CHAPTER TWO

Evidence of olfactory communication in workers of *Pachycondyla analis* (Hymenoptera: Formicidae)

Abstract

Pachycondyla analis (Latreille), a widespread Ponerine ant in sub-Saharan Africa feeds solely on termites mainly belonging to the sub-family Macrotermitinae that are of economic importance in Africa. *P. analis* capture termites by raiding their nests or galleries. The raiding behaviour is well coordinated and organised suggesting the use of several cues including intraspecific chemical communication. To investigate the mediation of intraspecific olfactory communication in *P. analis*, the responses of major and minor workers to conspecific volatiles were tested in Y-tube olfactometer bioassays. Major and minor workers were responsive to their own odours and to odours of mixed groups of workers. However, major workers were significantly less responsive to odours of minor workers than to their own odours. Coupled gas chromatography-mass spectrometry analysis showed that major and minor workers shared twenty one of the forty eight identified compounds. *(Z)*-12-Pentacosene, *n*-heneicosane, *n*-eicosane *n*-tricosane, *(Z)*-9-tricosene and *n*-pentadecane were the major components in the volatiles. The amounts of volatiles released by major and minor workers on their own were two and half times higher than those released by the mixed group of ants. This study provides the first detailed account of volatiles released by *P. analis* and, also evidence that *P. analis* uses olfactory cues as part of their communication system within and outside the nest during raids on termites.

Introduction

Termites are of economic importance from two different perspectives. They assist in nutrient re-cycling (beneficial), but destroy cellulose containing materials in their quest to acquire cellulose (Culliney and Grace, 2000) causing damage to crops, forests and wooden buildings worldwide. In Africa alone they account for 15-100 % losses in crops and in tree production (Janssen, 2006). Chemical communication plays a central role in the organisation of ant societies (Hölldobler and Wilson, 1990), bringing individuals in a colony together temporarily at relevant locations by recruiting colony members and also enabling efficient interactions and utilisation of available resources within the colony. Collective exploitation and aggressive/defensive behaviours are controlled by pheromone communication systems, and these traits are presumed to be crucial in ecological dominance, most especially in introduced ant species (Holway *et al*., 2002).

Pachycondyla analis (Latreille) is a specialised termite predator, widely distributed in sub-Saharan Africa (Lévieux, 1966). This ant species, commonly referred to as 'Matabele ants', organizes group raids on termite species which mainly belong to the sub-family Macrotermitinae (Longhurst *et al*., 1978). These raids are initiated when a scout ant detects a potential food source (Longhurst *et al.*, 1978, Lepage, 1981) and then recruits nestmates using trail pheromones (Longhurst *et al.*, 1979). Upon arrival at the food source, the ants spread out, break open termite galleries and then invade them to capture the termites. *P. analis* workers capture termites by stinging them, after which they carry the termites out of the gallery entrance and then return to continue hunting. After gathering enough termites they stop hunting, re-group in columns and start the return journey back to their nest (Longhurst *et al*., 1978). A major worker and a minor worker can grasp up to seven and three termites respectively between its mandibles. Others carry no termites but lead the columns of nest mates on the return journey back to the nest (Longhurst *et al*., 1978). The raiding process can last between 4 and 50 min depending upon the foraging distance and the termite species being raided.

Chemical communication within and outside the nest and during raids on termites has not been described in *P. analis*. Previous studies reported on trail laying signals released possibly from glandular sources (Longhurst *et al*., 1979; Hölldobler *et al*., 1994; and Janssen *et al*., 1995).

This chapter explores the use of intraspecific chemical communication in *P. analis*. Responses of major and minor workers to conspecific volatiles and to volatiles from mixed group of workers were tested. The composition of the volatiles was analysed and quantified using GC-MS.

Materials and methods

Collection of ants and rearing

Colonies of *P. analis* with representatives of all castes (workers), males, eggs, cocoons and larva were excavated in Mpala (0°17'N, 37°52'E), a research facility of Mpala Wild Life Foundation. Mpala is located in Laikipia district, Central Kenya 250 km north of Nairobi and about 50 km from the equator and 50 km north-west of Nanyuki (Figure 1.2). The excavated colonies were transported to the Animal Rearing and Containment Unit (ARCU) located on the *icipe* Duduville campus Nairobi, Kenya.

Excavations were carried out either in the mornings or late in the evenings by carefully digging around the perimeter of the nest after blocking all openings to the nest to prevent ants from escaping. Ant carrying boxes which were made from plastic food containers (Figure 2.1) were partially filled with soil from the excavated nests. Ants were carefully collected and placed in the ant box using a soft paint brush and entomological forceps.

In the laboratory, ant colonies were provided with nesting boxes (20 × 20 × 20 cm) made of aluminium with a lid which could be opened to observe the nest. The base of the nesting box was partially filled with soil collected at Mpala (which served as nesting site). This was attached to a 1.0 × 1.5 m foraging arena made of Perspex also partially filled with soil which was previously washed with double-distilled water and sterilised by drying in an oven overnight (Figure 2.2).

Ants were fed on termites collected from mounds or foraging galleries around *icipe* Duduville campus Nairobi, Kenya. Feeding was carried out twice daily (morning and evening). Conditions in the rearing room were kept between 50 - 60% relative humidity, 24-29^{o}C under a natural photoperiodic cycle.

Figure 2.1 Items used for the excavation of *P. analis* nest at Mpala, note the ants inside the plastic carrying box.

Bioassays

The olfactory responses of major and minor workers of *P. analis* to conspecific odours were tested in a Y-tube olfactometer (Figure 2.3). The odour source consisted of (a) 20 major (b) 20 minor (c) a mixture of 10 major and 10 minor workers. The bioassays were conducted at room temperature (24 ± 1°C) and 50 - 60% RH. In order to simulate ant foraging and raiding behaviour as observed in the field, all bioassays were carried out in the mornings and evenings during the period 0700 - 1000 hrs and 1600 - 1730 hrs, respectively local time, over a number of days using ants from different colonies.

