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Monomorium ant's trail pheromones: Glandular source, optimal concentration, longevity and specificity

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ABSTRACT

Many ants use pheromone trails to organize collective foraging. Trail pheromones are produced from different glandular sources and they may be specific to a single species or shared by a number of species. I investigated the source of trail pheromones in three *Monomorium* ant species: *Monomorium niloticum* (Emery), *M. najrane* (Collingwood & Agosti) and *M. mayri* (Forel). I also examined the optimal concentration, longevity and specificity of the pheromones. *M. niloticum* and *M. najrane* secrete trail pheromone from their venom glands, whereas *M. mayri* secrete trail pheromone from its Dufour's gland. The optimum concentration was 1.0 and 0.1 gaster equivalent (GE)/30 cm trail in *M. niloticum*, 1.0 GE in *M. najrane* and 5.0 GE in *M. mayri*. Longevity of the optimal concentration was about one day for all species. There is no species specificity among the three species of *Monomorium* in their trail pheromone.

Introduction

Pheromone trails illustrate how animal behavior can be modulated by pheromones. Trails deposited on a substrate are used for orientation and recruitment by many central place foragers, including ants, termites, social caterpillars, slugs and snails (Wyatt, 2003). Trail pheromone mixtures usually contain multiple pheromones that are derived from different glandular sources (Hölldobler and Wilson, 1990; Wyatt, 2003). Trail pheromones of ants can be employed with great efficiency for multiple purposes. For example, the odor trail pheromone of *Solenopsis invicta* Buren functions as an alarm pheromone, as well as an effective communicator of food location and an organizer of mass foraging (Wilson, 1962). Trail pheromones may help ants chose the more rewarding branch at a trail bifurcation, and may also allow more rapid changes in directing foragers to particular locations (Jackson and Ratnieks, 2006).

Trail pheromones vary in volatility and stability. They elicit different behavioral responses depending on concentration, context and relatives proportions in synergistic mixtures (Hölldobler and Wilson, 1990; Vander Meer et al., 1990). *Monomorium* is one of the most diverse ant genera, with more than 300 described species (Bolton, 1995; Heterick, 2001). Over 50 species of *Monomorium* have been recognized in the Arabian Peninsula (Collingwood and Agosti, 1996).

An improved understanding of communication via trail pheromones requires accurate investigation of their glandular source, optimal concentration, longevity and specificity in three *Monomorium* ants, *M. niloticum*, *M. najrane* and *M. mayri*.

Materials and methods

Insects

The three *Monomorium* colonies (containing three to five queen ants with brood, worker ants and males) were collected from their natural raiding column. They were brought immediately into the laboratory to dissect pheromone glands and to set up colony groups for laboratory tests. *M. niloticum* worker ants were collected near Al Ghat Governorate, North Riyadh, KSA. *M. najrane* and *M. mayri* were collected near Al Harek Governorate South Riyadh, KSA.

Colonies were housed in a climate room (temperature 28 ± 1 °C, relative humidity approximately 30%, a photoperiod of 12:12 (L/D) h). Each colony was housed in a plastic nest bottle within a large plastic box ($45 \times 30 \times 18$ cm) that was used as a foraging area. Colonies were given water, sugar syrup or honey, and segments of fresh mealworms three times per week. Ten days before the start of the experiment and during the experiment, they were not given

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sugar syrup or honey to ensure that they would readily form foraging trails to the syrup feeder. To prevent drying, a few drops of water were added to the soil as needed.

Source of trail pheromone

Following Pasteels and Verhaeghe (1974), venom glands and Dufour's glands were ground in a small glass tissue grinder in 100 µl hexane. The solutions were applied to the circumference of the circle (diameter 10 cm) which was drawn on a sheet of A4 paper with a 0.8 mm Standardgraph pen (Blundel Harling, Dorset). After application, the solutions were left to air-dry for 2 min for the solvent to evaporate. The paper was then presented to the ants.

