

Trail-laying behaviour during exploratory recruitment in the Argentine ant, *Iridomyrmex humilis* (mayr)

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RESUME

Comportement de marquage au cours du recrutement exploratoire chez la fourmi d'Argentine, *Iridomyrmex humilis* (Mayr).

L'accès des ouvrières d'*I. humilis* à un nouveau territoire entraîne une exploration collective par piste chimique. La piste chimique assure seule l'orientation des fourmis au cours du recrutement; de plus, les ouvrières fraîchement recrutées renforcent celle-ci au cours de leurs déplacements vers le nouveau territoire. La longueur et la fréquence des dépôts sont cependant plus importantes lors de la phase de retour au nid. Une approche théorique des distributions des longueurs des dépôts suggère que le comportement de marquage des ouvrières n'est pas un simple processus Markovien, bien qu'il constitue une bonne approximation du mécanisme réel.

La similitude de comportement de marquage observée au cours des recrutements alimentaires et exploratoires suggère l'implication d'une seule phéromone dans l'organisation spatiale des sociétés de la fourmi d'Argentine.

Mots-clés: *Iridomyrmex humilis*, fourmi d'Argentine, exploration, recrutement, comportement de marquage.

SUMMARY

Trail-laying behaviour during exploratory recruitment in the Argentine ant, *Iridomyrmex humilis* (Mayr).

The access of *I. humilis* workers to a new, chemically unmarked, terrain elicits a recruitment associated with a collective exploratory behaviour. This

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collective exploration is generated by the individual workers' chemical trail-laying and trail-following behaviour. Newly arriving workers orient themselves in accordance with the preceeding ones' chemical marks, and themselves lay more pheromone as they move along. Returning ants act similarly, albeit laying larger and more frequent marks. A theoretical analysis of the experimental streak lengths' distribution suggests that the workers trail-laying behaviour is not just a single-chain Markovian process, although this constitutes a good approximation.

The trail-laying behaviour exhibited by the ants strongly suggests that the same pheromone is being used both in food and exploratory recruitment. We propose that only one pheromone could be involved in the Argentine ants spatial organisation.

Key words: *Iridomyrmex humilis*, Argentine ant, exploration, recruitment, trail-laying behaviour.

INTRODUCTION

The Argentine ant *Iridomyrmex humilis* (Mayr) establishes large polygynic and polydomic societies of many thousands of individuals, performing food recruitment (mass recruitment) with the help of a trail pheromone produced by the ventral Pavan's gland (WILSON and PAVAN, 1959; COURET and PASSERA, 1979; ROBERTSON et al., 1980; VAN VORHIS KEY et al., 1981). Chemical analysis of the ventral gland showed (Z)-9-Hexadecenal (Z-9-16: Ald) to be an important component of the trail pheromone (CAVILL et al., 1979, 1980; VAN VORHIS KEY and BAKER, 1982a,b).

In a previous paper (DENEUBOURG et al., 1988, 1989), we showed that access to a new, chemically unmarked terrain elicits an exponential outbound surge of ants associated with a collective exploratory behaviour. The exploratory pattern consists of a column of ants extending from the nest, which fans out at its distal extremity into an "exploratory zone" where the workers explore individually in random directions. As this "exploratory zone" progresses into the area, a trail extends from it to the nest indicating a chemical trail formation (figure 1).

We describe in this paper laboratory studies on the Argentine ants' recruitment during exploration, the trail-laying behaviour of individuals moving either to or from a new unexplored terrain, and the trail pheromone involved in such a process.

METHODS

Colonies of *I. humilis* (Mayr) collected at Port-Leucate (Aude - France) were reared in laboratory nests (Brian, 1951) placed in plastic containers (20x30x4 cm). The ants were fed with brown sugar solution and cockroaches. Colonies were kept in a constant environment at $21^{\circ}\text{C} \pm 1^{\circ}\text{C}$, with a 12:12 hours light:dark regime.

Exploration was elicited by connecting a sand filled arena (80x80 cm) to the nest container by a cardboard bridge or a glass tube (see below). The sand and the bridge were changed before each experiment.

