Ins. Soc. *38*: 17–25 (1991)

1015–1621/91/010017-09 $ 1.50 + 0.20/0
© 1991 Birkhäuser Verlag, Basel

Trail-following responses of *Tapinoma simrothi* (Formicidae: Dolichoderinae) to pygidial gland extracts

T. Simon and A. Hefetz

Department of Zoology, Tel Aviv University, Ramat-Aviv, Israel

Key words: Trail pheromone; *Tapinoma simrothi*; pygidial glands; iridodials.

Summary

The pygidial (anal) gland was found to be the source of trail pheromone in the ant *Tapinoma simrothi*. Bioassays conducted with fractionated pygidial gland secretion indicated that the fraction containing iridodials and iridomyrmecin is responsible for the trail pheromone activity. Thus workers of *T. simrothi* may utilize the same glandular exudate for alarm and trail following. At high emission rates from a point source, the ants responded in alarm, e.g., rushed to the source with open mandibles and raised abdomen. When concentrations were low and drawn as a line, the ants followed the secretion calmly. Trails of *T. simrothi* are long-lived, having a biological half-life of 10 to 19 days. Quantitative studies of the evaporation rates of the iridodials by gas chromatography resulted in a half-life of 11 days, agreeing with the biological data. The implications of the use of the same glandular secretion for alarm and food recruitments are discussed.

Introduction

One of the most remarkable phenomena in the behavior of ants is the use of chemical trails as an orienting agent towards a food source. Extensive research has been conducted in the last 30 years concerning the glandular origins of trail pheromones, their chemical compositions and physical properties (Hölldobler, 1984; Attygalle and Morgan, 1985).

Dolichoderine ants are excellent subjects for the experimental analysis of trail pheromone properties, since they form long, conspicuous columns tightly bound to persistent odor trails (Wilson and Pavan, 1959). The source of the trail pheromone in this subfamily is considered to be an abdominal sternal gland, also called Pavan's gland (Pavan, 1955; Wilson and Pavan, 1959; Couret and Passera, 1979; Cavill et al., 1979, 1980). The gland is composed of a secretory epithelium situated on the 7th sternite, the cells of which empty into a reservoir located more anteriorly (Billen, 1986). It is not equally developed in all species. In *Iridomyrmex humilis* both the glandular epithelium and the reservoir are apparent, while in *Tapinoma nigerrimum* the epithelium is less developed and the reservoir is absent altogether.

While conducting laboratory observations on the recruitment behavior of *Tapinoma simrothi* Pheonicium (Emery, 1925) we noticed that the trail pheromone is apparently very stable. When food was offered to a colony that had been starved for several days, the ants used and overmarked the old track, although it had not been reinforced during the starvation period.

While the chemistry and glandular source of the alarm pheromone of this species had been studied earlier (Hefetz and Lloyd, 1983), nothing was known about either the chemistry or the glandular source of its trail pheromone. This study presents findings on the glandular source, chemical nature and some physical properties of the trail pheromone in *T. simrothi*.

Materials and methods

Colonies of *T. simrothi* were collected in Tel Aviv, Israel and transferred to artificial nests in the laboratory. Each nest was placed on a special foraging table on which the ants were allowed to forage for honey and housefly maggots. The foraging table was covered with sheets of filter paper that could be replaced when the experiments were done.

Glandular exudates were obtained by excising the pygidial glands from chilled ants under cold distilled water and then extracted with pentane. Analyses of the extracts were conducted by gas chromatography using a 50 m BP1 capillary column, programmed from 100–320 °C at 10°/min. Preparative fractionations of pygidial gland extracts were conducted using a packed 10% AT 1000 column that was temperature-programmed from 100–250 °C/min.

Iridodial-enriched extracts (IEE) were prepared by puncturing the dissected pygidial glands near the water surface; the oozing iridodials created a thin film on the water surface that was dissolved in pentane and dried over Na_2SO_4. The purity of IEE was determined by gas chromatography, confirming that the IEE extracts were almost free of the other aldehydes and ketones found in the pygidial gland (Hefetz and Lloyd, 1983).