The centimeters of arc the ants walked (counted before losing the trail) was measured. If an individual walked 1 cm or less, its walking distance was counted as a zero. After 30 individuals had explored the trail, the paper was removed and the activity was calculated as the mean length of arcs covered by all the 30 foragers. Before and after each test, the pen was cleaned thoroughly with hexane. The last washing was used in a blank trail test to ensure there is no residual activity from the last test. A pencil marking on the paper (with no chemical trail laid) was also presented to the ants, as well as different shapes and sizes of paper, to ensure these do not affect the results. Each test was replicated three times and the mean results were compared to hexane alone (control). This method has the advantage of providing a quantitative answer for the activity of each extract (Morgan, 2009).

Synergetic effect of abdominal glands

In an experiment to determine whether the glands have a synergetic effect, a mixture of both the Dufour's gland and the venom gland in 100 µl hexane was performed. This mixture was tested on workers for each ant species. Also the whole abdomen (gaster) in 100 µl hexane was applied in the same way. Worker ants were allowed in the foraging area for 20 min. The mean length of arcs covered by the 30 individuals was calculated and compared with hexane alone for each extract. Each test was replicated three times.

Optimal concentration of the trail pheromones

The optimal concentration of the trail pheromone of each species was determined using different concentrations of the source gland (0.001, 0.01, 0.1, 1, 5, 10, 20 and 40 glands equivalent per 30 cm trail) in 100 µl hexane. Worker ants were allowed in the foraging area for 20 min. The mean length of arcs run by 30 individuals was calculated for each concentration and compared to hexane alone (control). Each test was replicated three times.

Longevity of the trail pheromones

To determine the longevity of trail pheromones, the optimal concentration obtained from the previous experiment was used for each ant species. Worker ants were allowed in the foraging area for 20 min after different time periods from its initial application (0, 1, 2, 4, 8, 16, 32, 64 h). The mean length of arcs covered by the 30 individuals was calculated and compared with hexane alone for each time period. Each test was replicated three times.

Trail pheromones specificity

To test the specificity of trail pheromones between the three species, the hexane extract of the source gland of each species was tested against the other species using the method described above. The mean length of arcs covered by 30 worker ants was calculated.

Statistical analysis

Statistical analysis was undertaken using MINITAB software (MINITAB, State College, PA, Version 13.1, 2002). Data from experiments were first tested for normality using Anderson Darling test, and for variances homogeneity prior to any further statistical analysis. Data were normally distributed, and variances were homogeneous, thus, One-way ANOVA was used to determine overall effects of treatments followed by individual comparison using Tukey's Pairwise comparison.

Results

Source of the trail pheromone

The venom gland is the source of the trail pheromone of both *M. niloticum* (140.4 ± 14.26 cm mean($\pm$SD) length of arcs) and *M. najrane* (82.57 ± 15.46 arcs). The Dufour's gland of these two species induced activity similar to that of the control (Fig. 1). The Dufour's gland is the source of the trail pheromone in *M. mayri* (42.47 ± 5.43 arcs). The venom gland in *M. mayri* induced little activity compared with the Dufour's gland ($P<0.01$).

Synergetic effect of abdominal glands

There was no difference ($P<0.01$) between the activity induced by the source gland alone and that induced by the mixture of the two glands or for the gaster for any species (Fig. 1).

Optimal dose of the trail pheromones

In *M. niloticum*, the highest activity of worker ants evoked at the range of 0.1–1.0 gaster/30 cm trail. In *M. najrane* and *M. ayri*, the highest activity was evoked at 1.0 and 5.0 gaster/30 cm trail, respectively. The activity decreased significantly at concentrations below and above the optimal concentration ($P<0.01$) (Fig. 2).

Trail pheromone longevity

The activity of trail pheromone in all three species decreased significantly within one day, with a drop to <50% level (Fig. 3).

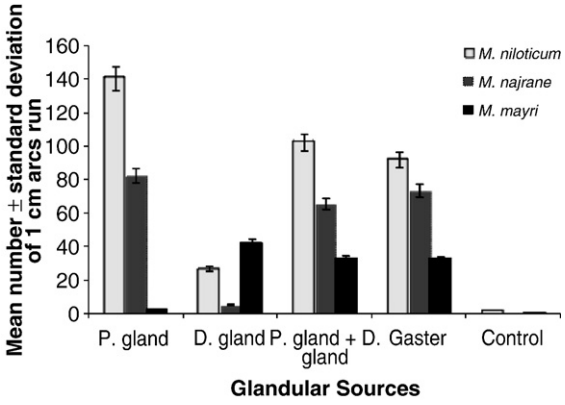

Fig. 1. Trail following activities evoked by different glandular sources of the three species of worker ants using the circular trail-following test. P. = Poison, D. = Dufour.