Exploratory recruitment to new terrain

The workers' orientation during recruitment was tested by experiments with a Y-shaped cardboard bridge (40 cm long, 1 cm large), as described previously (Aron et al., 1988). Briefly, the bridge had one fixed branch (20 cm long) connected to the nest entrance, and 1 or 2 interchangeable branches leading to the new arena, diverging from each other at an angle of 60° . The experiments were based on a binary choice between visual (environmental, surrounding laboratory cues) and chemical (trails) cues. They were performed in two successive periods.

Access period: *I. humilis* colonies were given access to a new terrain by connecting it with a single branch bridge to the nest.

Testing period: after a 30 min access period, the experimental set-up was modified (2-branched bridge) by displacing the first branch by 60° and replacing it by a new one. We recorded the choice of branch of each worker passing over the bridge during the next 10 minutes. Workers reaching the new terrain were immediately removed and kept apart until the end of the experiment.

In order to study possible recruitment interaction, careful observations of the workers' behaviour while returning to the nest were made.

Trail-laying behaviour

To study trail formation, the behaviour of the ants crossing a bridge either to or from the nest was monitored with a high-motion video recording coupled with a time-date generator (including stopwatch functions displaying hundredths of a second). A camera fitted with macrolenses was mounted on a mobile wagon moving in a horizontal plane parallel to the bridge, and positioned so as to observe the ants from side-on with a slight vertical perspective. This view allowed us to note when the workers' gaster touched the substrate, assuming that the ants lay chemical marks each time the abdomen meets its reflection on the bridge. Recordings were made for 1 hour after the colonies were given access to the new terrain. Ants crossing the bridge in both directions were chosen randomly, and kept in the field of view over 12 cm.

The workers' velocity moving either to or from the nest was measured for uninterrupted stretches of 5 to 12 cm.

For each ant during play back, a frame-by-frame analysis (one frame = 0.04 sec) was carried out and the gaster-substrate contacts reported. We measured the length of each pheromone streak (i.e. the distance between the point at which its gaster makes contact with the bridge and the point at which contact is

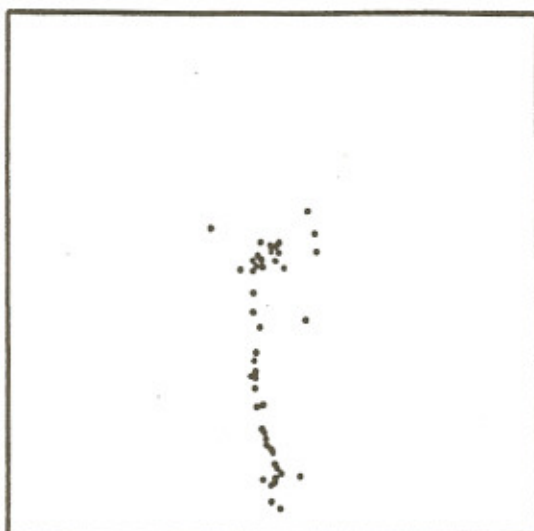


FIGURE 1: Exploratory pattern on a 0.8 x 0.8 m sandy virgin arena. Colony size: 450 workers. Each dot represents one ant.

FIGURE 1: Pattern exploratoire sur une aire couverte de sable de 0.8 x 0.8 m. Taille de la colonie: 450 ouvrières. Chaque point représente une fourmi.

broken); this was done by multiplying the average number of frames during which the ant marked continuously by 0.04 second and by the ants' velocity. The same operation was made for non-trailing streaks.

RESULTS

Exploratory recruitment to new terrain

Experiments carried out with the Y-shaped bridge indicated that most of the ants (52 out of 54) going to the arena travel along the branch initially used during the first period of access, even if shifted by 60° from its original position ($P < 0.001$, $n=3$; One sample X^2 test). This reveals that I. humilis workers orient along chemical trails during exploratory recruitment.

The observation of the first ants returning to the nest showed that they moved rapidly amidst quiescent nestmates, antennating them briefly and vigorously. This invitation behaviour stimulates inactive workers which leave the nest within seconds, and begin trail-following. Nevertheless, some ants were observed to leave the nest without having been touched by a recruiter, suggesting that the trail itself and/or the increasing activity in the nest could be sufficient to induce recruitment.

We are unaware of any chemically mediated invitation.

