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Queen retrieval in the Argentine ant

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Abstract. Queen retrieval recruitment in ants is the recruitment of workers towards queens which are outside the nest, using chemical trails. The odour trails enable the queens to orient rapidly and return to the nest. This behaviour, reported for the first time in the Argentine ant *Iridomyrmex humilis*, is briefly discussed with regard to its possible adaptative significance.
Key words. Iridomyrmex humilis; Argentine ant; queen retrieval; recruitment.

In ant societies, the queens often depend on workers to enable them to move between different sites. For example, they can be picked up and carried by workers. Such social carrying behaviour is common in ants, and the different modalities by which it proceeds have been reviewed for various ant species during emigration from one nest site to another[1,2]. The behavioural patterns of communication involved are often specific at the level of the ant subfamilies. In some species, reproductive females may be too large to be transported by relatively small workers. In *Camponotus pennsylvanicus*, workers grasp the queens by their mandibles and drag them to the target area[1]. In the Weaver ants, *Oecophylla longinoda*, queens leaving the nest move under their own power along chemical nest-moving trails, but are covered by a dense group of major workers[3]. This is also very characteristic of New World army ants when colonies emigrate to a new nest location during the nomadic phase. In this case, the queen running along nest-moving trails is most frequently surrounded by a retinue of soldiers and small workers; the size of the retinue varies according to the species and the society size[4]. It is not clear whether the retinue helps to guide the queen, or just to protect her as she follows the trail.

Colonies of the fugitive Argentine ant *Iridomyrmex humilis* frequently move to other nests in the course of a single season. This species establishes polygynous and polydomous societies of many thousands of individuals, and maintains a permanent contact between nests mediated by a network of chemical trails[5-7]. Workers and queens regularly move between the nests. In this species, queens rarely seem to be transported by the workers. When moving between colonies, as well as during emigration, queens are frequently observed travelling alone along chemical trails.

We describe here the use of a recruitment process in a new situation in the Argentine ant. This queen retrieval behaviour consists of the recruitment of workers by chemical trails towards queens isolated from the nest. These odour trails are followed by the queens, and lead them rapidly back to the nest.

Methods

Mature queens of *I. humilis* anaesthetized with CO_2 were placed 14 cm from the nest entrance of queenright laboratory colonies, in the centre of a circle (8 cm diameter) drawn on the floor of the rearing arena. The circle was at a distance of about 20 cm from the trail system leading the foragers to the food source. The circle was divided into twelve 30° sectors. The number of ants crossing each of the 12 arcs of the circle was counted between the moment when the queen was introduced and the moment she left the circle. The number was also recorded for an equivalent period of time before she was introduced. Four trials were conducted, lasting from 7 to 11 min.

The trail-laying behaviour of ants crossing a bridge towards an anaesthetized queen was monitored with a macro-video recording. I assumed that an ant deposited a chemical mark each time its abdomen touched the substrate, and considered a trail-laying passage as a passage on the bridge during which the ants made at least one mark [8].

Additional methodological details on the orientation of queens in the absence or in the presence of workers are presented in the text, with the description of individual experiments.

Statistical evaluations of the data were based on Chi-squared tests and Mann-Whitney U-tests for ranked observations [9]. Rayleigh tests and two-sample Chi-squared tests were used to evaluate the significance of the circular distributions [10].

Results

Introducing the queen elicits a rapid increase in the number of ants in the circle, followed by an increase in the traffic of workers crossing the different sectors. A few minutes later, a well-defined column of ants appears between the queen and the nest, and one sector becomes much more frequented than the others (fig. 1). In some experiments, up to 40% of the individual runs may be concentrated on one sector. The comparison of the circular distributions of the workers crossing the circle before and after the introduction of a queen shows that the two samples differ significantly from each other (table). This difference is explained by a shift of the mean population direction, which does not necessarily correspond to one of the sectors previously most frequented before introduction of the queen. Experiments performed under a red light gave similar results.

Many experiments were performed during the elaboration of the experimental method. In all cases but one the queen moved rapidly to the nest under her own power, crossing the most frequented sector. In one experiment the queen was still asleep under the anaesthetic. She was grasped by the petiole, lifted up, and carried by a worker towards the nest.

The increase of activity close to the isolated queen, and the use of directional information by the ants moving to and from the circle, suggest a recruitment process towards the queen, most probably relying on chemical

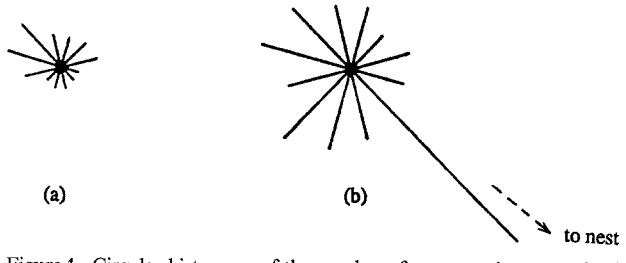

Figure 1. Circular histogram of the number of ants crossing on each of the 12 sectors before (*a*) and after (*b*) queen introduction.