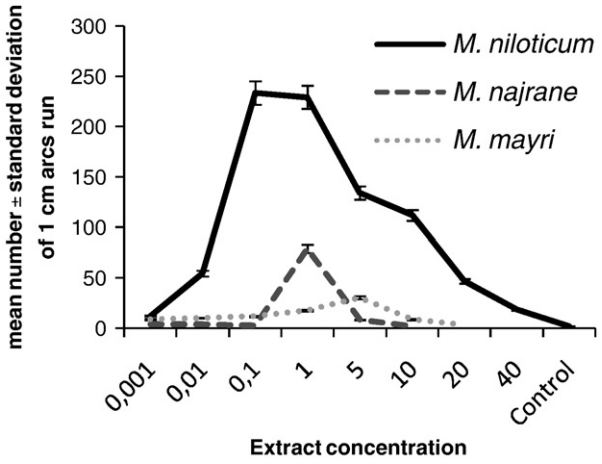

Fig. 2. Response of the three worker ants to different concentrations of gaster extract.

Species specificity

There is no species specificity among the three species (Table 1).

Discussion

In ants, several different anatomical structures are sources of recruitment and trail pheromones. It is assumed that these communication patterns have evolved independently several times in the different ant subfamilies (Hölldobler and Wilson, 1990). Morgan (2009) stated that sources of trail pheromones are the venom gland in Myrmicinae (except for the genera *Monomorium* and *Solenopsis* in which the Dufour's gland is involved). I found that the venom gland is the source of the trail pheromone in *M. niloticum* and *M. najrane*, which is similar to what is seen in *M. floricola* and *M. minimum* Buckley (Blum, 1966), *M. destructor* Jerdon (Ali, 1992), *M. lepineyi* and *M. bicolor* (Mashaly et al., 2009). The venom gland is the source of trail pheromones in many other genera, such as *Atta* (Moser and Blum, 1963; Cross et al., 1979), *Daceton* (Morgan et al., 1992), *Tetramorium* (Attygalle and Morgan, 1983; Cammaerts et al., 1994), *Leptothorax* (Möglich, 1979; Ali and Mashaly, 1997a), and *Pheidole* (Patel, 1990; Ali and Mashaly, 1997b; Jackson and Ratnieks, 2006). I found that *M. mayri* utilize the Dufour's gland as a source for trail pheromones, which is similar to what is seen in *M. pharaonis* (Hölldobler, 1973), *Solenopsis* species (Robert et al., 1989), *Ph. fallax* Mayr (Law et al., 1965), *Ectatomma ruidum* (Bestmann et al., 1995), *Polyergus rufescens* (Visicchio et al., 2001). *Gnamptogenys* species (Gobin et al., 2001; Blatrix et al., 2002).

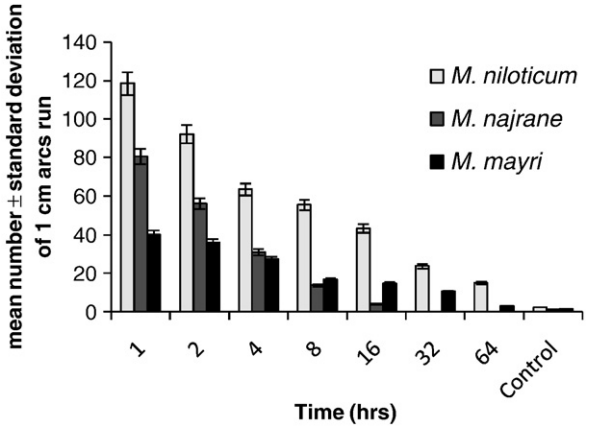

Fig. 3. Longevity of the trail pheromone of the three ant species.

Table 1
Inter specific responses of three *Monomorium* species to artificial trail extracts.