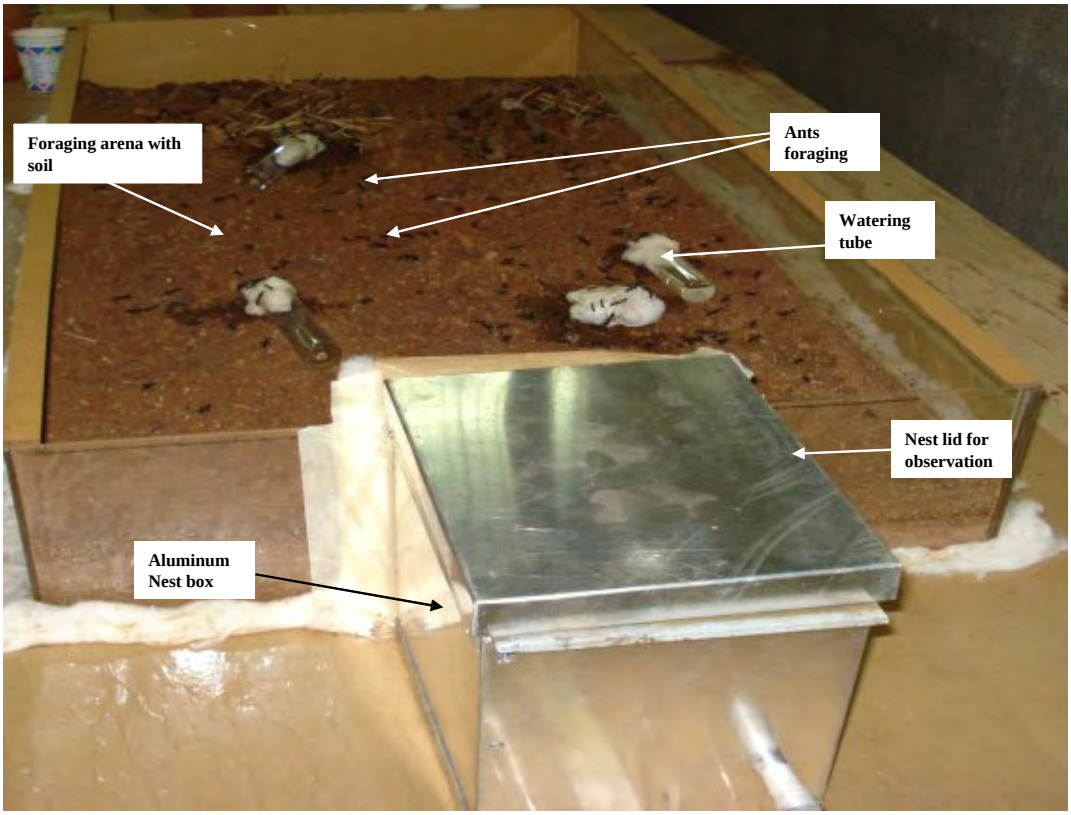

Figure 2.2 Ant rearing set-up in the laboratory, showing the ants nesting box, foraging arena, watering point and nest observation lid.

Y-tube olfactometer set-up

The olfactometer set-up (Figure 2.3) consisted of a glass Y-tube (base 7.5 cm long; Y-arms 7.5 cm long; internal tube diameter 10 mm). Each arm was extended with a small piece of Teflon tube of similar size, which was fitted to a long flexible Teflon hose that entered directly into the odour source. The base tube was also extended with a piece of Teflon tube of similar size to another flexible Teflon hose, leading to a vacuum pump. Air coming from the pump was directed outdoors to avoid contamination of the indoor air. At each end of the Y-tube a wire mesh was placed to prevent a test ant from getting out of the base or any of the Y-arms. Odour sources were placed in 200 ml glass jars (odour chambers) with screw tops containing inlets and outlets for air entering and odour to exit through the Y-tube. Each jar was connected to an air supply via flexible Teflon hoses. Charcoal-purified air was passed into the odour chambers at a flow rate of 250 ml/min. One of the Y-arms was connected to an odour source while the other was connected to an empty jar with only clean air (blank) passing through. The odours were

extracted through the base arm at 500 ml/min by a vacuum pump to ensure a steady flow and to prevent odours from building up in the Y-tube. A score line was drawn on the two arms of the olfactometer at 2 cm from the joint (Figure 2.3).

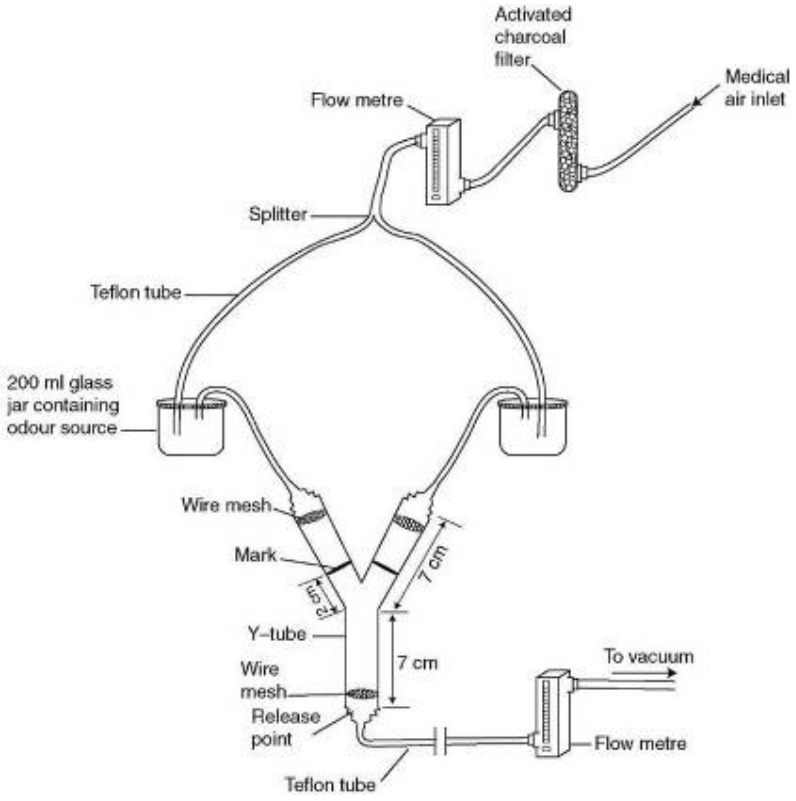

Figure 2.3. Schematic diagram of the Y- tube olfactometer bioassay set-up (not drawn to scale) used in the olfactometric bioassays of conspecific volatiles of *P. analis*.

