7 Bowden, S., Coll, J. C., de Silva, E. D., de Costa, M. S. L., Djura, P. J., Mahendra, M., and Tapiolas, D. M., Aust. J. Chem. *36* (1983) 371.
8 **1b**: oil. IR[ν_{max} cm^{-1}($CHCl_3$)] 1740, 1380, 1250, 1160. ^1H NMR [δ($CDCl_3$)] 1.56 (3H,s), 1.60 (3H, s), 1.65 (3H, s), 1.68 (3H, d, J = 1.5 Hz), 1.95-2.15 (8H, m), 2.03 (3H, s), 2.08 (6H, s), 2.10 (3H, s), 2.14 (3H, s), 3.51 (1H, dd, J = 2.5, 12.7 Hz), 3.65 (1H, dd, J = 1.9, 13.2 Hz), 3.81 (1H, d, J = 12.7 Hz), 4.02 (1H, dd, J = 7.3 ,12 Hz), 4.04 (1H, m), 4.13 (1H, d, J = 12 Hz), 4.14 (1H, dd, 7, 12 Hz), 5.05-5.4 (10H, m). Elementary analysis Calcd. for $C_{35}H_{52}O_{14}$ C, 60. 34; H, 7.47. Found C, 60.52; H, 7.40.
9 **1c** was prepared on treatment of **1a** with bis(trimethylsilyl)trifluoroacetamide (BSTFA) in pyridine.
10 Mizutani, K., Hayashi, A., Kasai, R., Tanaka, O., Yoshida, N., and Nakajima, T., Carbohydr. Res. *126* (1984) 177.
11 The hydrolysate followed by the reaction with BSTFA was analyzed by gas chromatograhy [2% OV-17 at gradient temperature (100–230 °C)].
12 **1d**: oil. $[\alpha]_D$ −204.2° (c = 1.78, $CHCl_3$). IR [ν_{max} cm^{-1} ($CHCl_3$)] 3560, 3550, 1640, 1500, 1370, 1210, 1140, 1060, 1015. EIMS (m/z) 259, 235, 217, 175, 157, 91.
13 Sivakumaran, T., and Jones, J. K. N., Can. J. Chem. *45* (1967) 2493.
14 Kuhn, M., and von Wartburg, A., Helv. chim. Acta *51* (1968) 1631.

0014-4754/92/070688-03$1.50 + 0.20/0

Trail-following responses of *Leptogenys diminuta* to stereoisomers of 4-methyl-3-heptanol [1]

S. Steghaus-Kovâc[a], U. Maschwitz[a], A. B. Attygalle[b,*], R. T. S. Frighetto[b,**], N. Frighetto[b,***], O. Vostrowsky[b] and H. J. Bestmann[b]

[a] *Zoologisches Institut, Fachbereich Biologie der Universität, Siesmayerstrasse 70, D-6000 Frankfurt (Federal Republic of Germany), and* [b] *Institut für Organische Chemie der Universität, Henkestrasse 42, D-8520 Erlangen (Federal Republic of Germany)*
Received 20 March 1991; accepted 11 February 1992

Abstract. Behavioral tests carried out with the four stereoisomers of 4-methyl-3-heptanol revealed that *Leptogenys diminuta* ants respond specifically only to the (3*R*, 4*S*)-isomer.
Key words. Trail pheromone; 4-methyl-3-heptanol; *Leptogenys diminuta*; Formicidae; Ponerinae; ant; chirality.

Leptogenys diminuta Smith, a common ponerine ant found in southeastern Asia, lays orientation trails by depositing the contents of the poison gland secretion. Recently we identified the trail pheromone of *L. diminuta* as (3*R*, 4*S*)-4-methyl-3-heptanol [2]. Precise preception of pheromonal signals plays a crucial role in the life of many insects. Although many models have been proposed, the olfactory perception of pheromonal messages at the molecular level is still poorly understood. Studies based on structure-activity relationships assist us to understand how odorants interact with the receptors. As very pure synthetic stereoisomers of 4-methyl-3-heptanol became available [3], we were able to conduct stereochemistry-pheromone activity studies on the workers of *L. diminuta* in order to verify whether the non-natural isomers are neutral or show some biological acitivity.

