SOIL-FREE COLLECTION OF ARGENTINE ANTS (HYMENOPTERA: FORMICIDAE) BASED ON FOOD-DIRECTED BROOD AND QUEEN MOVEMENT

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ABSTRACT

The movement of Argentine ant, *Linepithema humile* (Mayr), colonies was studied in the laboratory. Workers transported brood from the main colony to satellite nests within 2 h. Queens also moved into the satellite nests. Up to 70% of the brood were moved out of the main colony by 48 h. Although most of the brood and queens migrated to a satellite nest 60 cm from the main nest, a substantial number of brood and queens moved 12.2 m to a nest 60 cm from a food source. We subsequently employed these findings to induce a portion of a field colony of *L. humile* to enter artificial colony dishes within the laboratory. Workers, brood, and queens were collected continuously and effortlessly using this technique.

Key Words: Argentine ant, *Linepithema humile*, colony movement, soil-free, collection

RESUMEN

Se estudió el movimiento de colonias de la hormiga Argentina (*Linepithema humile* Mayr) bajo condiciones de laboratorio. Las hormigas trabajadoras comenzaron a transportar a las hormigas jóvenes desde la colonia principal a nidos satélite en menos de dos horas. Las reinas tambien se mobilizaron hacia los nidos satélite. Hasta un 70% de las hormigas jóvenes fueron sacadas de la colonia principal en un lapso de 48 horas. Aunque la mayoría de las hormigas jóvenes y reinas emigraron hacia nidos satélites ubicados a 60 cm del nido principal, una cantidad sustancial de hormigas jóvenes y reinas se mobilizó 12.2 m para ubicarse en un nido a 60 cm de una fuente de alimento. Estos resultados sirvieron para inducir a una porción de una colonia de campo de *L. humile* a penetrar en platos de colonias artificiales dentro del laboratorio. Esta técnica permitió colectar trabajadores, hormigas jóvenes y reinas con mucha facilidad y de manera contínua.

Colony migrations are common in a number of ant species. Entire colonies may move to a new location in response to a major disturbance to the nest, divide into subcolonies with a continuous exchange of foragers (satellite formation), or multiply when one or more fertilized queens depart the nest with a cadre of workers, ultimately establishing independent colonies (Holldobler & Wilson 1990). Colony migrations commonly occur in temperate woodland ant species without obvious provocation (Smallwood & Culver 1979, Smallwood 1982, Herbers 1985). However, colony emigration may also be induced by competition (Holldobler 1976), predation (Droual & Topoff 1981) and the need to find food (Topoff & Mirenda 1980).

The Argentine ant, *Linepithema humile*, is a pest of urban, agricultural and riparian woodland habitats which has spread widely, particularly in Mediterranean and subtropical regions (Markin 1970a, Ward 1987, Holldobler & Wilson 1990). The polygynous and polydomous colonies of *L. humile* occur over vast areas of California developed by human activity (Markin 1968a). Like other tramp ant species, *L. humile* tends to have fragile nest sites (Passera 1994) and has been reported to partially or completely abandon their nests in response to adverse conditions such as excess moisture or dryness (Markin 1968a). In addition, colony foundation via migration from the original colony has been reported in *L. humile* with new nests occasionally constructed near a food supply (Newell & Barber 1913).

Laboratory study of *L. humile* biology, behavior and control requires initial field collection of colonies, or colony fragments, containing one or more fertile queens, brood and workers. The standard collection process is fairly laborious, involving first the location of the colony followed by its excavation and transport to the laboratory. The final step, perhaps the most time-consuming activity, requires the separation of ants from the often large amounts of soil excavated with the ants (Markin 1968b, Baker et al. 1985, Knight & Rust 1990).

Movement of Argentine ant brood and queens was mentioned earlier (Newell & Barber 1913, Wilson 1971). Herein, we report more detailed experimental evidence about the movement of laboratory colonies of *L. humile* proximal and distal to a food source. Furthermore, we describe a continuous, soil-free method for collecting field ants which exploits brood and queen transport behavior. This method is simple and allows the investigator to procure large numbers of ants, which are more likely to express field characteristics than long-standing laboratory colonies.

