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Recruitment communication and pheromone trails in the Neotropical ants, *Leptothorax* (*Nesomyrmex*) *spininodis* and *L.* (*N.*) *echinatinodis*

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Abstract. *Leptothorax* (*Nesomyrmex*) *spininodis* and *L.* (*N.*) *echinatinodis* workers lay pheromone trails when returning from food sources to their nests. Workers from within the nest begin following the trail almost immediately upon the successful forager's return and do not require a leader. The trail remains effective for about four minutes under our laboratory conditions but it is unclear whether the trail alone is sufficient to induce recruitment or whether additional signals, perhaps emitted within the nest, are involved. Pheromone trails of this type have not previously been documented for leptothoracine ants.

Key words. Recruitment; trails; pheromone; foraging; *Leptothorax*; *Nesomyrmex*; ant.

Social insects recruit nestmates to food sources during nest emigrations and into battle, using various recruitment techniques[1]. Leptothoracine ants typically recruit nestmates by 'tandem running'[2-6], a pheromone mediated recruitment technique in which a recruiting ant leads a single follower to a particular target area. The recruiting ant secretes a pheromone from its poison gland onto its extruded sting and holds its gaster clear of the substrate, sometimes elevated in a characteristic 'tandem calling' posture. A recruit is attracted by this pheromone and follows the recruiting ant. The leader is stimulated to move forward by the antennations of the follower on its gaster or back legs and stops moving when this contact is interrupted. Adult transport also occurs during nest emigrations where it appears to serve a different function from tandem running[3,7]. Pheromone trails have been reported during foraging and nest emigration in some leptothoracine species, but these trails only appear to assist in orienting workers already familiar with the target area, and tandem running is required to recruit additional nestmates[8-10]. In addition, certain slave-making social parasites in this group use 'processions' (or group recruitment) to recruit nestmates during slave raids[1,11]. These processions are thought to have evolved from tandem running and involve the recruiting ant dragging its sting along the substrate and secreting a short-lived pheromone trail as it leads up to 40 followers to the nest to be raided[4-6,12]. In the present paper, we report the discovery of another kind of pheromone trail in this group of ants, a trail that is laid by successful foragers of *Leptothorax* (*Nesomyrmex*) *spininodis* Mayr and *L.* (*N.*) *echinatinodis* Forel as they return from a food source to the nest. This trail guides new recruits to the food in the absence of a leader and is effective for about four minutes under our experimental conditions. Although these trails are clearly part of the recruitment process, additional signals might also be involved.

