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ORIGINAL ARTICLE

Source, optimal dose concentration and longevity of trail pheromone in two *Monomorium* ants (Formicidae: Hymenoptera)

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Abstract Workers of *Monomorium lepineyi* and *Monomorium bicolor* secrete trail pheromones from their venom glands. The Dufour's gland and the hind gut secretions have no effect on the trail pheromone activity in *M. lepineyi*, while in *M. bicolor* the secretions have an inhibitory effect. The optimal dose of the trail- following in each of the mentioned two species was found to be 1.0 and 0.1 poison gland equivalent/30 cm trail, respectively. Although the concentration of the optimal dose in the two species is different, the longevity of each is nearly the same as the pheromone lasts 120 min.

1. Introduction

Species of the genus *Monomorium* are very diverse in size and habits, ranging from very small generalist scavengers to large, polymorphic seed harvesters. The genus is one of the most diverse ant genera of the family Formicidae, with more than 300 described species (Bolton, 1995; Heterick, 2001).

Diversity of exocrine glands are specialized for the production of chemical signals and are associated with particular cuticular structures which permit the release and dispersion of the secretion (Blum, 1985; Billen and Morgan, 1998). At least 39 different exocrine glands have been described in ants so far (Hölldobler and Wilson, 1990; Billen, 1994). Several of these represent a 'standard exocrine set', while others are thought to have only a limited distribution. In almost all species studied, trail pheromones originate from organs located in the gaster of the ants. The use of multiple trail pheromones by a single ant species means that foraging communication can be more complex than is possible with a single pheromone (Jackson and Ratnieks, 2006). Sources of trail pheromones are the venom gland, Dufour's gland and the hind tibia in subfamily Myrmicinae, pygidial gland in subfamily Ponerinae, Pavan's gland in subfamily Dolichoderinae, postpygidial gland in subfamily Aenictinae, and the hind gut in subfamily Formicinae (Billen and Morgan, 1998).

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The present study aimed to investigate the source, optimal dose concentration and longevity of the trail pheromone in two *Monomorium* ants, *Monomorium lepineyi* and *Monomorium bicolor*.

2. Materials and methods

Monomorium ants comprised of few queens with brood, workers and males were collected from their natural raiding column (moisturized soil in a field) and immediately brought into the laboratory for trail-following experiments. The available species, *M. lepineyi* and *M. bicolor* were collected from a place 3 km north to Minia University, EL-Minia, Egypt.

Each ant species was kept in a plastic bottle containing moisturized soil. The bottle was placed in a plastic bowl with 25 cm internal diameter and 60 cm vertical wall to serve as a foraging area. Ants were fed with tiny droplets of bee's honey placed on a piece of paper and offered fresh dead insects at least once a week. Glass test tube filled with tap water and sealed with cotton piece was placed in the foraging area for ant drinking. To compensate moisture loss of the soil granules, few drops of water were added on alternate day.

The experimental determination of the glandular origin of trail pheromone involved presentation of extracts of the glands to ants. The behavioral response of 30 ants has been quantified through a circular trail test where the distance followed is equivalent to trail activity according to Pasteels and Verhaeghe (1974). Consequently, each of the dissected hind gut, venom gland and Dufour's gland was crushed in 100 µl hexane using a glass tissue grinder and immediately transferred by a Hamilton micro-syringe into the reservoir of a standard-graph pen, then the solution applied uniformly along the circumference of a circle with a diameter of 10 cm drawn on a sheet of white photocopying paper. The circumference of the circle was divided into arcs of 1 cm each. Hexane was used in the same manner as a control. Then the sheet of paper containing the extract was introduced into the foraging area and the mean number of arcs run by ants was calculated and used as a measurement of activity.

To determine the optimal dose of the trail pheromone of each species, different concentrations of the source gland were prepared (0.01, 0.1, 1, 5, 10, 20 and 40 glands equivalent per 30 cm trail). Each concentration in 100 µl hexane was allowed for workers in the foraging area for 20 min and the mean number of arcs run by individuals was calculated as in the previous test.

For determining the longevity of the trail pheromone, the optimum dose obtained from the previous experiment of each ant species was applied for workers after different time period from its initial application (0, 15, 30, 45, 60, 75, 90, 105, 120 min) and the mean number of arcs run by individuals was calculated.

Analysis of variance using one-way ANOVA (DSL) test was used to analyse the obtained data.