Test ants were introduced individually into the apparatus by disconnecting the Y-tube at its base and allowing the ant to walk into the olfactometer. Subsequently, the tube was reconnected to re-establish airflow from the odour sources through the arms and out at the base towards the vacuum pump. An ant was allowed to settle down for 5 min, after which its behaviour was monitored. A choice was recorded when an ant chose and arm and stayed there for at least 1 min, or when it frequently visited an arm. A no-choice was recorded when the ant remained in the base arm for more than 5 min after the start of the test. Each test was terminated after 10 min from the introduction of the ant into the Y-tube. Sixty replicates were carried out for each treatment (30 minor and 30 major workers). All ants were tested against odours of their nestmates. Overall ants from three

different colonies were used for the experiment. To avoid positional bias, odour chambers were rotated for every replicate. A clean Y-tube was used for each replicate in order to avoid carryover of trail following pheromones. Parts between the Y-tube, vacuum and odour sources were changed or cleaned with soapy water, rinsed with dicholoromethane and acetone after each bioassay to remove traces of trail pheromones and contaminants. All glassware were cleaned with Teepol® laboratory detergent Kent UK, rinsed with acetone and dried for five hours at 160°C in an oven. Teflon parts were rinsed with acetone and water to remove volatiles and then flushed with a stream of nitrogen to dry them.

Volatile collection and analyses

A pull-push volatile collection system (Figure 2.4) was used to collect volatiles from ants. Charcoal-purified and humidified air was continuously passed through a 2 litre volatile collection chamber (Analytical Research Systems INC, Gainesville, FL, USA) containing ~200 ants and through a filter containing Super-Q (30 mg, Analytical Research Systems INC, Gainesville, FL, USA). Volatiles released in the chamber were pulled through the filter by vacuum at 500 ml/min. Before connecting the adsorbent traps; the set-up was purged by passing humidified air through it for 20 min. This period allowed the ants to settle down in the containers. Volatiles were collected overnight for 14 hours (N = 12).

The Super-Q traps were eluted with 100µl of dichloromethane (DCM) under ice and the eluent was pushed through the trap using a gentle stream of charcoal-filtered nitrogen (N_2) (Figure 2.5). To this eluent, 2 µg of ethyl nonanoate (98% purity, Sigma-Aldrich) were added as an internal standard. The volatiles were analysed on an Agilent Technologies 7890A gas chromatograph equipped with a capillary column HP-5 MS (30 m × 0.25mm ×0.25µm, ID and film thickness) and coupled to 5795C mass spectrometer.

One µl of each sample was injected in the split less mode (Inlet temperature = 250°C, Pressure = 6.83 psi), and helium was used as the carrier gas at 1.0 ml/min. The oven temperature was held at 35°C for 5 min, increased to 250°C at 10°C/min, and then held at this temperature for 15 min.

Ethylbenzene, ethyl propanoate, *p*-xylene, *o*-xylene, nonane, decane, α-pinene, 1-undecene, *n*-pentadecane, *n*-hexadecane, *n*-eicosane, *n*-tricosane and *n*-heneicosane were identified by comparison of their mass spectral data with those from the NIST 08 library, and by their retention indices with those of authentic compounds. Other components in the volatiles were tentatively identified based on comparison of their mass spectral data with the NIST 08 library data. Individual compounds (ng) were quantified relative to the amount of internal standard added.

Chemicals.

Ethylbenzene, ethyl propanoate, *p*-xylene, *o*-xylene, nonane, decane, α-pinene, 1-undecene, *n*-pentadecane and *n*-hexadecane, with purity of > 99% were obtained from Aldrich, Gillingham, Dorset, UK. *n*-Eicosane, *n*-tricosane and *n*-heneicosane were provided by Dr. Peter Teal, USDA/ARS-CMAVE, Florida, USA, while (*Z*)-9-tricosene was provided by Dr. Antony Hooper, Rothamsted Research, Harpenden, UK.