Trail-laying behaviour

The observation of the ants crossing the bridge between the nest and the new arena showed a trail-laying behaviour by workers both moving from and returning to the nest. Macro-video recordings revealed that, in both situations, the gaster is bent downwards and the ventral side periodically and lightly pressed in contact with the support, breaking the trail into streaks of different lengths.

Samples of individuals crossing a 12 cm-bridge, either to or from the new terrain, indicate that, whatever their direction, about 40% of the workers laid no trail whatsoever.

The analysis of the workers' velocity on the bridge showed no statistical difference between trail-laying and non trail-laying ants, either moving to or from the arena. Table I indicates that ants crossing the bridge toward the nest lay significantly longer marks than those moving in the opposite direction, and furthermore the average distance between two streaks is shorter.

Pooling all streaks respectively both for ants moving to and from the arena, we estimated the probability q that a worker having marked k times lays one mark more (the number of pairs of frames during which the ants lay trail $(++)$ divided by the number of frames during which trailing is observed $(+)$); and $(1-q)$ that the ant stops marking (the number of pairs of frames during which the ant stops trailing $(+-)$ divided by the number of frames during which trailing is observed $(+)$). Both q and $(1-q)$ are assumed to be independent of k . This hypothesis implies that the probability of an ant interrupting trailing remains constant with the length of streaks previously laid. The fraction of the number of streaks of length n is given by

$$F(n) = q^{(n-1)}(1-q) \quad (1)$$

Figure 2 shows the experimental and theoretical (according to equation (1) with estimated values of q) distributions of the streak lengths for the ants moving to and from the arena. The comparison between the experimental and theoretical distributions suggests a strong analogy in both directions, despite the fact that they are not statistically identical. There is a discontinuity between the frequency of streaks of length 1, and those of a superior length. This could be due to an over-estimation of their number, as it is not always

Trail-laying behaviour	towards the nest	towards the area	Student's t test
Length of streaks (cm)	0.19 \pm 0.03 n=13	0.10 \pm 0.01 n=17	t=-2.69; df=28 p<0.05
Distance between two streaks (cm)	0.31 \pm 0.09 n=13	0.57 \pm 0.07 n=17	t=2.30; df=28 p<0.05
Workers' velocity (cm/sec)	1.09 \pm 0.09 ^a n=13	1.01 \pm 0.07 ^a n=17	
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No trail-laying behaviour			
Workers' velocity (cm/sec)	1.11 \pm 0.07 ^a n=10	1.15 \pm 0.08 ^a n=13	

TABLE I: Means and standard errors of the streak lengths, the distance between two streaks, and the workers' velocity on the bridge during exploratory recruitment. a: means are not statistically different (Students' t test; P>0.05).

TABEAU I: Moyennes et erreurs standards des longueurs des dépôts, de la distance entre deux dépôts, et des vitesses de déplacement des ouvrières au cours du recrutement exploratoire. a: pas de différence significative entre les moyennes (Student's t test; P>0.05).

clear if the ant is actually laying when only one frame in a row shows the gaster to be in contact with the bridge. Nevertheless, equation (1) can be seen as a first approximation of the experimental streaks distribution.

DISCUSSION

Recruitment to new, unexplored terrains near the nest has been reported for a number of ant species: e.g. *Solenopsis saevissima* (WILSON, 1962), *Solenopsis geminata* (JAFFE and PUCHE, 1984), *Oecophylla longinoda* (HOLLOBLER and WILSON, 1978), *Leptothorax unifasciatus* (ARON et al, 1986), and different army ants (TOPOFF, 1972; CHADAB and RETTENMEYER, 1975; TOPOFF et al., 1980). The mechanisms involved in such a process varying between mass, group or tandem-running recruitment. In most cases, recruited ants entering the new field