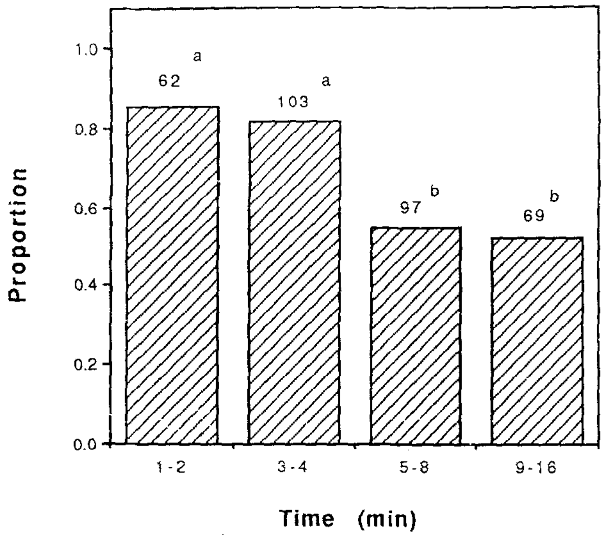
Materials and methods

L. spininodis (*Ls*) and *L. echinatinodis* (*Le*) are very similar and closely-related arboreal ant species[13]. We collected colonies of both species, often in close proximity to one another, nesting in hollow twigs and other cavities in small trees and bushes along roads near El Pilar on the Paria Peninsula in northeastern Venezuela and in the Arima Valley of northern Trinidad during the period 1–16 May 1988. The number of workers in nests was generally small (*Ls*, $N = 51$ nests, mean = 39.9, SD = 33.77; *Le*, $N = 31$ nests, mean = 22.2, SD = 17.41). Nests were either queenless (*Ls*, 64.7%; *Le*, 45.2%), or contained one (*Ls*, 29.4%; *Le*, 38.7%) or more queens (*Ls*, 5.9%; *Le*, 16.1%). Multiple queen nests contained 2–4 queens, and functional polygyny was confirmed through dissection[14]. The many queenless nests suggest a high frequency of colony fission and might be indicative of polydomy[7,15]. Laboratory tests indicated that workers in both queenright and queenless nests were generally aggressive toward workers from other nests of either species, but that alien workers could be adopted both intra- and interspecifically when they eclosed from pupae which we transferred among nests. These characteristics are similar to those of certain temperate zone *Leptothorax* species[15-17].

In the laboratory, the ants were maintained in plastic nests and culture dishes and were fed an artificial diet supplemented with frozen *Drosophila*[7]. Experiments were conducted in plastic arenas which had a surface area of ca. 46 × 46 cm and walls that were 6 cm high and lined with petroleum jelly. Initial foraging and nest emigration experiments were conducted as in previous studies[18] but the absence of tandem runs during these experiments suggested that these ants might use pheromone trails to recruit nestmates. This hypothesis was tested by conducting the following experiment. An arena was subdivided into three portions by petroleum jelly barriers with half of the arena (46 × 23 cm) designated the home area, and the other half of the arena divided into two equal portions (23 × 23 cm) designated foraging areas. A colony's nest was placed in the center of the home area and workers could access the two foraging areas across two plastic bridges (1 × 7 cm). The bridges were supported on three plastic boxes (8 × 12 × 1 cm), one in each portion of the arena. The bridges met at a 45° angle to form a choice point on top of the box in the home area, directly in front of and ca. 12 cm from the nest entrance. Additional replacement bridges (see below) were scattered in the colony's home area but were not connected to the foraging areas. A bottle (6.0 cm long × 0.7 cm diameter) filled with water and stoppered with cotton was located in each portion of the arena. Food was placed in both foraging areas between experiments, and the colony was deprived of food for several days prior to each experiment. During each experiment, a food source containing artificial diet was placed in one randomly-chosen foraging area at a distance of approximately 24 cm from the nest. When a worker began feeding, the bridges connecting the home area to the foraging areas were exchanged with the replacement bridges referred to above. When the worker left the food and crossed the bridge toward the nest, the two bridges connecting the home area to the foraging areas were exchanged with one another. The time at which the successful forager crossed the bridge on its return to the nest, and the time at which workers crossed either bridge leading to the foraging areas during the next 16 min were recorded. No other workers were allowed to return across the bridges during this period. This experiment was conducted a total of 54 times with two *L. spininodis* colonies and one *L. echinatinodis* colony. These colonies had brood but no queens, and contained 22, 23, and 47 workers respectively. The results for different colonies and species were very similar (e.g., across species, 2 × 4 contingency tables, heterogeneity chi-square = 3.64, df = 3, $p > 0.05$) and are pooled in the present analysis to permit better resolution of the patterns in the data.