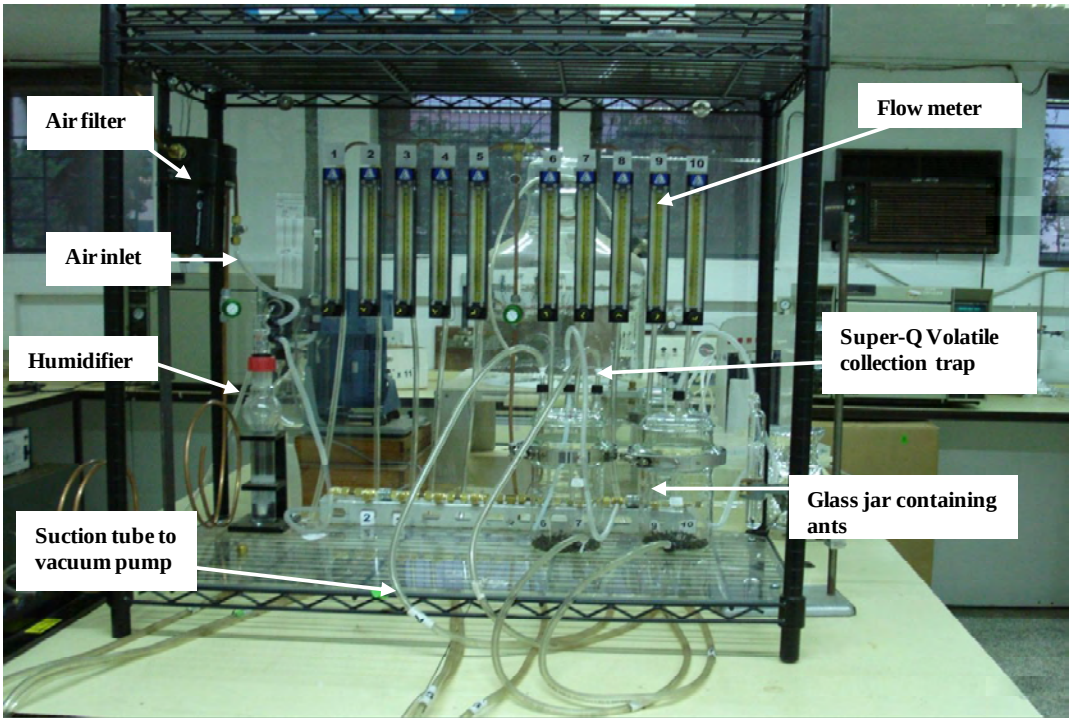

Figure 2.4 A push-pull volatile trapping collection system used to trap chemical volatiles from ants.

Volatile extracts not analysed immediately were stored in the freezer at - 20 °C until used. Controls were also trapped in a similar way using only blank jars/cylinders. After trapping, all glassware were soaked and washed with industrial detergent (Teepol®), rinsed with acetone (purity > 90%) prior to baking overnight at 250 °C in an oven. This was to avoid carryover or contamination which could arise from the glassware.

Statistical analysis

Data obtained from Y-tube olfactometer were analysed using Chi square (χ^2) $P = 0.05$, to test for differences between odours. Analysis was carried out using SAS statistical software version 9.1. (SAS Institute, 2001).

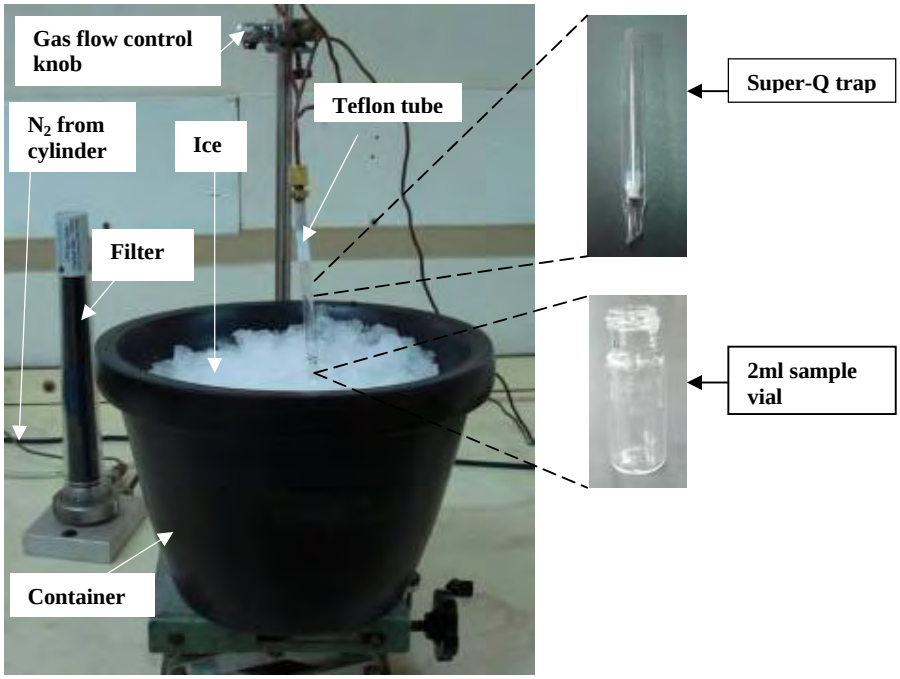

Figure 2.5 Set-up for eluting volatiles from Super-Q traps in the laboratory after collecting volatiles from ants.

Results

Y-tube bioassays

Between eighty and ninety percent of all tested ants made a choice. Both major and minor workers did not differ in their responses to odours. Overall, when an odour cue

was offered opposite to clean air, 70% of ants chose an odour source. Both major (χ^2 = 9.8, $P < 0.01$) and minor (χ^2 = 14.4, $P < 0.001$) workers showed preference for odours from mixed ants compared to the blank (Figure 2.6a). Similarly, both majors (χ^2 = 6.8, $P < 0.01$) and minors (χ^2 = 5.5, $P < 0.05$) preferred odours from majors than the blank (Figure 2.6b). This was the same when majors (χ^2 = 5.3, $P < 0.05$) and minors (χ^2 = 14.29, $P < 0.001$) were given a choice between odours from minors and a blank. (Figure 2.6c).

Identification of ant volatiles

Forty eight components were identified by GC-MS in the volatiles of *P. analis* (Table 2.1). Thirty four of these were identified from odours of mixed ants (Figure 2.7), 27 from odours of major ants (Figure 2.8a) and 32 were identified from minor workers (Figure 2.8b). Total volatile emissions (ng) were; major (55.88 ± 14.05) and minor (57.86 ± 16.91) workers. In general, the amount of volatiles released by the different worker groups was 2.5 fold higher than those released by the mixed major and minor (20.14 ± 7.05) worker ants (Table 2.1). Compounds identified from mixed workers included two esters, an aromatic compound, terpenes, and hydrocarbons (alkanes, alkenes, methyl branched alkanes) with chain lengths between C_{10} – C_{30}. Ten compounds in the odours of mixed groups of ants were not present in the odours of both major and minor workers (Table 2.1, Figure. 2.7. and 2.8). Undecane (23.3%), (*Z*)-12-Pentacosene (13.45%), pentadecane (10.44%), 5-methylundecane (7.04%) and *n*-tricosane (5.01%) were the most abundant compounds in the odours of mixed ants.