Materials and methods

Chemicals. Samples of (3*RS*, 4*R*)-4-methyl-3-heptanol and (3*RS* ,4*S*)-4-methyl-3-heptanol were synthesized as diastereomeric mixtures by Frighetto in our laboratory [4]. Four stereoisomers of 4-methyl-3-heptanol were a gift of Prof. Matteson (Washington State University). Purity of the chemicals and concentrations of the solutions were determined by capillary gas chromatography.

Ants. Several colonies of *L. diminuta* were collected from sites near Ulu Gombak Experimental Station in Malaysia. The colonies were transferred to Frankfurt and maintained on a diet of live arthropods (nymphs of *Blaberus discoidalis* (Blattoidea) and *Acheta domestica* (Saltatoria), and larvae of *Tenebrio molitor* (Coleoptera)).

Quantification. Individual poison glands excised from worker ants were analyzed by capillary gas chromatography as described previously [2]. A solution of authentic 4-methyl-3-heptanol was used as an external standard for quantification.

Trail-following tests. The trails were drawn with a lead pencil on a white piece of cardboard and the test chemicals were applied, as ethanol solutions, by disposable glass capillary tubes (10 µl, Brand). A standard trail test consisting of two intercrossing S-shaped lines (each 20 cm) was used when two substances were being tested for relative activity. One of the lines was streaked with one test chemical and the other with another test chemical or solvent as control. The test was repeated 15 times and the choice response of the first worker that followed the trail was noted. Samples prepared by Frighetto [4] containing (3*RS*, 4*R*)-4-methyl-3-heptanol and (3*RS*, 4*S*)-4-methyl-3-heptanol were tested in this way at 50 pg/cm level.

In order to determine the trail-following threshold of *L. diminuta*, different amounts of (3*RS*, 4*S*)-4-methyl-3-heptanol were applied to a trail (100–0.01 pg/cm) and tested against a control trail streaked with solvent ethanol. The response of each ant that followed the 20-cm trail to the end was considered positive; incomplete or

no trail following was considered negative. For each bioassay, the response of 10 worker ants was noted and the test was repeated ten times for each concentration. Similarly, the activity of a range of concentrations of (3 *RS*, 4 *S*)-4-methyl-3-heptanol was tested against artificial trails made of a poison gland extract. A standard solution of 10 poison glands was made in ethanol (70%, 1 ml) and for each test 10 µl was applied to one arm of the intercrossing trail (10 µl/20 cm; 0.1 GE). The other arm was treated with different amounts of (3 *RS*, 4 *S*)-4-methyl-3-heptanol (0.05–10 ng) as ethanol solutions.

When synthetic samples of all four stereoisomers of 4-methyl-3-heptanol became available (Matteson samples), the standard trail test was modified as follows in order to test more than two chemicals at a time. The modified bioassay comprised four common regions (only 3 are shown in fig. 3) and three two-arc choice regions. A solution containing a test isomer was applied on common regions and one arc (10 cm) of each choice region. The other arc of each choice region (10 cm) was treated with a solution of a different stereoisomer (fig. 3).

Preliminary tests were conducted at 0.5 ng/cm level. A more rigorous series of evaluations were done at a concentration of 5 ng/cm. In these experiments, each isomer solution (175 µl, 1 ng/µl, total trail length 35 cm) was tested against those of the other three isomers (50 µl, 1 ng/µl; trail length for each arc = 10 cm). To avoid directional effects, the choice of left or right arc for a test chemical was varied in such a way that all the possible combinations of left and right directions were realized. The ants were sucked into an aspirator and allowed to come out through a plastic tube (1 cm ID). Each ant was placed at the beginning of the trail and its trail-following behavior was observed. Only the ants which ran to the end of the trail, selecting clearly only one branch at each point of dichotomy, were taken into consideration for evaluations. The chi-square test was used for statistical evaluations.