Artificial trails that were used for the bioassays were created by applying 20 µl of the test solution on one arm of an ellipse (20 cm long) drawn on a filter paper. The other arm was applied with pentane as a solvent control. Ten minutes after application of the trail, while the ants were still foraging towards the food source, the paper was placed on the foraging table, interrupting the original trail and allowing the ants to choose between the two routes, or to create a new trail. A response was considered as positive when the ants followed the route in which the tested material was applied, and a trail was created within 10 min ($n = 10$). The positions of the tested material and the control were reversed after each test. The results were expressed as the relative number of trails followed by the ants, out of the total examined.

Scoring was slightly different when fractions of the pygidial gland secretion were tested in order to obtain higher sensitivity. The number of ants following the route in which the tested material was applied were counted every 10 seconds (during a 5 min period). The cumulative number of ants per min was selected as the basic unit

for comparison. Thus, the results are expressed as the average number of ants following the tested material per min.

The alarm response of the ants was characterized by fast recruitment to the source with aggression (open mandibles and elevated abdomen). Quantification of the alarm response of workers of *T. simrothi* to pygidial gland extracts was done using serial dilutions of glandular exudates (0.01, 0.1, 1, 5, 10 glands/ml). The ants (15 ants per assay) were transferred to a petri dish ($r = 7.5$ cm) and after a "calm down period" of about 30 minutes, a disk (0.25 cm^2) impregnated with 10 µl of the tested extract was placed in the middle of the dish. The responses were quantified by counting the number of alarmed ants contacting the test disk within the first minute after the application of the test solutions.

In order to assess the longevity of a trail, pygidial gland extracts were applied (0.1; 0.2; 0.4 ant equivalents/20 cm) onto absorbing paper and then aged at room temperature ($22° \pm 1$) for up to 40 days. Every 4 or 7 days 10 trails were assayed as before and the results were expressed as the percentage of trails followed by the ants.

$T_{1/2}$ of the iridodials fraction was also assessed chemically. Twenty µl of pygidial gland extracts (10 glands/ml) were applied to filter paper disks (1×2 cm) that were aged at room temperature ($22° \pm 1$) for up to 30 days. Disks were extracted every few days in pentane containing 0.5 µg of C-11 as internal standard and the amount of iridodials was quantified by gas chromatography using a BP1 capillary column. The recovery efficiency for the iridodials was nearly 100%. The half life ($T_{1/2}$) of the trail pheromone was calculated from the transformed data ($Ln(x)$) using a linear regression test.

Results

Trail-following responses of workers of *T. simrothi* to various body parts and gland extracts are presented in Table 1. Head extracts or glandless abdominal extracts were completely devoid of activity even at the high concentration of 0.2 ant equivalents/trail. Whole gasters, on the other hand, were fully active even at the low extract

Table 1. Trail-following response of ant workers to head, gaster and abdominal glands extracts of *T simrothi*

Extract	% trail following* ($N = 10$)	
	0.2 gland equivalents/trail	0.005 gland equivalents/trail
Head	0	–
Gaster	100	100
Glandless gaster	0	–
Pygidial glands	100	90
Sternal gland	100	0
Venom + Dufour's gland	90	0

* Activity is expressed as the % of trails followed out of trails assayed

concentrations of 0.005 ant equivalents/trail. In an effort to locate the glandular origin we further assayed isolated abdominal glands. While at high extract concentrations all the glands induced trail following, at low extract concentrations only the pygidial gland exudates induced biological activity (Tab. 1). The trail-following responses detected in extracts of the sternal glands, or the complex of Dufour's and poison glands, at high concentrations were probably caused by pygidial gland contamination. Gas chromatographic analysis of these extract revealed the presence of about 3% of contaminating iridodials.