3. Results

3.1. The source of the trail pheromone

Data presented in Fig. 1 indicate that the poison gland is the source of the trail pheromone in *M. lepineyi* and *M. bicolor* where the mean number of arcs run by ant individuals was 23.37 ± 1.77 and 40.77 ± 2.98, respectively, whereas the Dufour gland or the hind gut of the two species induced a little activity and nearly similar to that evoked by a control blank bioassay.

In a trial to find out whether the Dufour's gland or the hind gut content has a synergetic effect for the venom secretion, a mixture of both the Dufour's and the poison gland or a mixture of both the hind gut and the poison gland was tested against workers. Results showed that the Dufour's gland and the hind gut secretions had no significant difference ($P > 0.05$ one-way ANOVA) in the trail pheromone activity in *M. lepineyi* while in *M. bicolor* the activity was significantly ($P < 0.01$ one-way ANOVA) decreased being less than that of poison gland extract alone (Fig. 1).

3.2. Effect of concentration on trail-following activity

Data presented in Fig. 2 indicate that workers of *M. lepineyi* and *M. bicolor* utilize a concentration of 1.0 and 0.1 gaster extract/30 cm trail, respectively, to evoke their highest activity. The activity was significantly decreased at a concentration below and above 1.0 and 0.1 gaster ($P < 0.01$ one-way ANOVA), respectively.

3.3. The longevity of the pheromone

To determine how long the pheromone of *M. lepineyi* and *M. bicolor* can persist, the optimum dose obtained in the previous experiment was allowed for workers after different period of time after its initial application. It was found that the activity of workers toward the optimum dose of *M. lepineyi* (1.0 gaster) and *M. bicolor* (0.1 gaster) decreased gradually by the time until reached its lowest level after 2 h (Fig. 3).

4. Discussion

In this study the poison gland has been identified as a source of the trail pheromone in *M. lepineyi* and *M. bicolor* as in other *Monomorium* species, *Monomorium floricola*, *Monomorium minimum* Buckley and *Monomorium pharaonis L.* (Blum, 1966). Ritter et al. (1973) reported that *M. pharaonis* has been found to produce two trail substances from the poison gland; which were identified to be Monomorine I and Monomorine III. However, the true pheromone was identified as faranal secreted from the Dufour's gland and shown to be much more active than the monomorines (Ritter et al., 1977). When a mixture of both the poison gland and the Dufour's gland or hind gut was tested as a trail for *M. lepineyi*, no significant difference in activity of workers was obtained as in some *Pheidole* spp. (Ali and Mashaly, 1997a) whereas in *M. bicolor* workers, the activity was significantly decreased. In this case is probably due to the presence of certain substance (s) in the Dufour's gland or the hind gut which acts as an inhibitor as in *Tetramorium simillimum* (Ali and Mashaly, 1997b).

Ants are able to optimize their foraging behaviour by selecting the most rewarding source, due merely to a modulation of the quantity of pheromone laid on a trail (Hangartner, 1969; Hölldobler and Wilson, 1990; Traniello and Robson, 1995). Data in this study indicate that workers of *M. lepineyi* and *M. bicolor* utilize a concentration of 1.0 and 0.1 gaster

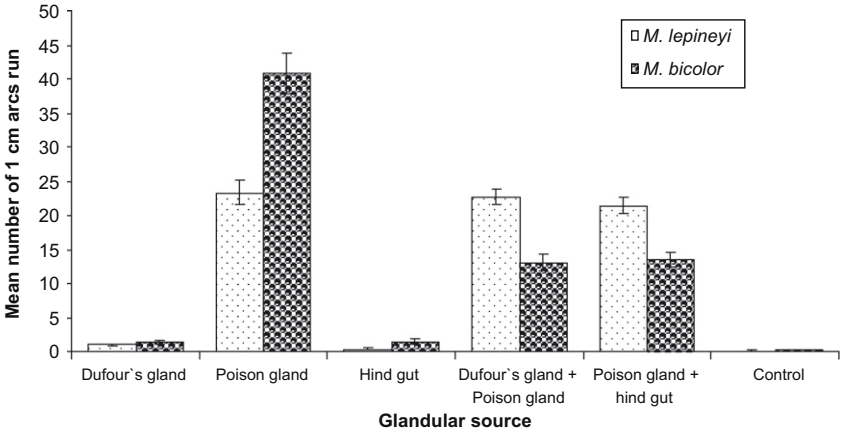

Figure 1 Trail-following activity evoked by different glandular sources of *M. lepineyi* and *M. bicolor* workers using the circular trail-following test.