Similar bioassays as described above were conducted with the (3 *R*, 4 *S*) isomer at a trail concentration level of 0.67 pg/cm against 100, 250, 500, and 1000 times more concentrated trails of (3 *S*, 4 *R*), (3 *S*, 4 *S*), and (3 *R*, 4 *R*) isomers.

Results and discussion

4-Methyl-3-heptanol contains two chiral centers and may therefore exist as four stereoisomers. Only one of these stereoisomers, (3 *R*, 4 *S*), is found in the poison gland of *L. diminuta* and employed as the trail pheromone[2].

The average total amount of 4-methyl-3-heptanol in the poison gland of individual workers ants was found to be about 10–20 ng per ant. This value which was quantified from the GC peak areas showed a significant variation from ant to ant.

Preliminary structure-activity tests were conducted with diastereomeric mixtures of (3 *RS*, 4 *R*)-4-methyl-3-heptanol and (3 *RS*, 4 *S*)-4-methyl-3-heptanol[3] (Frighetto samples; purity shown in table 1). In this dual-choice bioassay, the ants were able to differentiate clearly between the two trails at 50 pg/cm level, and nearly all the ants selected the branch streaked with (3 *RS*, 4 *S*)-4-methyl-3-heptanol ($n = 15$, $p < 0.001$). The results evidently showed the significance of the *S* configuration at C-4 for trail-following activity.

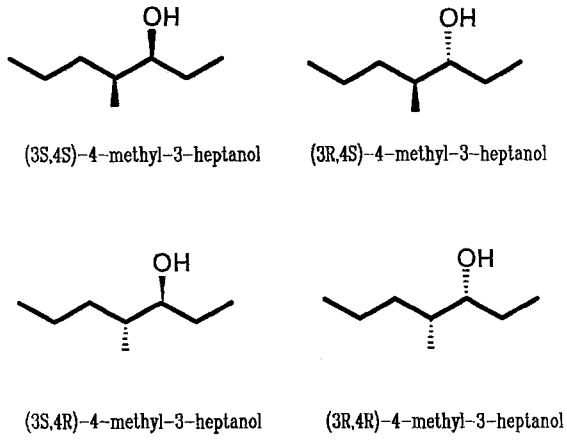

Table 1. Analytical data of (3 *RS*, 4 *R*)-4-methyl-3-heptanol and (3 *RS*, 4 *S*)-4-methyl-3-heptanol (Frighetto samples)*

Sample	Percentage composition			
	(3 *R*, 4 *R*)	(3 *S*, 4 *S*)	(3 *S*, 4 *R*)	(3 *R*, 4 *S*)
(3 *RS*, 4 *S*)	5.3	41.2	3.8	49.7
(3 *RS*, 4 *R*)	47.1	1.9	48.5	2.5

* Purity was checked by gas chromatography on Ni(II)-bis-[3-heptafluorobutyryl-(1 *R*)-camphorate]and Mn(II)-bis-[3-heptafluorobutyryl-(1 *R*)-camphorate] chiral capillary columns.

A range of concentrations of (3 *RS*, 4 *S*)-4-methyl-3-heptanol was presented to workers ants to determine the trail-following threshold of *L. diminuta*. The results illustrated in figure 1, show that the number of ants eliciting positive responses increased as the trail concentration was raised. At a concentration of 1.0 pg/cm, only 20 out of 100 ants tested were able to follow the test trail to the end. However, this result and those observed for higher concentrations were nevertheless statistically very significant ($p < 0.001$) compared to the values obtained for solvent control. Even at a concentration of 0.1 pg/cm a significance of $p < 0.01$ was observed. Only the responses observed for the lowest concentration (0.01 pg/cm) were non-significant over the control values. From these data, we can deduce that the concentration of (3 *RS*, 4 *S*)-4-methyl-3-heptanol required to evoke 50% positive responses is about 1.0–10 pg/cm and the minimum concentration for any significant response is about 0.01–0.1 pg/cm.