Table 2. Trail-following responses of ant to different fractions of pygidial gland extract. Means having no letters in common are significantly different (Duncan's New Multiple Range Test, $P < 0.05$)

Fraction		Means ± SD*	*N*
1	4-heptanone 2-methyl-4-heptanone	5.4±3.3	10 b
2	6-methyl-5-hepten-2-one 4-hydroxy-4-methyl-2-pentanone	1.6±1.3	10b
3		1.9±1.3	10b
4	Iridodials, Iridomyrmecin	13.2±4.2	10a
	Control	1.7±0.9	10b

* Expressed as the number of ants travelling on the trail per min

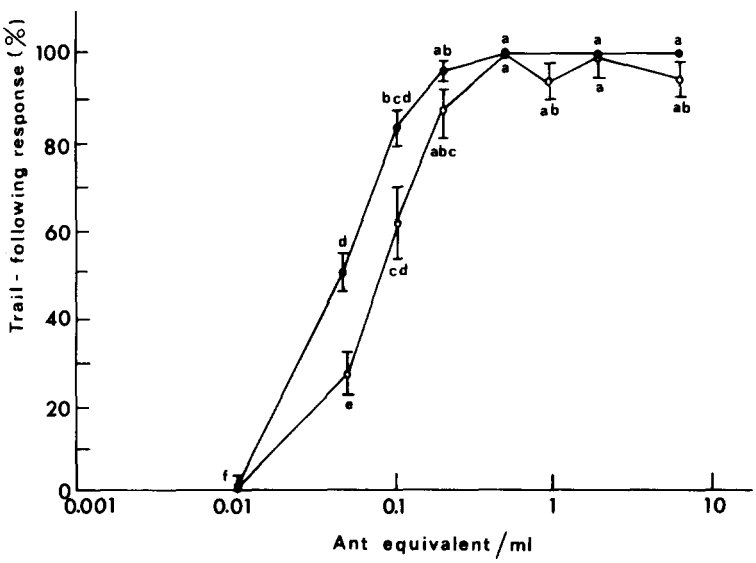

Figure 1. Trail-following response (mean ± s.e.) of *T. simrothi* to pygidial gland extracts (closed circles) and to IEE (open circles) ranging in concentration from 0.01 to 5 ant equivalents/ml, (0.2×10^{-3} to 0.1 ant equivalents/trail) measured as the percentage of marked trails followed by the ants. Means having no letters in common are significantly different according to Duncan's New Multiple Range Test ($P < 0.05$, DOF = 46)

Since the more volatile components of the pygidial gland of *T. simrothi* act as an alarm pheromone (Hefetz and Lloyd, 1983), in order to separate the trail-following substance from the alarm-elicitor compounds the secretion was divided into four fractions (Tab. 2). All fractions were tested against pentane as control and the trail-following activity was calculated as the number of ants travelling on the trail per minute. The results of these assays demonstrated that only fraction 4, containing the iridodials and iridomyrmecin, had an activity that was significantly higher than the control.

Table 3. Recruitment of alarmed *T. simrothi* workers to different concentrations of pygidial gland exudates. The results are expressed as the number of ants contacting disk within the initial 60 seconds (mean ± se). Means having no letters in common are significantly different (Duncan's New Multiple Range Test, $P < 0.05$)

Pygidial gland equivalents/test	Response	*N*
10^{-1}	32.6±6.9	10a
5×10^{-2}	26.7±8.2	10a
10^{-2}	22.7±9.9	10a
10^{-3}	7.4±7.5	10b
10^{-4}	5.6±4.5	10b
Control (pentan)	8.4±8.5	10b