Results and discussion

The results of the experiments indicate that successful foragers of these species often lay short-lived pheromone trails on their return from a food source to the nest. Typically, when a successful forager entered the nest, a group of 5–10 workers exited the nest almost immediately and began following the trail while the successful forager remained inside the nest and engaged in trophallaxis with nestmates. Workers following the trail appeared to function independently of one another and apparent recruits sometimes lost the trail before reaching the bridges to the foraging areas. Returning foragers did not always appear to lay effective trails or to induce recruitment. Nonetheless, nearly half of the 331 workers that crossed bridges to the foraging areas in these experiments, crossed within the first 4 min after the successful forager had crossed a bridge; and 83.0% of these workers selected the bridge taken by the successful forager (fig.). This result is significantly different from random expectation ($X^2 = 72.01$, df = 1, $p < 0.001$). During the remaining 12 min of the experiments, only 53.6% of the workers chose this bridge, a proportion which is significantly different from that observed during the first 4 min (2 × 2 contingency table, $X^2 = 31.71$, df = 1, $p < 0.001$), and which is not significantly different from random expectation ($X^2 = 0.352$, df = 1, $p > 0.05$). This evidence indicates the existence of a pheromone trail that is effective for about four minutes under these experimental conditions. The source of the trail pheromone and the method of laying the trail were not determined. No apparent invitation behavior by recruiting workers inside the nest was observed and there was little opportunity for such behavior to occur since recruits exited the nest so quickly. However, additional chemical inducement near the nest entrance cannot be ruled out. Thus, it is unclear whether these trails are sufficient to induce a full recruitment response or whether any additional signals are involved.



Proportion of workers choosing the bridge used by a successful forager rather than an alternative bridge at various time intervals after the forager crossed the bridge on its return from the food source to the nest. The total number of workers crossing the bridges is given above each bar. The overall difference among these proportions is highly significant (2 × 4 contingency table, $X^2 = 33.45$, df = 3, $p < 0.001$) but proportions identified with the same letter are not significantly different (2 × 2 contingency table, X^2-test with continuity correction, $p > 0.05$).

Pheromone trails of the type reported here would be much more efficient than tandem running for quickly recruiting numerous nestmates to a nearby food source, since several recruits could immediately follow the trail to the food rather than waiting to be led in tandem runs. The existence of such trails in these tropical species but not in their temperate zone relatives might relate to the relative abundance and distribution of their food sources, or the level of competition that they encounter[1]. Moreover, the arboreal foraging and nesting habits of these ants might contribute to the relative efficiency and evolution of trails by imposing constraints on possible deviations from the appropriate path during recruitment. The pheromone trails reported here were relatively short lived but the plastic substrate used in our laboratory test might have had a negative impact on trail longevity, and similar trails in nature might last longer and be effective over greater distances than would be predicted from our results. Furthermore, trail reinforcement by additional successful foragers might also occur in nature and enable modulation of the colony's response with respect to the size, quality, or proximity of food sources[1]. Trail-laying in these ants probably involves poison gland secretions and probably evolved from tandem running but further studies of these and related species are necessary to test these hypotheses.

This is the first study to investigate recruitment in the *Leptothorax* subgenus *Nesomyrmex*, and the pheromone trails described here further underline the distinctness of this subgenus as indicated by morphological studies[19]. To date, collections of tropical leptothoracine ants remain sparse and no tropical social parasites have yet been discovered in this group of ants[1]. This apparent absence contrasts with the situation among temperate zone *Leptothorax* species which show an amazing propensity for the evolution of various kinds of socially-parasitic relationships[1,11]. Nonetheless, in these *Nesomyrmex* species, we found facultative polygyny, a high frequency of small queenless nests, aggressive colony defence, and the ability to adopt unrelated workers from eclosing brood. These characteristics are similar to various temperate zone *Leptothorax* species and have been suggested as factors that might contribute to the evolution of various forms of social parasitism[1,5,6,11,15,20]. Indeed, the discovery of trails during recruitment in these tropical leptothoracine species might be construed as another factor which could predispose them to the evolution of slavery in particular since increased efficiency in recruitment (e.g., processions) appears to have evolved repeatedly among leptothoracine slave makers in the temperate region[1,5,6,11]. Ultimately, if the paucity of social parasites among tropical leptothoracines is confirmed through further collecting then it might be explained by various other factors that might distinguish these ants from their temperate zone relatives. These factors include differences in species diversity, colony density, intra- and interspecific competition, and aspects of seasonality[1,11,20].

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