Similarly, the activity of a range of concentrations of (3 *RS*, 4 *S*)-4-methyl-3-heptanol was tested against that evoked by 0.1 gland equivalent (0.1 GE) of a natural extract by a dual-choice bioassay. The concentration 0.1 GE was selected because a poison gland contains

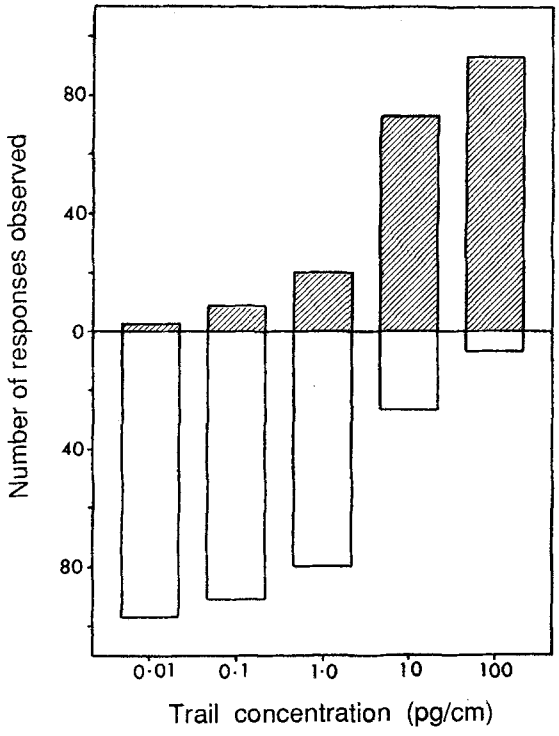

Figure 1. Trail-following responses evoked by different concentrations of (3 *RS*, 4 *S*)-4-methyl-3-heptanol. Hatched bars represent positive responses (complete 20-cm trail following) and open bars negative responses (incomplete or no trail-following). See "Materials and methods" for details.

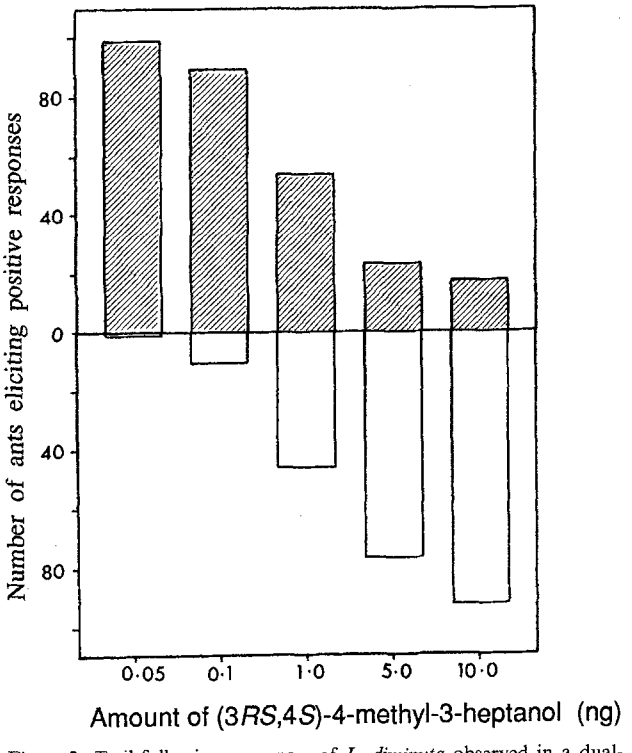

Figure 2. Trail-following responses of *L. diminuta* observed in a dual-choice bioassay. A poison gland extract (0.1 GE) was applied to one arm of the intercrossing trail and the other arm was streaked with different amounts of (3 *RS*, 4 *S*)-4-methyl-3-heptanol. The total number of ants that selected the glandular extract trail is represented by hatched bars and that of the synthetic chemical trail by open bars.