Materials and Methods

Collection of Original Colonies

Laboratory colonies were established from nest sites in soil excavated from an open field bordering a suburban housing development in Alameda County, California, using a method similar to Markin (1968b) and Knight & Rust (1990). Soil containing *L. humile* workers, brood and queens was spread out in a 55 cm by 40 cm by 30 cm plastic container coated with teflon (E. I. Du Pont de Nemours Co. Wilmington, Delaware) to prevent ant escape. Artificial colony dishes were constructed in a manner similar to Markin (1970b). Plastic petri dishes (9 cm dia.) were filled with casting plaster to a depth of 2 cm and affixed to the lid of a 200 ml glass jar. A 13 mm dia. hole was drilled through the dish and jar lid and a piece of plastic tubing (20 mm length) was glued to the hole. The petri dish-jar lid was fitted to a jar with water, and a length of cotton dental wick was inserted through the plastic sleeve. The plastic sleeve allowed the sealed plaster chamber housing the ants to maintain a high humidity without the plaster becoming saturated. Grooves were etched into the plaster to increase the surface area of the artificial nest. Two nest entrances were fitted with truncated plastic pipette tips so that tubing could be attached to the nest for experiments. An opaque cover was placed over the nests and they were placed within the container holding the ants and soil. As the soil dried, the colony moved into the constructed nests within 14 days.

Laboratory Investigation of Brood and Queen Movement

We determined the rate of queen and worker-assisted brood migration from their original colony dish to satellite nests in the absence of an environmental disturbance. A nest containing ca 2000 workers, 1000 brood pieces and 25 queens was placed inside a teflon-coated 243 cm by 150 cm by 5 cm arena (Fig. 1). Plastic tubing (12.2 m by 1.2

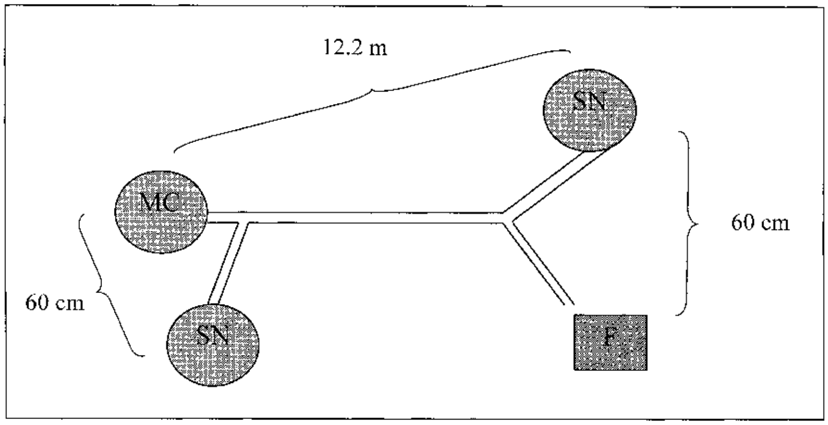

Fig. 1. Diagram of the nest relocation experiment showing the locations of the main colony (MC) satellite nests (SN) and food (F).

cm inside dia.) was attached to one nest entrance, while the other was blocked with modeling clay. Food (0.25 M sucrose plus cockroaches [*Blattella germanica*]) was provided at the tubing port distal to the colony. A 30 cm length of tubing was attached via a y-connector to the main foraging line 30 cm from the colony entrance and 30 cm from the food. Satellite nests (see colony dish description above) were attached to each of the ends of these branches. Access to the satellite nests was blocked for three days while a foraging trail was established between the main colony and the food. Thereafter, ants had access to the satellite dishes. The number of brood and queens in the satellite nests were counted at 2, 4, 8, 16, 24 and 48 h intervals. Afterwards, the number of brood and queens remaining in the main colony were counted and the percent of brood and queen movement over time was calculated. Ten replicates (colonies) were conducted. Near-colony v near-food brood and queen distributions at 48 h were compared with paired Wilcoxon signed-rank tests (BBN Software Co. 1995).

Establishing Continuous Recruitment of Field Ants into the Laboratory

A *L. humile* trail from a field colony was first noted September, 1996 along the outside edge of a threshold at a remote corner of our insectary. We attempted to induce *L. humile* workers to trail just inside the insectary for the purpose of conducting fairly realistic food acceptance studies for ant bait development, and collecting all stages of field ants for other laboratory studies. Synthetic cis-9-hexadecenal (Aldrich Chemical Co., Milwaukee, Wisconsin), the major trail pheromone component of *L. humile*, was applied at a rate of 200 ng/100 cm (Key & Baker 1982) in a continuous line between the existing trail onto the top of a wooden table (80 cm by 80 cm by 100 cm high). Droplets of 0.25 M sucrose were placed sequentially along the chemical trail every 10 cm to encourage recruitment. Workers reaching the table top were provided freshly killed cockroaches (*B. germanica*) along with 0.25 M sucrose in a cotton-stoppered vial. A moistened, plaster-filled petri dish with an opaque cover was placed along the trail in an effort to attract and capture queens and brood. A study was initiated in December, 1998 to quantify *L. humile* movement indoors. The number of workers, brood and queens moving into each of three dishes within a 24-h period was determined once on each of 10 successive weeks.