(*Z*)-12-Pentacosene (35.53%) was the most abundant compound in the odours of major workers followed by *n*-heneicosane (9.66%), *n*-tricosane (9.38%), (*Z*)-9-tricosene (9.21%) and *n*-pentadecane (4.61%). In minors (*Z*)-12-pentacosene (38.64%), (*Z*)-9-Tricosene (11.28%), *n*-tricosane (10.13%), *n*-eicosane (8.30%) and *n*-pentadecane (7.05%) (Table 2.1). Twenty one compounds were common to both major and minor workers of *P. analis* (See italicized columns Table 2.1). Six compounds found in the odours of major workers were not present in those of minor workers whilst eleven compounds present in minors were absent in majors (Figure 2.8a; b).

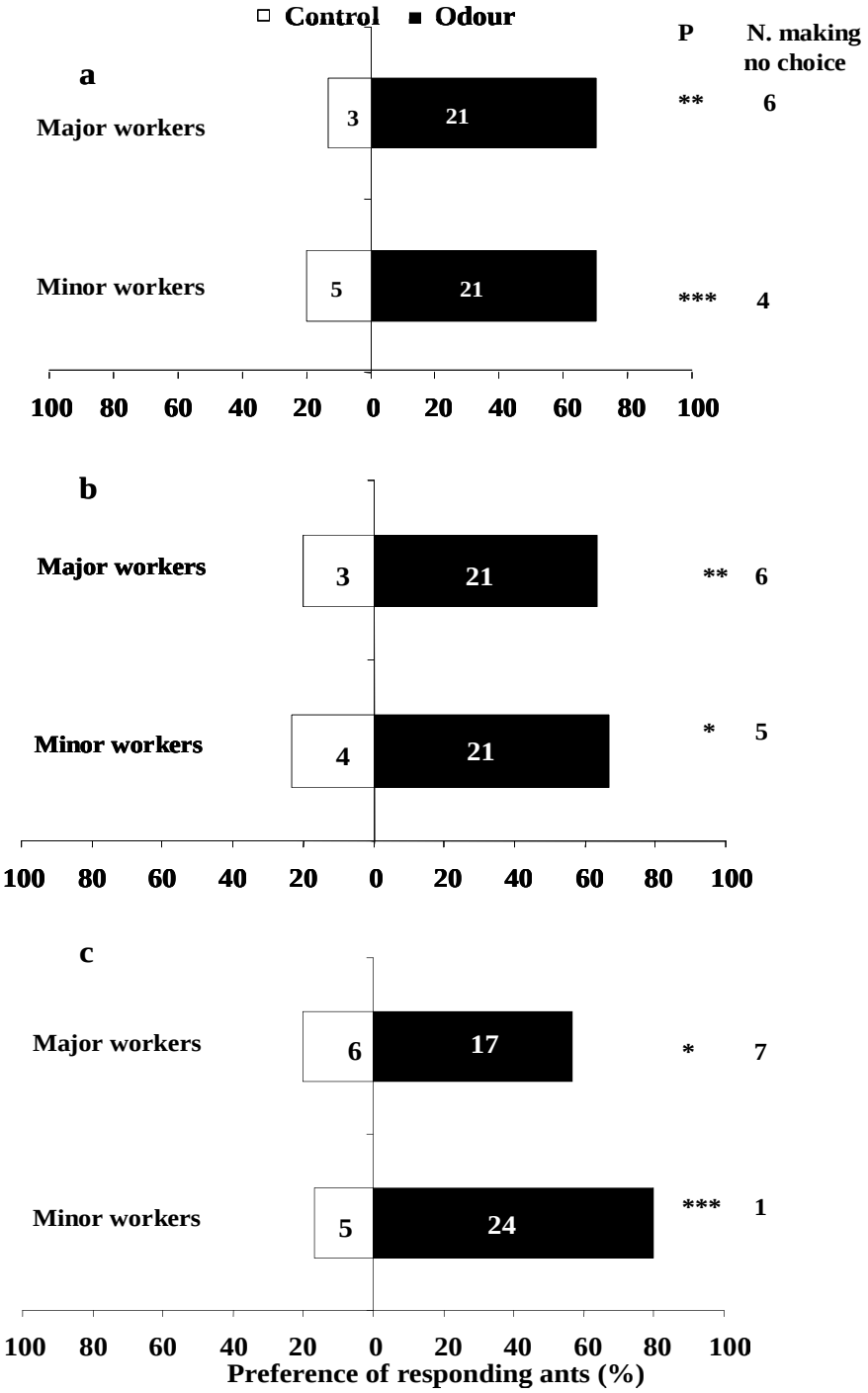

Figure 2.6 Preference of major and minor workers *P. analis* to odors of (a) Mixed ants (major and minor), (b) Major and (c) Minor workers respectively. Numbers within bars refer to the number of ants making a choice, while numbers outside bars refer to ants that made no choice. (*N*= 30 each for major and minor workers in each treatment, χ^2 test, * = significant at *P* < *0.01*,**= significant at *P* < 0.05 and ***= significant at *P* < 0.001).