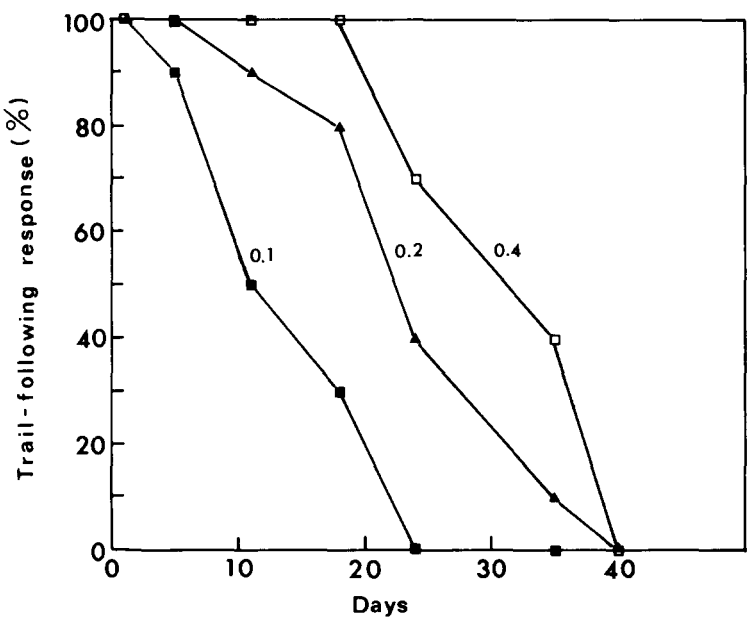

Figure 2. Activity loss of aged pygidial gland extracts of 0.1, 0.2 and 0.4 ant equivalents/trail (5, 10 and 20 ant equivalents/ml). Activity was assessed for trails which had been aged at room temperature (22 °C ± 1) for up to 40 days. Every 4 or 7 days, 10 trails were assayed and the results were expressed as the percentage of trails followed by the ants.

Table 4. The regression equations of evaporation rates and the calculated $T_{1/2}$ of iridodials fraction from pygidial glands of *T. simrothi*

Ln (*x*) Transformation	r^2	$P<$	DOF	$T_{1/2}$ (days)
1. $Y = 943.3 - 0.0025X$	0.906	0.001	10	11.5
2. $Y = 963.5 - 0.0028X$	0.894	0.001	17	10.3

A dose-response study using pygidial gland or IEE extracts as trail-following elicitors demonstrated that the threshold concentration for induction of trail following was around 0.05 ant equivalents/ml (10^{-3} gland equivalents/trail) (Fig. 1). Responses then increased linearly with dosage and leveled out at a trail concentration of 0.2 ant equivalents/ml (4×10^{-3} gland equivalents/trail).

Since the pygidial gland exudate functions also as an alarm pheromone (Hefetz and Lloyd, 1983), it was important to assess also the threshold concentration for alarm eliciting function of the secretion. Using serial dilutions of pygidial gland secretion in an arena test, it was found that the threshold response of the ants is at an approximate concentration of 10^{-2} gland equivalents (Tab. 3), at least 10 times higher than that for the trail following response.

The longevity of applied trails constituting pygidial gland extracts was tested at 3 concentrations: 0.4 glands/trail (20 glands/ml), 0.2 glands/trail (10 glands/ml), and 0.1 glands/trail (5 glands/ml) (Fig. 2). The half-life of the trail pheromone was found to be between 10 and 19 days. Evaporation rates of the iridodial components of the pygidial gland secretion were also evaluated chemically, resulting in a half-life of 11 days (Tab. 4).

Discussion

Dolichoderine ants are notable in constructing conspicuous trails to food sources. In all species investigated to date, the Pavan's gland was found to be the trail-pheromone source (Attygalle and Morgan, 1985). The only identified compound acting as a trail pheromone from this subfamily is (*Z*)-9-hexadecenal which was purified from the Pavan's gland of *Iridomyrmex humilis* (Cavill et al. 1979, 1980). Although synthetic (*Z*)-9-hexadecenal was shown to elicit prolonged and intense trail following behavior (Van Vorhis Key and Baker, 1982), the authentic pheromone was considered to be multicomponent, since gaster extract trails containing 100 times less (*Z*)-9-hexadecenal were comparable in activity to the synthetic trails (Van Vorhis Key and Baker, 1982; Attygalle and Morgan, 1985).

According to Fanfani and Dazzini Valcurone (1984) and Billen (1986), the Pavan's gland is a complex of a secretory epithelium located on the 7th abdominal sternite and a reservoir that opens between the 6th and 7th sternites. Workers of *T. simrothi* lack the reservoir part and their glandular epithelium thickness is between 15–17 μm (unpublished data). A similar condition was found in *Liometopum microcephalum* and in *Tapinoma nigerrimum* (Miradoli Zatti and Pavan, 1957; Billen, 1986).

Our findings with *T. simrothi* demonstrate that the trail pheromone in this species originates from the pygidial glands. The residual activity exhibited by extracts of the 7th sternite (the location of Pavan's gland), in *T. simrothi*, was apparently due to pygidial gland contaminants. The fact that the ants were induced to follow the route applied with pygidial gland extracts along 20 cm, suggests that the secretion does not function merely in recruitment, but has also an orienting component to it. Optimal trail following by workers of *T. simrothi* was obtained at extract concentrations that were equivalent to 5×10^{-4} glands/cm. This is at least one order of magnitude lower than was found for *Iridomyrmex humilis*, in which 0.1–1.0 ant equivalents/50 cm trail elicited optimal responses.