Comparison of the circular distributions of the workers crossing the circle before and after queen introduction. The two-sample Chi-squared test is used to evaluate the statistical significance of the orientation data between both situations [10]. The total number of ants crossing the circle, and the proportion of ants travelling along the sector which will be the most frequented once the queen is introduced, are indicated before (Q−) and after (Q+) the introduction of the queen.

	Total no. of ants		% of ants on most frequented sector		Chi-square test (df = 11)
	Q−	Q+	Q−	Q+	
1	228	600	3.9	23.6	$\chi^2 = 66.0$***
2	179	1213	9.5	40.3	$\chi^2 = 98.6$***
3	216	730	13.4	33.0	$\chi^2 = 48.9$***
4	249	704	4.0	27.0	$\chi^2 = 81.4$***

*** : $p < 0.001$

trails. The hypothesis is supported by observations on the workers' trail-laying behaviour. The first workers to discover the queen in the centre of the circle touch her with their antennae several times, and run excitedly in short looping circles around the queen, repeatedly dragging the gaster over the substrate – apparently laying a chemical trail. This behaviour summons nearby workers to collect around the queen. Afterwards, some of the workers head for the nest, keeping the tip of the abdomen in close contact with the ground.

As shown in figure 2, trail-laying behaviour analyses of ants crossing a bridge towards an anaesthetized queen reveal that workers lay trails more or less equally when moving to and from the nest. However, whereas only a few trail-laying passages occur before the introduction of the queen (14.3% and 17.7%, away from and towards the nest, respectively) this proportion is more than quadrupled for both directions after the queen is introduced (64.1% and 76.1%) ($\chi^2 = 58.39$, df = 1,

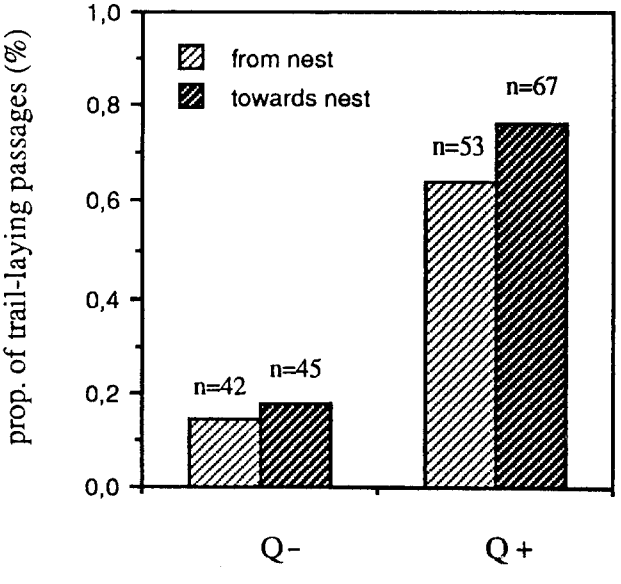

Figure 2. Proportion of trail-laying passages made by Argentine ants moving to and from the nest, before (Q−) and after (Q+) queen introduction. The total number of passages is indicated on the top of each column.

$p < 0.001$). This result, associated with the rapid increase of the number of workers around freshly-introduced queens, shows a process of recruitment of workers towards queens outside the nest, using trail pheromone. The queens then follow the trail to orient towards the nest. Such behaviour, reported here for the first time, may be identified as queen retrieval recruitment.

The first recruiters heading towards the nest most probably use visual cues to orient. This ability has been shown during foraging when Argentine ants move towards a food source[11]. As supported by the following experiments, queens are unable to orient towards the nest if they are not guided by odour trails of workers. The circular distribution of queens crossing a circle, and the time spent to reach the nest, were reported in the absence or in the presence of workers. Whereas in the first situation the circular distribution of queens around the circle is random ($r = 0.37$, n.s., $n = 8$; Rayleigh test), in the presence of workers a highly significant mean orientation of the queens appears which corresponds to the trail laid by the ants ($r = 0.96$, $p < 0.001$, $n = 8$; Rayleigh test). In addition, the time spent by isolated queens in reaching the nest is greater than that observed in the presence of foragers ($p < 0.0001$, $n_1 = n_2 = 8$; Mann-Whitney U-test). In four experiments queens had not found the nest after 20 min, whereas in the presence of workers the average time was about 18.5 s (s.d. = 4.5 s).

Based on these results, I carried out some experiments to determine whether the queens are able to orient alone along the trail. Recruitments were induced towards a queen placed in the centre of a circle drawn on a sheet of paper. Once the trail was well established in one sector and the queen had crossed the circle heading to the nest, the paper was removed to a new worker-free arena. The experiment was repeated with a new freshly-anaesthetized queen placed in the centre of the circle on which the trail had been laid. In all the experiments performed ($n = 12$), the new queen rapidly left the circle by the same sector as was used by the first queen, thus showing that queens are able to orient along odour trails, even in the absence of workers. Nevertheless, observations suggest that a minimal trail is not enough for queens to find the way back to the nest, and that they need a strengthened trail in order to orient. This was supported by the following experiments, performed with a Y-shaped bridge. The bridge had two replaceable branches (the fork of the Y) pointing towards the nest, diverging at an angle of 60°, and one elevated blind branch, which ended in a footplate on which a queen was placed. The first three trail-laying workers leaving the queen towards the nest were allowed to cross on one branch of the fork, and all other ants were removed from the bridge. I then recorded the choice of the queen crossing the fork in the following 3 min. The results obtained show that queens chose more or less equally the marked and unmarked branches of the fork leading to the nest (33 queens were tested; 15 chose the branch on which the three foragers passed and 10 chose the unmarked branch; 8 queens had not crossed the bridge after 3 min).