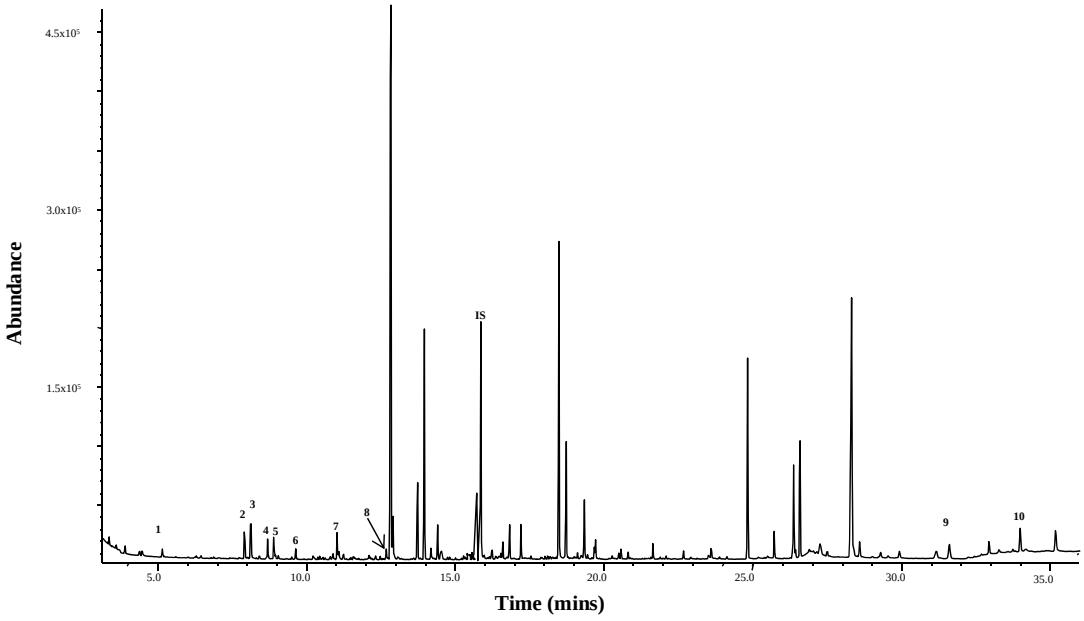

Figure 2.7 Representative GC-MS profile of odours collected from mixed (100 major and 100 minors) worker ants of *P. analis* by trapping for 14 hours overnight. Numbered peaks are compounds unique to volatiles of mixed workers. 1) Ethyl propanoate, 2) ethylbenzene, 3) p-xylene, 4) o-xylene, 5) Nonane, 6) 1R-.alpha. pinene, 7) Decane, 8) 1-undecene, 9) *n*-pentacosane, 10) squalene. IS, internal standard (ethyl nonanoate).

A comparison of the main components shared by the mixed, major and minor workers showed that, when workers were mixed they produced quantitatively less volatiles, with major workers releasing more volatiles than the minors. But, minor workers produced significantly more quantities of (Z)-9-tricosene and *n*-tricosane compared to the major workers (Figure 2.9).

Table 2.1 Chemical compounds tentatively identified by GC-MS from body odours of mixed (major and minor), major and minor workers of *P. analis*

S/no	Chemical name	Mixed		Major		Minors	
		Mean (ng) ± SE*	**Percent Total**	**Mean (ng) ± SE***	**Percent Total**	**Mean (ng) ± SE***	**Percent total**
1	Ethyl propionate	0.43 ± 0.30	0.32				
2	Etylbenzene	0.35 ± 0.13	1.08				
3	p-xylene	0.60 ± 0.23	1.83				
4	o-xylene	0.06 ± 0.05	0.71				
5	Nonane	0.08 ± 0.06	0.88				
6	1R-.alpha.Pinene	0.77 ± 0.58	0.35				
7	Decane	0.10 ± 0.08	1.16				
8	1-Undecene	0.04 ± 0.03	0.42				
9	*n*-Undecane	3.46 ± 0.78	23.13	*3.49 ± 0.89*	*8.07*	*2.14 ± 0.47*	*4.51*
10	Nonanal	0.51 ± 0.03	3.17			0.62 ± 0.05	0.94
11	5-methylundecane	1.07 ± 0.11	7.04	*0.40 ± 0.04*	*0.75*	*0.62 ± 0.05*	*0.62*
12	3-methylundecane,	0.19 ± 0.08	0.52			0.36 ± 0.01	1.18
13	2-methyl-1-hexene	0.23 ± 0.02	1.05	*0.39 ± 0.03*	*0.72*	*0.16 ± 0.05*	*0.27*
14	*n*-Dodecane	0.51 ± 0.03	0.79	*0.20 ± 0.13*	*0.33*	*0.19 ± 0.06*	*0.30*
15	Methyl benzoate	0.22 ± 0.09	0.70			0.63 ± 0.37	0.30
16	5-methyltridecane	0.13 ± 0.10	0.58	*0.15 ± 0.09*	*0.26*	*0.52 ± 0.26*	*0.33*
17	3-methyltridecane	0.22 ± 0.04	1.17	*0.25 ± 0.03*	*0.47*	*0.40 ± 0.17*	*0.52*
18	*n*-Tetradecane	0.87 ± 0.56	1.17	*0.39 ± 0.04*	*0.73*	*0.67± 0.25*	*0.60*
19	1-Pentadecene			*0.17 ± 0.11*	*0.32*	*0.45 ± 0.09*	*0.57*
20	*n*-Pentadecane	2.53 ± 0.66	10.44	*4.61 ± 0.31*	*8.37*	*3.60 ± 0.46*	*7.05*
21	Butylated Hydroxytoluene	0.74 ± 0.06	3.95	*0.14 ± 0.01*	*0.25*	*0.12 ± 0.08*	*0.21*
22	3-methylpentadecane			*0.68 ± 0.58*	*1.13*	*0.49 ± 0.29*	*0.92*
23	3, 8-dimethyldecane	0.43 ± 0.25	1.89	0.92 ± 0.04	1.63		
24	*n*-Hexadecane					0.59 ± 0.32	0.33
25	8-Heptadecene	0.09 ± 0.02	0.35	*0.68 ± 0.58*	*0.25*	*0.78 ± 0.55*	*0.18*

Table 2.1 contd…..

