food patch for the first time deposited a pheromone, causing ants that arrived at the same patch in subsequent food collection attempts to touch fewer food items. Pheromone secretion involves the process of food collection by ants. An ant that collects a food item marks the collection site with a pheromone and afterward returns to the nest while marking the returning route with a pheromone trail that is used later by its nest-mates to find the food source (Hölldobler and Wilson, 1990). Bradshaw *et al.* (1986) found that leaf-cutter ants of the species *Atta cephalotes* Wheeler mark leaf fragments that they collect with pheromone and that other ants collect these marked leaf fragments 3–4 times more frequently than unmarked leaf fragments.

Another possible explanation is related to short-term memory. Ants that have not collected wheat before may encounter wheat that other nest-mate ants inside their nest collected. Consequently, those ants can possibly recognize wheat as a food source. However, in both experiments, ants that collected a food item from the patch were marked and prevented from returning it in subsequent food collection attempts, making short-term memory a less applicable explanation. Furthermore, suppose the differences were due to short-term memory. In that case, we should expect the proportion of times ants touched both food items in the patch with their antennae to be higher in the first than in subsequent food collection attempts, also when replacing the sand/soil after each collection attempt. Since this was not the case, it is more likely that those differences are due to the pheromone effect.

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