Results and Discussion

Laboratory Investigation of Brood and Queen Movement

Workers explored the satellite nests within 5 minutes, once access was allowed. Within 2 hours, both brood and queens were observed in both satellite colony dishes, with numbers increasing in the new nests through 48 hours (Fig. 2). Although more brood were transported to the new nest closer to the colony than to the food, the counts were not significantly different at 48 h (Wilcoxon statistic = 14; critical value = 8; $P > 0.05$). Also, the distribution of queens between the 2 new nests was not significantly different (Wilcoxon statistic = 12.5; critical value = 8; $P > 0.05$). Mechanical disturbance, flooding, or light or air currents entering the brood chambers generally causes an immediate response by the colony, either in the form of a retreat into the nest interior or emigration from the nest (Holldobler & Wilson 1990). We allowed a 3-d acclimation period, and were careful not to disturb the colonies or foraging conduits, yet some brood and queens exited the main nest within a relatively short period of time. We considered that brood and queen relocation in our study may have been in response to high nest density and/or suboptimal environmental conditions such as humidity or moisture. Smallwood & Culver (1979) speculated that colony emigration may be in response to the build-up of waste products in the nest. This was based on the laboratory observation that ants will abandon a fouled artificial nest in favor of an alternative clean nest. However, if the nest environment was unfavorable, we would have expected complete, or nearly complete, evacuation of the primary nest. In our studies, no more than 70% of the brood was transported from the primary nest, and in 8 of the 10 colonies less than 50% of the brood was moved. Therefore, it appears that *L. humile* inherently exploits new habitats, including those close to a reliable, abundant food source.

Establishing Continuous Recruitment of Field Ants into the Laboratory

Worker ants from the field colony followed the pheromone trail into the laboratory, along the door frame, and onto the top of the table. Recruitment was rapid and foraging activity was intense once the food was located (Fig. 3a, b). Although we used both trail pheromone and food to effect recruitment, it is quite possible that one or the other could have directed workers to the table top. Consequently, this approach may

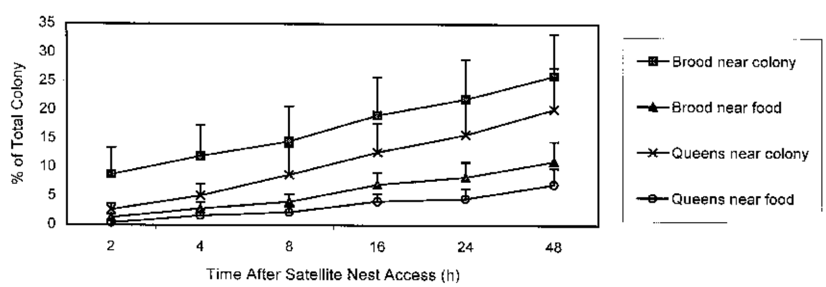

Fig. 2. Distribution of *L. humile* brood and queens in satellite nests 60 cm (near colony) from the main nest and 12.2 m (near food) from the main nest. Each data point represents the mean (+SEM) fraction of the total brood and queen population (n = 10 colonies) in the satellite nests.