Source species	Test species		
	M. niloticum	*M. najrane*	*M. mayri*
	No. of arcs run (mean ± S.E.)		
M. niloticum	98.24 ± 2.17	77.13 ± 0.27	71.03 ± 1.12
M. najrane	38.21 ± 0.47	39.47 ± 3.18	29.23 ± 1.63
M. mayri	73.16 ± 3.04	64.42 ± 2.49	83.19 ± 2.31

I found that neither the Dufour's gland nor the venom gland had effect on the source gland in the three ant species. The same results were found in case of *M. lepineyi* by Mashaly et al. (2009) and in cases of *Ph. jordanica* and *Ph. sinaitica* by Ali and Mashaly (1997b).

To optimize their foraging behavior, ants select the most rewarding source. This selection is due to a modulation of the quantity of pheromone laid on a trail (Hölldobler and Wilson, 1990). A specific concentration of trail pheromones is important since concentrations that are too high or too low elicit either no response or repellency (Barlin et al., 1976). The optimum concentration of the trail pheromone for *M. niloticum* ranges from 0.1 to 1.0 gland per 30 cm trail as previously found by Mashaly et al. (2009) in both *M. lepineyi* and *M. bicolor*, In *Iridomyrmex humilis* Mayer, the optimal activity was in response to a trail containing of 0.1–1.0 ant (gaster) equivalent per 50 cm. This activity dropped when the concentration was lower or higher than the optimal concentration (Van Vorhis et al., 1981). In *T. impurum* Foerster, the highest activity was reported at 0.1 poison gland equivalent/30 cm trail. The activity decreased at a concentration of 1.0 and 0.01 poison gland/30 cm trail, and totally disappeared at a concentration of 0.001 gland/30 cm trail (Morgan et al., 1990). In *M. mayri*, the highest activity evoked at 5.0 glands, which is similar to what is seen in *L. angulatus* Mayr and *T. simillimum* Smith (Ali and Mashaly, 1997a). *Ph. jordanica*, *Ph. sinatica* and *Ph.* sp. utilize a wide range of pheromone concentrations, and the highest activity occurred between 1 and 5 gaster equivalent/30 cm trail (Ali and Mashaly, 1997b). The same range was also observed in *Crematogaster inermis* Mayr, which utilizes the tibial gland as the source of trail pheromones (Ali and Mashaly, 1997a).

In ants, trail pheromone longevity varies between 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 are be more suited to persistent or recurrent food sources (Fitzgerald and Underwood, 1998). The activity of trail pheromone in all three species decreased to half of the original activity level after a single day. Little activity occurred after 2 days for *M. niloticum* and *M. mayri*. In *M. pharaonis* Linnaeus, the trail remained active for about a day, while those of *M. minimium* Buckle were hardly active after 2.5 h (Blum, 1966). At the optimal concentration of the trail pheromone of *M. lepineyi* and *M. bicolor*, the activity of worker ants decreased to its lowest level after 2 h (Mashaly et al., 2009). Pheromones are released mainly from exocrine glands as liquids that evaporate into the surrounding air and form a cloud of vapor about the signaling animal (Bossert and Wilson, 1963). The chemical nature and distance through which a pheromone may transmit a message is a function of the volatility of the compound, its stability in air, its rate of diffusion, olfactory efficiency of the receiver, and, wind currents.

Cross-attraction among these three species (Table 1) strongly suggests that they use the same chemicals in their trail pheromone. *M. minimum* Buckley worker ants will follow artificial trails prepared with *M. pharanis* Linnaeus venom extracts in addition to their own. However, *M. pharanis* worker ants will not follow artificial *M. minimum* trails. *M. floricola* also does not follow artificial *M. minimum* trails (Blum, 1966). *At. texana*, *At. cephalotes*, and *At.*

sexdens follow each other's artificial trails, and *Acromyrnmx octospinosus* follow artificial trails of the three *Atta* species. *Ac. octospinosus* trail pheromones induced activity in only *At. texana* and *At. cephalotes* (Robinson et al., 1974).

In conclusion, long lasting trail pheromones are secreted from the venom gland in *M. niloticum* and *M. najrane*, and from the Dufour's gland in *M. mayri*. Each pheromone had its own persistence. Also, the concentration of the pheromone had a strong effect on worker activity. Therefore, these results can suggest the use of trail pheromone in order to increase the rate of food uptake which contains ant baits. This in turn, can facilitate ant control process.

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