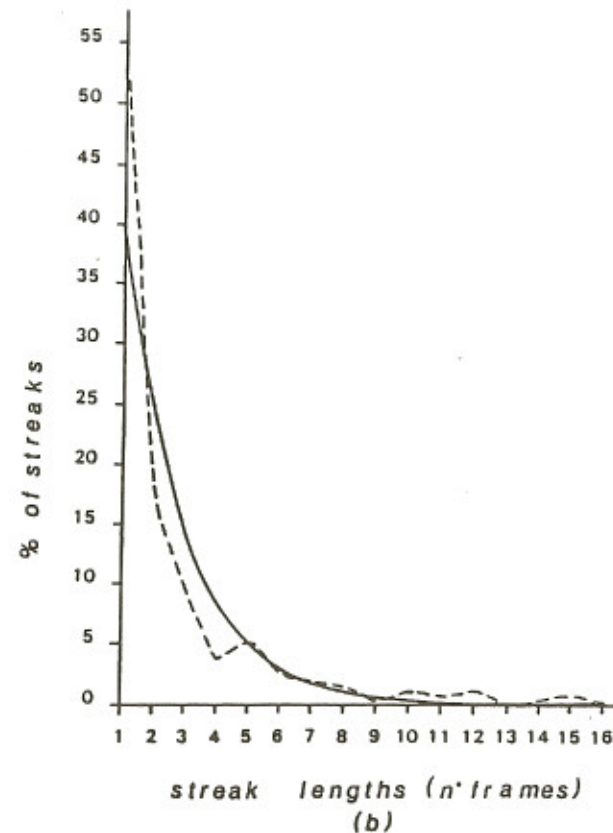
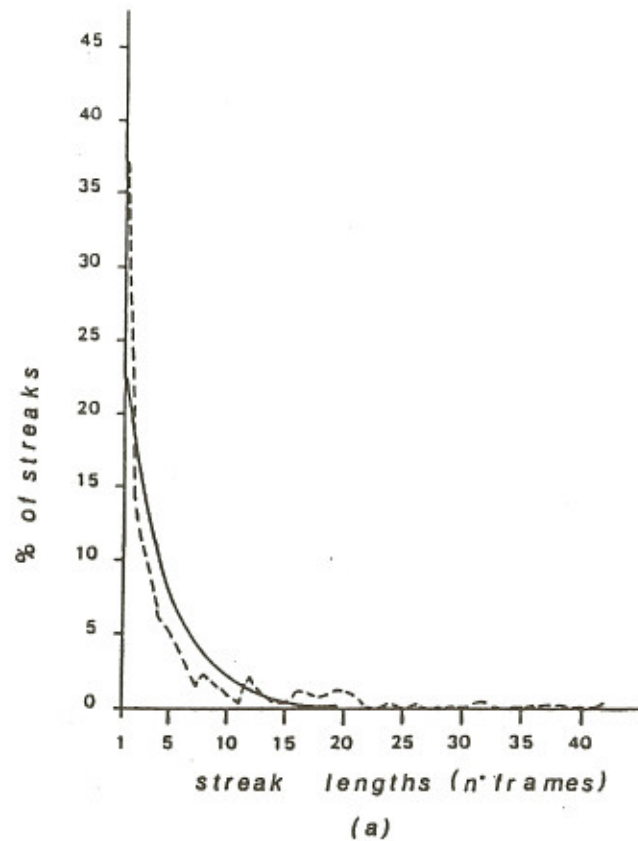


FIGURE 2: Experimental (dashed curve) and theoretical (solid curve) streak length distributions for the ants moving towards the nest (a) and towards the area.

FIGURE 2: Distribution expérimentale (trait discontinu) et théorique (trait plein) de la fréquence des longueurs des dépôts pour les fourmis se déplaçant vers le nid (a) et se rendant sur le nouveau territoire (b).

wandered off in individually random searching patterns. In contrast, recent observations showed that exploration of new territories in I. humilis proceeds according to a well structured characteristic pattern: a collective exploratory behaviour during which the ants keep permanent contact with each other through the medium of chemical trails (DENEUBOURG et al., 1988, 1989). A minimal model showed how the individual explorers' marking and trail-following behaviour may generate this collective exploratory pattern.

The experiments reported here demonstrate that I. humilis workers perform a typical mass recruitment (sensu WILSON, 1962) to a newly encountered terrain. The recruiters exhibit rapid locomotion and accelerated antennal beatings which undoubtedly serve to arouse nestmates to leave the nest and follow the trail. Similar invitation behaviour has been described by VAN VORHIS KEY and BAKER (1986) during food recruitment. Ants encountering natural chemical trails in the absence of any invitation process may also begin trail following. This behaviour and the occurrence of workers leaving the nest without any direct contact with the recruiters lead us to suspect that the trail itself can alert and activate quiescent workers to trail following. However, VAN VORHIS KEY and BAKER (1986) observed that, during foraging, uninvited ants leaving the nest followed the trail for a significantly shorter distance than invited ones.