The pygidial glands of dolichoderine ants are known as the source of terpenoids that constitute the alarm defense system of these ants (Blum and Hermann, 1978; Hefetz and Lloyd, 1983; Attygalle and Morgan, 1984; Tomalski et al., 1987). In *T. simrothi* the more volatile constituents of the gland were shown to be the alarming substances. Iridomyrmecin was implied to have insecticidal properties, but no function was assigned for the iridodials (Pavan, 1959). Iridodials were also reported to be the consituents of Pavan's gland in *T. erraticum* in addition to their presence in the pygidial glands. In view of the behavioral response of *T. simrothi* to artificially applied trails, we suggest that the iridodial-containing gas chromatographic fraction function as a trail pheromone of this species. Using the same glandular exudate both for alarm and trail following in *T. simrothi* is possible since the threshold concentrations for alarm behavior was about 10 times higher than the threshold for trail-following behavior.

The fact that *T. simrothi* workers use the same glandular exudate as alarm pheromone and trail pheromone is not entirely surprising. The information encoded within a trail pheromone includes at least two components: recruitment and orientation. These may be encoded within two different glandular exudates as in *Myrmica rubra* and *Leptogenys chinensis* (Cammaerts, 1984; Maschwitz and Schönegge, 1977), or within a single gland secretion as in *Solenopsis invicta* (Vander Meer et al., 1988).

We suggest that the system operating for *T. simrothi* is similar to that in *Solenopsis invicta* in that a single secretion serves both to recruit workers and to orient them to a food source. Workers that returned from the food source released small quantities of the pygidial gland secretion while dragging their abdomen on the substrate. Workers that were alarmed, on the other hand, released large quantities of the secretion from a point source while raising their abdomen. These unusually high concentrations evoke in ants the dramatic alarm behavior of both recruitment and elevated aggression. Comparable results were obtained for *Atta texana* in which the alarm pheromone at low doses acts also to attract and recruit workers to a point source (Blum et al., 1968; Moser et al., 1968).

Having a half-life of 11 days makes the iridodials an especially good orienting agent. *Tapinoma simrothi* utilizes in nature a relatively stable food source: aphids' honey-dew. Since the iridodials persist on the trail overnight, the relocation of the food source on the next foraging day by the scout ants is facilitated. This hypothesis may also explain the results obtained for *I. humilis*. Trail following was stopped after 8 hrs, while the longevity of 1000 ng/50 cm of synthetic (*Z*)-9-hexadecenal was 2 hrs. It was possible that this aldehyde constitutes only the recruiting component of the

trail pheromone, while the orienting factor remains elusive. Likewise it is also possible that the sternal gland secretion in *T. simrothi* act as a short-distance recruiter while the iridodials fraction act as an orientation factor. Until further studies are performed, this point remains speculative.

The durability of a trail is probably also related to the foraging strategy of each species (Bradshaw and Howse, 1984). For example, the trail laid by *Solenopsis invicta* (= *saevissima*), a mostly predator species, is short-lived (Wilson, 1962), while that of *T. simrothi* is long-lived. Short duration trails are adaptive and can be predicted for any opportunistic species, while in species with more stable food sources, trails should predictably be long lasting. Long-lived trails not only enable the ants to relocate their food source on the next foraging day but also allow the scout ants to expand their exploratory trips.

References

Attygalle, A. B. and E. D. Morgan, 1985. Ant trail pheromones. *Adv. Insect Physiol. 18*:1–30.

Billen, J., 1986. Morphology and ultrastructure of the abdominal glands in Dolichoderine ants (Hymenoptera, Formicidae). *Ins. Soc. 33*:278–295.

Blum, M. S. and H. R. Hermann, 1978. Venoms and venom apparatuses of the Formicidae: Dolichoderinae and Aneuretinae. In: *Arthropod Venoms* (S. Bettini, Ed), Berlin: Springer-Verlag, pp. 871–894.

Blum, M. S., F. Padovani and E. Amante, 1968. Alkanones and terpenes in the mandibular glands of *Atta* species (Hymenoptera: Formicidae). *Comp. Biochem. Physiol. 26*:291–299.