S/no	Chemical name	Mixed		Major		Minors	
		Mean (ng) ± SE*	**Percent Total**	**Mean (ng) ± SE***	**Percent total**	**Mean (ng) ± SE***	**Percent Total**
26	*n*-Heptadecane			*0.09 ± 0.04*	*0.16*	*0.28 ± 0.14*	*0.19*
27	Hexadecanal			*0.22 ± 0.01*	*0.35*	*0.36 ± 0.17*	*0.24*
28	5-Octadecene	0.05 ± 0.04	0.55			0.09 ± 0.03	0.16
29	*n*-Eicosane					4.49 ± 1.41	8.30
30	*n*-Heneicosane	1.02 ± 0.34	6.28	*5.79 ± 0.25*	*9.66*	*1.37 ± 0.50*	*1.25*
31	3-methylheneicosane					0.13 ± 0.07	0.22
32	*n*-Docosane	0.18 ± 0.01	0.96	1.00 ± 0.16	1.69		
33	9-octyldocosane,					0.28 ± 0.05	0.36
34	(*Z*)-9-tricosene	0.64 ± 0.33	3.86	*5.11 ± 0.52*	*9.21*	*8.08 ± 1.62*	*11.28*
35	*n*-Tricosane	1.16 ± 0.25	5.01	*5.13 ± 0.37*	*9.38*	*6.67 ± 2.09*	*10.13*
36	*n*-Tetracosane			*0.22 ± 0.11*	*0.39*	*0.36 ± 0.28*	*0.45*
37	Cyclotetracosane	0.11 ± 0.09	0.91	*1.35 ± 0.54*	*2.26*	*2.20 ± 0.39*	*2.86*
38	*(Z)*-12-pentacosene	1.86 ± 0.87	13.45	*19.25 ± 7.55*	*35.53*	*16.95 ±5.12*	*38.64*
39	*n*-Pentacosane	0.30 ± 0.13	0.85				
40	13-undecylpentacosane,					0.30 ± 0.04	0.58
41	9-Nonadecene	0.07 ± 0.06	0.81	0.21 ± 0.15	0.35		
42	1-Hexacosene					1.64 ± 0.96	2.85
43	9-Hexacosene			1.28 ± 0.67	2.15		
44	*n*-Heptacosane	0.62 ± 0.36	1.04			1.42 ± 0.11	2.14
45	1-Heptacosanol			1.44 ± 0.15	2.12		
46	*n*-Octasocane	0.47 ± 0.26	0.88	*0.94 ± 0.40*	*1.67*	*0.90 ± 0.40*	*1.51*
47	Squalene	0.03 ± 0.02	0.32				
48	*n*-Triacontane			1.38 ± 0.25	1.79		
	Total	**20.14 ± 7.05**	**100**	**55.88 ±14.05**		**57.86 ± 16.91**	**100**

Odours were collected overnight. Italics indicate compounds that are common to both major and minor workers, ng= nanogram per µl, *= Average for four replicates.

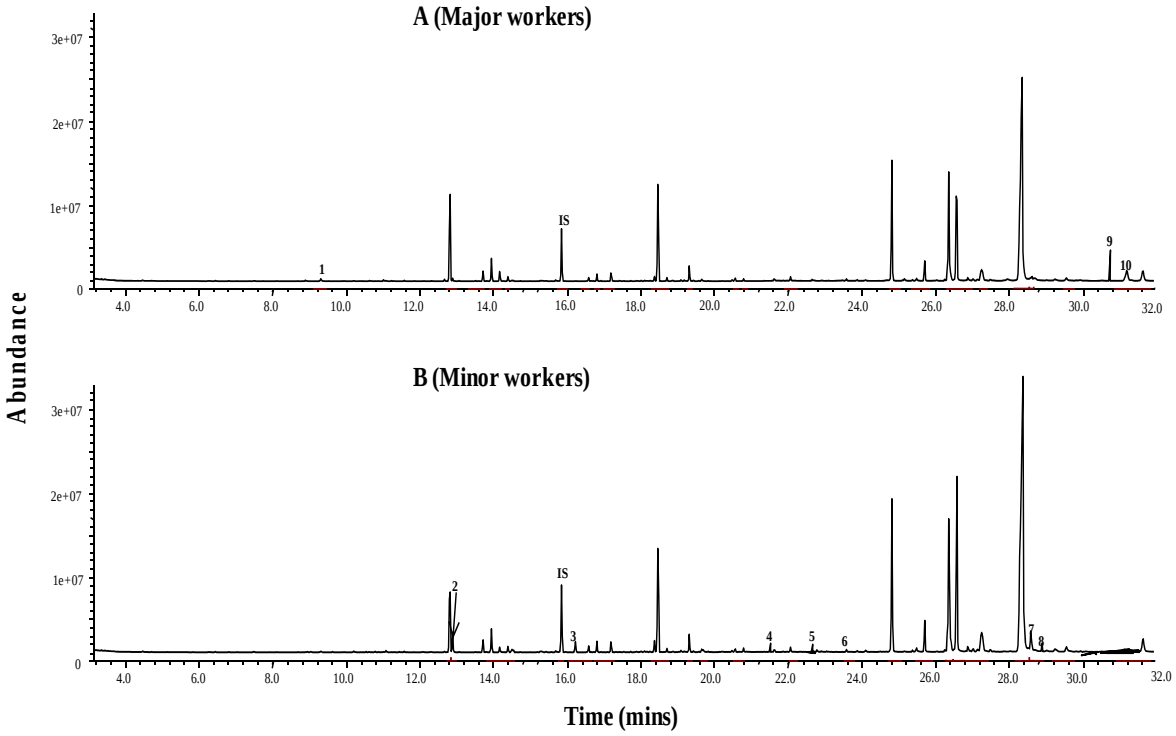

Figure 2.8 Representative GC-MS profiles of odours collected from 200 ants (a) major workers and (b) minor workers of *P. analis* by trapping for 14 hours overnight. Numbered peaks are compounds unique to each. Peaks numbered are 1) p-benzoquinone, 2)9-hexacosene, 3) 1-heptacosanol, 4) 1-hexacosene 5) *n*-hexadecane, 6) n-eicosane, 7) 3-methylheneicosane, 8) 9-octyl-docosane, 9) 13-undecylpentacosane, 10) *n*-triacontane.

Discussion

Results from the Y-tube bioassays showed that both major and minor workers of *P. analis* were attracted to odours from either mixed groups of workers or workers of the same group (minor or major) showing that they responded to the chemical cues from other workers. The use of olfactory cues in communication is very important during recruitment, and raiding of termite nests, most especially when ants are not within visual distance from each other. These cues could either be from a co-worker of the same size or another worker of different size class. There seems to be a specialised coding of olfactory cues from members of the same size (e.g. major to major or minor to

minor, Figures 2.6 b and c), which could be for the purpose of recognition of colony members and task allocation within the colony or during raids. Olfactory cues could also be used to signal when to start or stop a raid, and on the return journey back to the nest.