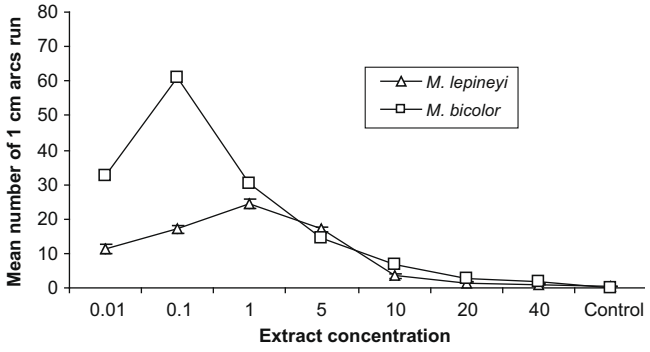

Figure 2 Response of *M. lepineyi* and *M. bicolor* workers to different concentrations of gaster extract.

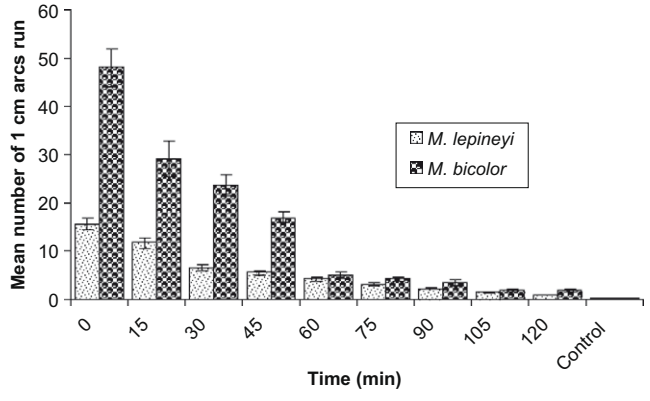

Figure 3 Longevity of the trail pheromone of the ant *M. lepineyi* and *M. bicolor* (1 and 0.1 gaster equivalent/30 cm trail, respectively).

equivalent per 30 cm, respectively, to evoke their highest activity. Van Vorhis Key et al. (1981) demonstrated that the optimal activity was found in response to a trail containing of 0.1–1.0 ant equivalent per 50 cm for *Iridomyrmex humilis* Mayer. In *Tetramorium impurum* Forester, the highest activity was reported at a concentration of 0.1 poison gland equivalent/30 cm trail (Morgan et al., 1990). The optimal dose of trail-following activity of *Pheidole teneriffana* which induced the highest activity was only one gaster equivalent/30 cm (Ali, 1996). *Pheidole jordanica, Pheidole sinatica* and *Pheidole sp.*, utilize a wide range of pheromone concentration as the highest activity was fallen in between 1 and 5 gasters equivalent/30 cm trail (Ali and Mashaly, 1997a). Also the same range was observed with *Crematogaster inermis* Mayr, which utilizes the tibial gland as the source of the pheromone, while *Leptothorax angulatus* Mayr and *T. simillimum* Smith, utilize moderately higher concentration as the optimal dose was found to be five gasters for the same distance of trail (30 cm) (Ali and Mashaly, 1997b).

A parameter critical to the function of a trail is its persistence, and trail longevity must be matched to the foraging ecology of a particular species. Indeed, in ants trail longevity varies from minutes in *Aphaenogaster albisetosus* (Hölldobler et al., 1995) to several weeks in some Eciton species (Torgerson and Akre, 1970). Short-lived trails can rapidly modulate recruitment to ephemeral food sources, whereas long-lived trails will be more suited to persistent, or recurrent, food sources. At the optimal dose of the trail pheromone of *M. lepineyi* and *M. bicolor*, the activity of workers decreased to its lowest level after 2 h (Fig. 3).

The trail of *M. pharaonis* Linnaeus remained active for about a day, while those of *M. minimium* Buckle, were hardly active after 2.5 h (Blum, 1966). Ali (1996) found that, in *Ph. teneriffana* Forel, the optimal dose of the trail completely disappeared after 1 h. In *Ph. jordanica*, *Ph. sinatica* and *Ph. sp.*, the activity of workers decreased to its lowest level after 75–90 min from its initial application at a concentration of two gasters, the activity recorded just a trace after 105–120 min at a concentration of five gasters (Ali and Mashaly, 1997a).

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