As previously reported by VAN VORHIS KEY and BAKER (1986) during foraging activities, we noted that freshly recruited explorers not only follow existing trails but also reinforce them as they travel towards the new territory. The trail-laying behaviour exhibited by the ants during exploration is similar to that observed by these authors during foraging recruitment. This strongly suggests that the same trail pheromone (Z-9-hexadecenal) could be used during both activities.

Our results show that the length and frequency of the streaks are significantly higher when ants return to the nest from the new territory. While we have no evidence to attribute to this quantitative difference to any specific function, it could be that the returning ants' role is more to stimulate the recruitment and to provide a certain degree of directional information, while the advancing ants' role is more related to structuring the collective recruitment (i.e. forming the trail and exploratory front).

The strong similarity between the theoretical and experimental streak length distributions suggests that the probability of an ant stopping or continuing marking is probably not a single-chain Markovian process, although this constitutes a good first approximation of the real process.

Iridomyrmex humilis' exploratory behaviour is remarkable in that they mark continually and explore collectively. It is (to our knowledge) the first report of such a behaviour in the sub-family of the dolichoderine ants. These "exploratory swarms" resemble to army ant type hunting raids, albeit on a much smaller scale (RETTEMEYER, 1963; SCHNEIRLA, 1971; CHADAB and RETTEMEYER, 1975; TOPOFF, 1972; TOPOFF et al., 1980; FRANKS and FLETCHER, 1983). A similar collective exploration has been reported in another ant, outside the Dorylinae, the myrmicine Pheidologeton diversus (MOFFETT, 1984, 1988).

The massive and collective exploration observed in Iridomyrmex humilis could constitute, as in army ants, a food searching strategy. The discovery of new food sources in the Argentine ant elicits a foraging mass recruitment in which chemical trails connect to the exploratory trails. In such circumstances, columns of ants moving to a new terrain are diverted to rich food sources (ARON and PASTEELS, 1988). The genesis of a collective exploratory behaviour in Iridomyrmex humilis corresponds to their highly agonistic and opportunistic behaviour. The chemical trails can lead to a rapid mobilization of a large number of individuals to freshly discovered sources and/or to colonization of new sites, and constitute a massive and permanent defense against other competing ant species (see also MOFFETT, 1988).

Colonies of the Argentine ant parcell into sub-colonies ("divisional migrations") of hundred workers, some queens and brood establishing their nest near the sources, and stay interconnected by continuous trails of workers (NEWELL, 1909; SKAIFE, 1955; DECHENE, 1970; MARKIN, 1970). Nevertheless, unpublished data show that the discovery of new terrains (and potentially favorable nest locations?) are sufficient to induce nest-migration along exploratory trails, leading to the subdivision of the colonies. Iridomyrmex humilis' polydomous nests are not permanent structures, and hence, movements of populations because of unfavorable environmental conditions such as food-resource depletion, flooding, dessication, or extremes of temperature, are frequent in this species (MARKIN, 1970; WILSON, 1971). Nest moving corresponds most frequently to seasonal migrations. The ants may then fuse with adjacent colonies, leading to the formation of one massive colony in a favorable location (NEWELL, 1909; NEWELL and BARBER, 1913; CHOPARD, 1920; SKAIFE, 1955; MARKIN, 1970; BENOIS, 1973). This overall scenario is very similar to that of Solenopsis invicta (TSCHINKEL, 1987), which is also an opportunistic, invasive, polygynic, polydomic pest species. Furthermore, some Solenopsis sp. form collective explorations similar to those of I. humilis (HOLLOBLER and WILSON, pers. com. of HOLLOBLER).

The probable use of the same trail pheromone (Z-9-hexadecenal) both in exploratory and food recruitment trails suggests that the entire spatial organization of Argentine ant societies (according to the temperature, relative humidity, nest site location, food source availability, ...) could be mediated by only one pheromone (possibly a mixture of compounds). Upon finding new sources, exploratory trails became food recruitment trails. As divisional migrations proceed, foraging trails are then used for nest moving near to the sources. With time, and with the evaporation/deactivation of the (Z)-9-hexadecenal, these trails no longer recruit but remain as guidelines used for traffic between the different sub-colonies.

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