about 10–20 ng of 4-methyl-3-heptanol. It is evident from the data presented in figure 2 that trails made of 1 ng of (3 *RS*, 4 *S*)-4-methyl-3-heptanol can compete with those made of 0.1 GE of natural extract. At amounts over 5 ng/trail, the synthetic trails were even more active than those made of natural extract. Our earlier experiments have shown that the other compounds identified in the poison gland release no trail-following activity or any synergistic enhancement to that of 4-methyl-3-heptanol[2]. Thus, we may conclude that 4-methyl-3-heptanol alone, at concentrations approximately comparable to those found in natural trails, can evoke trail-following behavior similar to that released by poison gland extracts. Furthermore, we have observed no significant differences between poison gland trails and synthetic trails when evaluated for many other ethological features such as aging of natural and synthetic trails and the ability of ants to select a fresh trail from an old one (a publication on these ethological aspects is in preparation).

In a second series of experiments each sample of a stereoisomer was subjected to a trail-following bioassay against that of another stereoisomer (Matteson samples; purity shown in table 2). When tested at a concentration of 500 pg/cm it was visibly very clear that the ants preferred to follow the (3 *R*, 4 *S*) isomer trail without any hesitation (fig. 3). In order to evaluate the activities of non-natural isomers (3 *R*, 4 *R*), (3 *S*, 4 *R*), and, (3 *S*, 4 *S*), more concentrated test trails were used. At a concentration of 5 ng/cm, even the non-natural isomer samples

Table 2. Diastereomeric purities of 4-methyl-3-heptanol isomers (Matteson samples)*

Sample	Percentage diastereomeric impurity*
(3 *S*, 4 *S*)	0.13
(3 *R*, 4 *S*)	0.32
(3 *S*, 4 *R*)	0.58
(3 *R*, 4 *R*)	0.21

* Determined by gas chromatography on a Carbowax capillary column; Reference [3] provides independent NMR analytical data.

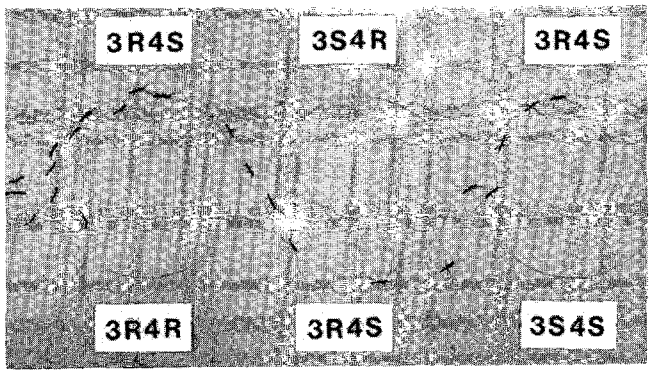

Figure 3. Trail-following responses evoked by stereoisomers of 4-methyl-3-heptanol. The (3 *R*, 4 *S*) isomer was applied on common regions and one arc of each choice region (175 µl, 1 ng/µl). The other arc of each choice region (10 cm) was streaked with a different stereoisomer as labeled (50 ml, 1 ng/µl).

showed a reasonable degree of activity when tested against control trails made with ethanol. The data obtained from the bioassay distinctly demonstrated the ant's preference for the trails of (3*R*, 4*S*) isomer ($p < 0.0001$) (table 3). Although the purities of the test compounds were nearly the best a modern synthetic chemist may achieve nowadays, and significantly better than those used for earlier behavioral tests in general, nevertheless all the isomers tested contained a small amount of impurities (table 2). The significance of these impurities is evident from the slight but significant activities shown by the non-natural isomers (table 3) which may contain trace amounts of the active (3*R*, 4*S*) isomer as an impurity. For example, among the non-natural isomers the (3*S*, 4*R*) isomer sample showed the highest activity (table 3) and this was the most impure stereoisomer tested (diastereomeric impurity 0.58%, table 2). Furthermore, the (3*S*, 4*S*) sample which was the purest of all the samples tested showed the least activity.