Discussion

In New World army ants, which are all monogynous, clusters and retinues of workers around the queen are considered to be adaptative for protecting the reproductive females[4]. This permanent contact between workers and the queen could reduce the risk of the queen getting lost along emigration trails. In *I. humilis*, the proportion of queens to workers may reach more than 1% in summer[12]. In such a highly polygynous situation the use of a retinue of ants to accompany every reproductive female seems rather unlikely.

Under natural conditions, queens of *Iridomyrmex humilis* readily move unescorted along the chemical trails leading to new nest sites or other colonies of the society[5,13]. The results showing that queens are able to orient themselves alone along odour trails are in accordance with these observations. Nevertheless, they show that a strengthened trail is necessary for the queen to find her way back to the nest, and that in the absence of chemical cues she is unable to orient. This last point may be relatively trivial since queens are probably most of the time inside the nest, and are therefore unlikely to be familiar with the external environment.

We may expect that during trail-following, running individuals could be disturbed by wind, falling twigs or other natural hazards, and this could lead to some queens losing their way. Moreover, as they usually travel alone, the risk of their getting lost is probably greater than in species where the queens are escorted by workers. Consequently, queen retrieval recruitment may have two advantages. Firstly, the mobilization of a large number of workers around isolated queens ensures a rapid protection against potential predation. Secondly, the chemical trail formed during recruitment guides the queens rapidly to the nest. Similar queen retrieval behaviour has been seen in *Atta cephalotes*, *Meranoplus* spp., and *Camponotus pennsylvannicus* (Hölldobler, pers. comm.). We may thus tentatively conclude that queen retrieval recruitment could occur in other ant species in which queens are rarely carried or accompanied by a retinue of workers, but instead move by themselves along chemical trails during emigration or inter-nest exchanges.

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Identification of an oviposition-regulating pheromone in the European grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae)

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Abstract. The oviposition of the European grapevine moth (EGVM) *Lobesia botrana* can be deterred by an extract of conspecific eggs corresponding to 20 egg equivalents. The reduction of the oviposition behavior is dose-dependent. Nine chemicals have been extracted from the eggs and identified as straight chain fatty acids and esters of fatty acids. A mixture of these rather simple molecules induces the same levels of deterrence as the total extract. It might be possible to use oviposition regulating pheromone in the future for the control of EGVM populations.
Key words. Oviposition-deterring pheromone; Lepidoptera; Tortricidae; *Lobesia botrana*; eggs; fatty acids; esters of fatty acids.

The European grapevine moth (EGVM) *Lobesia botrana* (Lepidotera: Tortricidae, Olethreutinae) is a major pest in vineyards in Europe. The yield reductions caused by this insect are due both to damage by the larvae and to further attack by fungi. The reduction varies according to the grape vine cultivars. It can attain more than 70% in Greek vineyards [1], while almost 100% of grapes have been reported to be attacked in Italian vineyards [2]. Like other Olethreutinae species, *L. botrana* females typically lay isolated eggs on the flower buds of the grapevines or on the grapes, depending on the adult generation involved, and the plant phenology [3]. Because of the low density of eggs naturally observed on the flowers or the grapes in vineyards, we hypothesized that the eggs carry an epideictic pheromone which influences the oviposition of conspecific females and regulates the egg spacing in *L. botrana*. Biological evidence has recently been presented for an oviposition-deterring pheromone (ODP) in this species, extracted from the egg surface [4]. We present here the chemical identification of nine constituents of this pheromone. The constituents are linear fatty acids and methyl esters of fatty acids.

Materials and methods

The insects used for egg extraction and bioassays originated from a stock culture, annually infused with insects collected in the field, reared on a semi-artificial diet [5]. The extraction method was adapted from that developed for the Lepidopteran Pyralidae *Ostrinia nubilalis* [6]. We collected about 21300 eggs from 386 two-day-old mated females individually placed for 24 h in glass tubes (diam. = 1 cm, L = 8 cm). After removing the females, the eggs were washed for 12 h with purified methanol (GC purity > 99.99%) (ca 2 ml/tube). Scales were removed by filtration on millipore filters (type GW, 0.22 µm), and the extract was concentrated under a stream of purified nitrogen. The oviposition assays were performed on artificial oviposition substrates (cardboard) providing a choice between treated areas (32 application circles of 1.1 cm diam., centers 2.1 cm apart) and non-treated areas (between the application circles). The treated area corresponded to 1/3 of the non-treated area (see Gabel and Thiéry [4] for a detailed description). Different dilutions of the extract (expressed in egg equivalents [e.e.]) were offered to groups of 10 mated females which had already