Bradshaw, J. W. S. and P. E. Howse, 1984. Sociochemicals of Ants. In: *Chemical ecology of insects* (W. J. Bell and R. T. Cardé, Eds), London, Chapman and Hall, pp. 429–473.

Cammaerts, M. C., 1984. Probable functions for a previously unrecognized pheromone in ants of the genus *Myrmica*. *Behav. Processes 9*:135–145.

Cavill, G. W. K., P. L. Robertson and N. W. Davies, 1979. An Argentine ant aggregation factor. *Experientia 35*:989–990.

Cavill, G. W. K., N. W. Davies and F. S. McDonald, 1980. Characterization of aggregation factors and associated compounds from the Argentine ant *Iridomyrmex humilis*. *J. Chem. Ecol. 6*:371–384.

Couret, H. and L. Passera, 1979. Marquage des pistes alimentaires chez trois espèces de Dolichoderinae (Hym. Formicidae): *Tapinoma erraticum* (Latr.) *Tapinoma nigerrimum* (Nyl.) et *Iridomyrmex humilis* (Mayr.). *Ann. Sci. Nat. Zool. 1*:39–48.

Emery, C., 1925. Révision des espèces paléarctiques du genre *Tapinoma*. *Rev. Suisse Zool. 32*:45–64.

Fanfani, A. and M. Dazzini Valcurone, 1984. Nuovi Dati Relativi alla "Glandola di Pavan" in *Iridomyrmex humilis* Mayr. (Formicidae Dolichoderinae). *Pubbl. Ist. Entom. Univ. Pavia, 28*:1–9.

Hefetz, A. and H. A. Lloyd, 1983. Identification of new components from anal glands of *Tapinoma simrothi* Pheonicium. *J. Chem. Ecol. 9*:607–613.

Hölldobler, B., 1984. Evolution of insect communication. In: *Insect Communication* (T. Lewis, Ed), London, Academic Press, pp. 349–378.

Maschwitz, U. and P. Schönegge, 1977. Recruitment gland of *Leptogenys chinensis*. *Naturwissenschaften 64*:589–590.

Miradoli Zatti, M. A. and M. Pavan, 1957. Studi sui Formicidae. III. Nuovi Reperti dell'organo ventrale nei Dolichoderinae. *Boll. Soc. Ent. Ital. 87*:84–87.

Moser, J. C., R. C. Brownlee and R. Silverstein, 1968. Alarm pheromones of the ant *Atta texana*. *J. Insect Physiol. 14*:529–535.

Pavan, M., 1955. Studi sui Formicidae. I. Contributo alla conoscenza degli organi gastrali dei Dolichoderinae. *Natura (Milano) 46*:135–145.

Pavan, M., 1959. Biochemical aspects of insect poisons. *Trans. Fourt Int. Congr. Biochem. Vienna, 1958 12*:15–36.

Tomalski, M. D., M. S. Blum, T. H. Jones, H. M. Fales, D. F. Howard and L. Passera, 1987. Chemistry and functions of exocrine secretions of the ants *Tapinoma melanocephalum* and *T. erraticum*. *J. Chem. Ecol. 13*:253–263.

Van Vorhis Key, S. E. and T. C. Baker, 1982. Trail-following responses of the Argentine ant, *Iridomyrmex humilis* (Mayr), to a synthetic trail pheromone component and analogs. *J. Chem. Ecol. 8*:3–14.

Vander Meer, R. K., F. Alvarez and C. S. Lofgren, 1988. Isolation of the trail recruitment pheromone of *Solenopsis invicta*. *J. Chem. Ecol. 14*:825–838.

Wilson, E. O., 1962. Chemical communication among workers of the fire ant *Solenopsis saevissima* (Fr. Smith). 1. The organization of mass foraging. 2. An information analysis of the odour trail. 3. The experimental induction of social responses. *Anim. Behav. 10*:134–164.

Wilson, E. O. and M. Pavan, 1959. Glandular sources and specificity of some chemical releasers of social behavior in Dolichoderine ants. *Psyche 66*:70–76.

Received 28 July 1989;
accepted 5 January 1990.