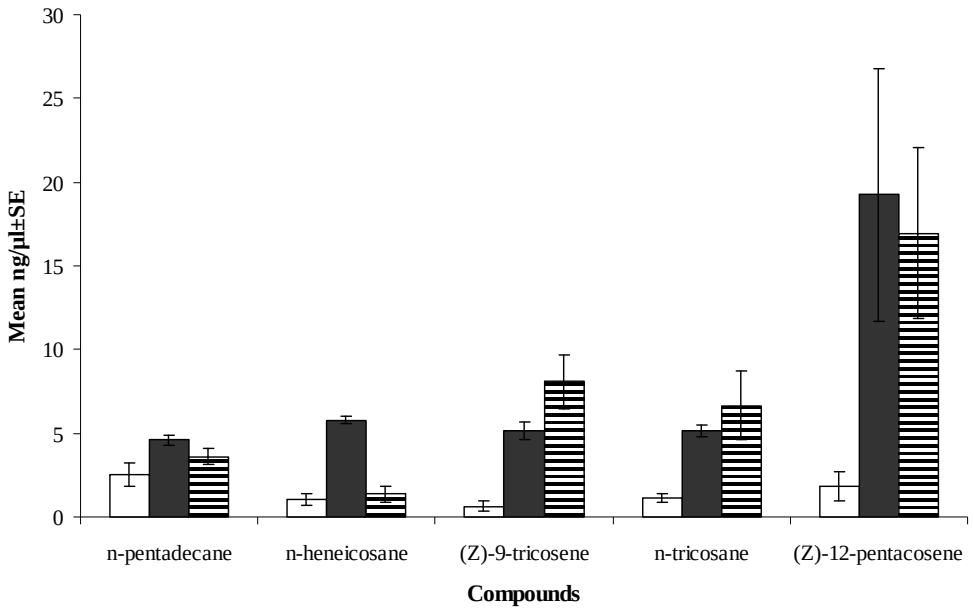

Figure 2.9 Mean concentrations in µg/µl ± SE of the most abundant components emitted in the volatiles of *P. analis* workers. Compounds are represented in ascending order with starting from the least to the most abundant,.□= mixed workers, ■=Major workers and ▤ minor workers.

It appears that clear coordination exists within raiding parties of *P. analis* when they carry prey after a raid, which strongly points to the use of olfactory cues. Glancey and Dickens (1988) had shown that workers of the red imported fire ant (*Solenopsis invicta*) were attracted to volatiles of both sibling larvae and those of heterocolonial origin. Positive response to volatiles cues from familiar founding queens and the ability to recognise each other was also reported by D'Ettorre and Heinze (2005) in queens of *P. villosa*.

These results revealed that the volatile chemical profile of *P. analis* workers contained different blends of compounds. The absolute volatile emissions from mixed workers were 2.5 fold less than those from major and minor workers when trapped alone. Ideally one would expect the volatiles from mixed groups to be the sum of those from major

and minor workers trapped separately. This unexpected result could be explained by the fact that when major and minor workers are together they release compounds that are representative of the colony odour as they interact with each other for the purposes of task allocation, kin recognition and colony cohesion. Upon separation, workers produce compounds that are unique for their group (major or minor). However, this needs to be investigated to ascertain whether the proportion of majors and minors in a group of workers has an influence on the chemical profile of the group.

Volatiles from mixed major and minor workers contained terpenes, esters and hydrocarbons. Longhurst *et al.* (1979) reported 13 unidentified compounds in the mandibular glands, 43 hydrocarbons (*n*-alkanes) from the Dufour's glands and 10 minor components with ester like odours from the Poison glands secretions of *P. analis* workers. Terpenes and hydrocarbons had also been reported in the secretions from Dufour and mandibular glands of *P. striata* and *P. indica* (Morgan *et al*. 1999; 2003). When the volatiles of major and minor workers were analysed, there were qualitative differences in the constituent compounds. Quantitatively there was no significant difference in the total quantity of compounds released by both major and minor workers of *P. analis.* Longhurst *et al*., (1979) did not find qualitative or quantitative differences in the amount of chemicals from the Dufour's glands of major and minor workers of *P. analis*. Though, volatile emission as a whole was not quantitatively different between worker groups. There exist quantitative differences in the amounts of certain common compounds within the volatile profiles of major and minor workers (Table 2.1). This variation indicates the presence of a chemical profile signature which is unique to the two worker groups in *P. analis*. Based on these chemical differences, it is possible therefore to identify or group workers of *P. analis* into their size groups (major and minor workers). Existence of a chemical signature between these two worker groups could have application as task allocation cues in and outside the nest. In social ants, variations in glandular secretions most especially from the Dufour's glands are important for creating specific colonial or individual labels used to produce trail, nest recognition, egg marking and nest marking pheromones (Abdalla and Cruz-Landim 2001). The greater chemical diversity in the profile of minor workers could be explained by the fact that they stay mostly within the nest; and do not scout for food (termites), but participate in the raiding process as described earlier. Their multi-tasking nature may

require possessing the ability of detecting and, or releasing a diverse array of chemical compounds for effective communication with co-workers of the same or different size. Presence of certain compounds in the volatile profiles of the major workers could also be attributed to specific tasks like foraging, care of brood etc which they undertake within and outside the colony.

In summary, this chapter provides an insight into understanding the chemical communication of *P. analis* within and outside the nest during raids on termites. Five main conclusions can be drawn from this chapter (1) *P. analis* uses olfactory cues in their communication system within and outside the nest; (2) volatiles released by *P. analis* are mainly composed of hydrocarbons; (3) major and minor workers emit volatiles which are 2.5 fold higher than those emitted when majors and minors are mixed; (4) thirty four volatiles were identified from mixed group of ants, twenty seven from major workers, and thirty two from minors; (5) twenty one compounds were common to both major and minor workers; (6) *(Z)*-12-pentacosene, *n*-heneicosane, *n*-eicosane *n*-tricosane, *(Z)*-9-tricosane and *n*-pentadecane were the most abundant compounds in the odours of major and minor workers.

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