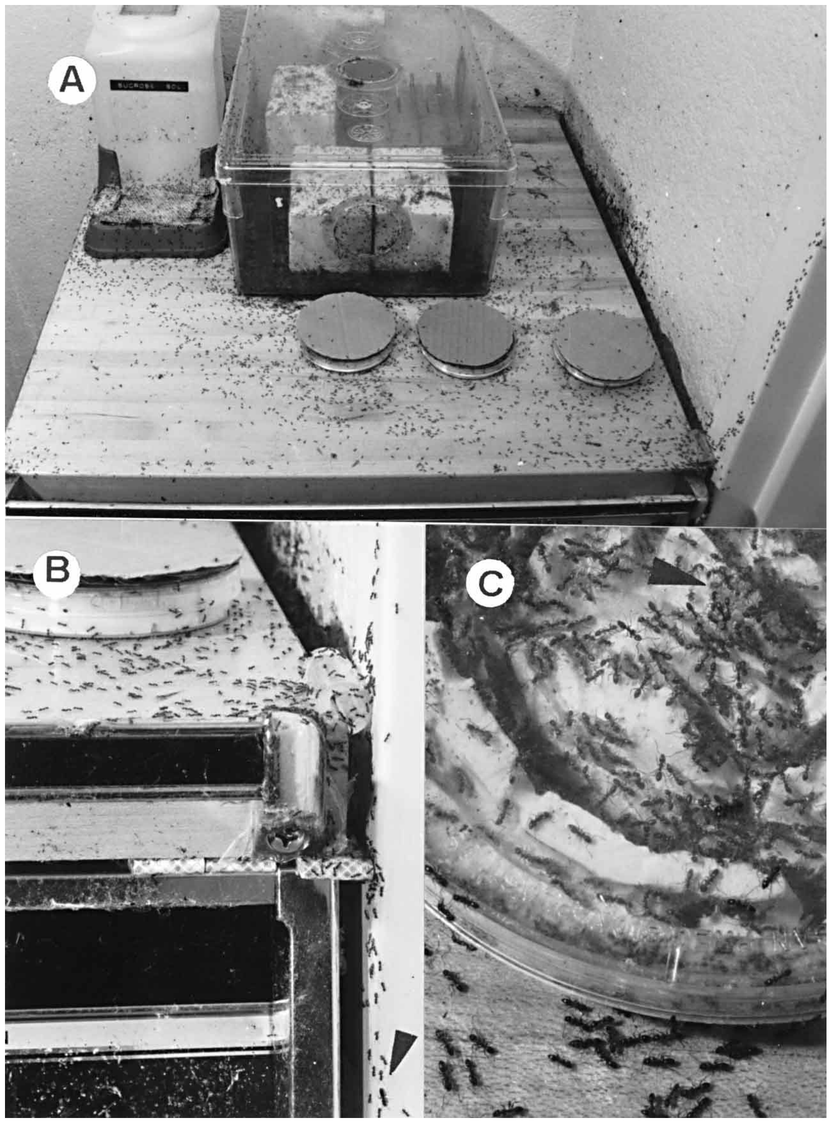

Fig. 3. Continuous movement of a *L. humile* field colony between an outdoor nest and a table top within the laboratory. A) Ant foraging surface with food (0.25 M sucrose [back left], German cockroaches [back center]) and collection dishes (foreground). B) *L. humile* trail along door frame, including queen (arrow). C) Collection dish containing workers and brood (arrow).

be effective in ant species for which no synthetic trail pheromone is available. Worker activity was restricted to the table top as long as cockroaches and sucrose were continuously provided.

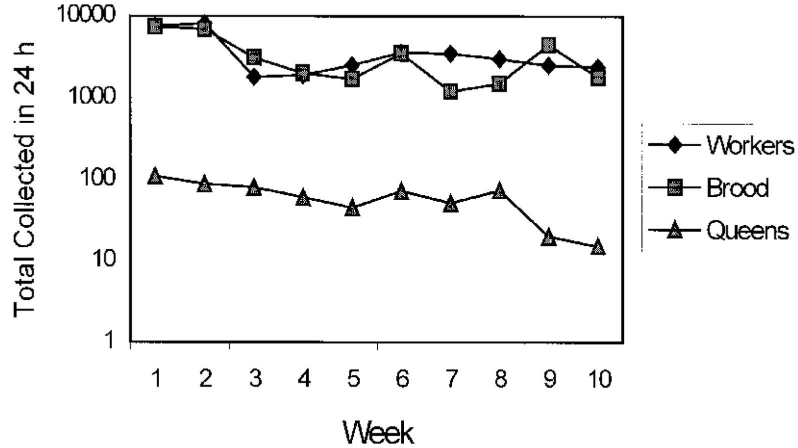

Fig. 4. Total *L. humile* field workers, brood and queens collected in 3 petri dishes within 24 h at each of 10 weekly time points.

De-alated queens and workers carrying brood were observed on some occasions (Fig. 3b) moving between the colony and food source. Initially, workers entered and explored the artificial harborage, but neither queens nor brood were collected. However, after several weeks, queens, brood and workers moved into the harborage. This system was established in September, 1996. Since that time, we have continuously collected workers, queens and all stages of brood (Fig. 3c). Ten collections, each over a 24-h period between December, 1998 and March, 1999 revealed several thousand workers and brood, and over 10 queens (Fig. 4). When the plaster collection dishes were filled, the ants were transferred to larger holding containers until used in behavioral and efficacy experiments.

Our findings corroborate earlier observations of Newell & Barber (1913) that *L. humile* transported colony members close to a continuous food source. To some degree this behavior resembles the habit of a nomadic ponerine ant, *Amblyopone pallipes*, where larvae are often transported to raided food instead of vice versa (Wilson 1958). This food procurement strategy would appear to be more energetically efficient than food transport in species which construct temporary nests and where the food supply is reliable. A collection method which exploits this behavior should apply broadly to *L. humile* in other settings and possibly to other polygynous ant species that expand their colonies through partial emigration.

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