In some earlier ethological studies with synthetic chiral pheromones, activities shown by non-natural antipodes containing small but very significant quantities of active compound have been often interpreted as activity shown by the antipode itself although it was the activity of the sample as a whole that was being monitored. In order to check the significance of the impurities, we increased the concentration of the non-natural isomer trails by a factor of 100 to 1000, and tested against (3*R*, 4*S*)-isomer trails of 0.67 pg/cm concentration. A hundred times more concentrated trails made of (3*S*, 4*R*), (3*S*, 4*S*) and (3*R*, 4*R*) were still significantly less active than that of (3*R*, 4*S*) sample at a concentration of 0.67 pg/cm ($p < 0.0001$) (fig. 4). The trails made of (3*S*, 4*R*) and (3*R*, 4*R*) samples were able to compete well with the (3*R*, 4*S*) trails at a concentration of 250 times more than that of the latter. Interestingly when the concentration was increased by 500 times, the trails made of (3*S*, 4*R*) and (3*R*, 4*R*) samples were even more active than those made of (3*R*, 4*S*)-isomer sample (fig. 4). Furthermore, the purest (3*S*, 4*S*) samples (diastereomeric purity 0.13%) were able to compete with those of (3*R*, 4*S*)-isomer trails only at a concentration of 500 times more than that of the latter (fig. 4). In order to become significantly more active than that of (3*R*, 4*S*) sample, the concentration of the (3*S*, 4*S*) trail required a concentration of 1000 times more. One might justifiably argue that until we synthesize 100% pure isomers, a task beyond the capabilities of synthetic and analytical chemists, we cannot interpret that the activities shown by the non-natural isomer samples used in this study were entirely due to the minute contaminations of the highly active (3*R*, 4*S*) isomer. However, there is sufficient circumstantial evidence for us to believe this may well be true.

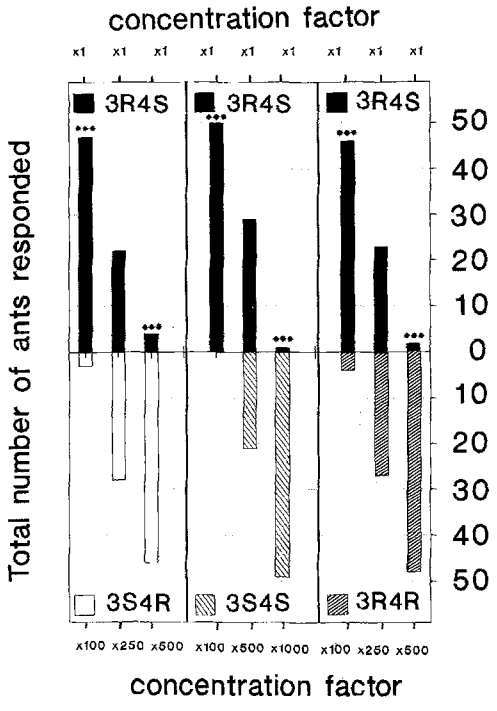

Figure 4. Trail-following responses of *L. diminuta* workers to (3*R*, 4*S*)-4-methyl-3-heptanol (0.67 pg/cm) tested against trails made of higher concentrations of (3*R*, 4*R*)-, (3*S*, 4*R*)- and (3*S*, 4*S*)-isomer samples.

Table 3. Trail-following responses of *L. diminuta* workers to stereoisomers of 4-methyl-3-heptanol determined by a dual-choice bioassay*

Isomer pair	Choice	Chi^2	p
(3*R*, 4*S*):(3*S*, 4*R*)	143:17	99.23	$p < 0.0001$
(3*R*, 4*S*):(3*S*, 4*S*)	151: 9	126.03	$p < 0.0001$
(3*R*, 4*S*):(3*R*, 4*R*)	159: 1	156.03	$p < 0.0001$
(3*S*, 4*R*):(3*S*, 4*S*)	104:56	14.40	$p < 0.001$
(3*S*, 4*R*):(3*R*, 4*R*)	93:67	4.23	$p < 0.05$
(3*R*, 4*R*):(3*S*, 4*S*)	100:60	10.00	$p < 0.01$

* each isomer was tested at a trail concentration of 5 ng/cm.

The (3*S*, 4*S*) stereoisomer of 4-methyl-3-heptanol is produced and used as a pheromone component by elm bark beetles [5]. However, in this case no significant differences have been observed in the response of *Scolytus scolytus* to different stereoisomers of 4-methyl-3-heptanol in laboratory bioassays conducted with samples of purities ranging from 94.4% to 98% [6]. It is well known that conducting bioassays with beetles is rather tedious. On the contrary, trail pheromone bioassays, compared to many other insect bioassays, are easy to perform and give unambiguous results. Hence, trail-following bioassays are ideally suited for structure-activity and dose-response studies.

Of the several hundred insect pheromones identified only about 100 are chiral [7]. In a review on stereoisomerism in insect pheromones, Silverstein [8] has defined nine possible response categories that may be encountered with chiral pheromones. According to this classification the responses elicited by *L. diminuta* fall into the most common category where one of several optical isomers evokes maximum pheromonal activity and is uninhibited by the others.

Acknowledgment. We are indebted to Prof. D. S. Matteson (Washington State University) for making the stereoisomers of 4-methyl-3-heptanol available to us. ABA, RTSF, and NF thank the Alexander von Humboldt-Stiftung, CNPq and EMBRAPA, respectively, for fellowships. We also thank Prof. T. Eisner for his helpful comments on the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft.

* Baker Laboratory, Chemistry Department, Cornell University, Ithaca, NY 14853-1301 USA. (Author to whom correspondence should be addressed)
** EMBRAPA/CNPDA, C.P. 69, 13820 Jaguariúna-SP, Brazil.
*** CPQBA/UNICAMP, C.P. 6171, 13083 Campinas-SP, Brazil.

1 Pheromones, 81; Pheromones, 80: Martischonok, V., Melikyan, G. G., Mineif, A., Vostrowsky, O., and Bestmann, H. J., Synthesis (1991) 560.
2 Attygalle, A. B., Vostrowsky, O., Bestmann, H. J., Steghaus-Kovâc, S., and Maschwitz, U., Naturwissenschaften *75* (1988) 315.
3 Tripathy, P. B., and Matteson, D. S., Synthesis (1990) 200.
4 Frighetto, N., Ph. D. thesis, University of Erlangen-Nürnberg, 1988.
5 Silverstein, R. M., in: Insect Communication, p. 105. Ed. T. Lewis. Academic Press, London 1984.
6 Blight, M. M., Wadhams, L. J., and Wenhan, M. J., Insect Biochem. *9* (1979) 525.
7 Mori, K., Tetrahedron *45* (1989) 3233.
8 Silverstein, R. M., in: Chemical Ecology: Odour Communication in Animals, p. 133. Ed. F. J. Ritter. Elsevier, Amsterdam 1979.

0014-4754/92/070690-05$1.50 + 0.20/0

Queen retrieval in the Argentine ant

S. Aron*

Laboratoire de Biologie Animale et Cellulaire, C.P. 160, Université Libre de Bruxelles, B-1050 Bruxelles (Belgium)
Received 30 September 1991; accepted 11 February 1992

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.