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Termite raiding by the Ponerine ant *Pachycondyla analis* (Hymenoptera: Formicidae): Behavioural and Chemical Ecology

by

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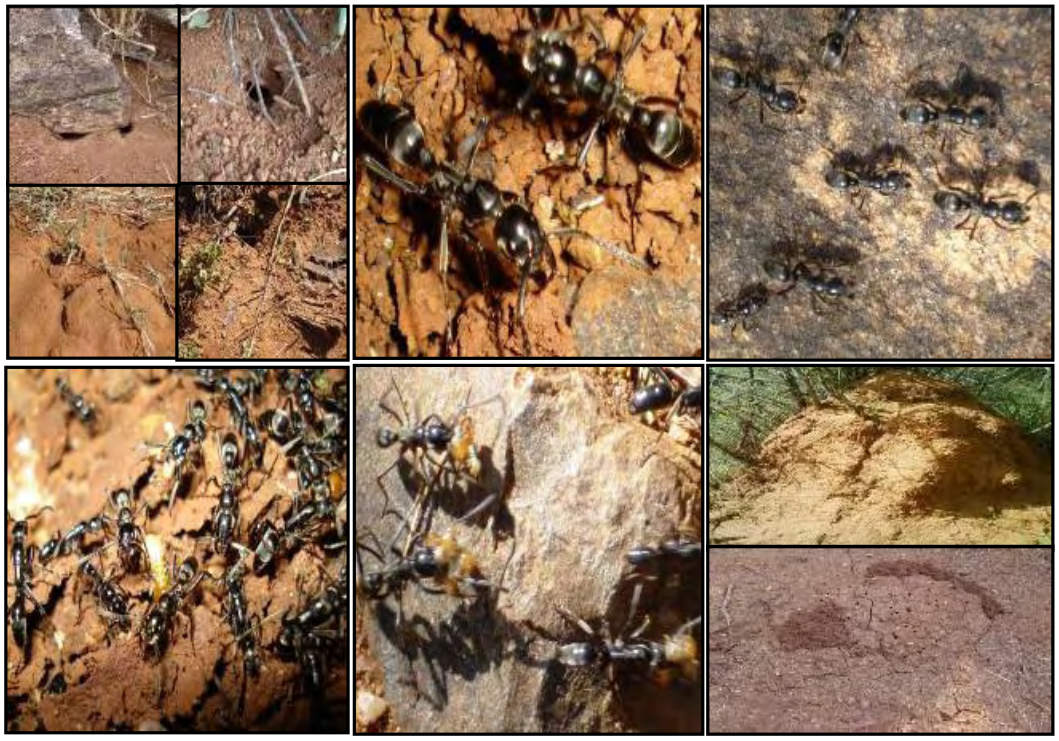
Declaration

I, Abdullahi Ahmed Yusuf declare that the thesis, which I hereby submit for the degree *Philosophiæ Doctor* Entomology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature:…….

Date:…….

To my parents Alhaji Ahmed Rufa'i Yusufu, Hajiya Hadiza Laraba Ahmed and my lovely wife Fatima Isa Abdullahi, for the immeasurable love, care and affection. And to my daughter Khadijatul-Kubra who was born in my absence



"Until when they came to the valley of the ants, a **she ant** exclaimed, "O **ants**, enter your houses may not Solomon and his armies crush you, unknowingly". He therefore smiled beamingly at **her** speech*, and submitted, "My Lord, bestow me guidance so that I thank you for the favour which You bestowed upon me and my parents, and so that I may perform the good deeds which please You, and by Your mercy include me among Your bondmen who are worthy of Your proximity.""**"***(Prophet Solomon heard the voice of the she ant from far away)*

[Qur'an, Chapter *27* (*Al-Naml*-The ant):18-19]

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This thesis is based partly on the following publications:

Note: Chapters 1 to 4 were written as separate publications and submitted to appropriate international journals, consequently there is some overlap of information and references in the thesis.

Chapter 1: A. A. Yusuf, I. Gordon, C. W. W. Pirk, R. M. Crewe, P. G. N. Njagi and A. Hassanali (2009). Termite raiding behaviour of *Pachycondyla analis* at Mpala, a Kenyan savannah, (Manuscript).

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Termite raiding by the Ponerine ant *Pachycondyla analis* (Latreille) (Hymenoptera: Formicidae): Behavioural and chemical Ecology

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Abstract

The ant *Pachycondyla analis* (formerly *Megaponera foetens*, commonly known as the Matabele ant) is a widespread ponerine in sub-Saharan Africa. It feeds solely on termites of economic importance belonging to the sub-family Macrotermitinae. These termites are captured during organised raids on their nests and galleries. Previous studies mostly concentrated on certain aspects of the raiding behaviour and trail laying pheromones in this species. Thus the detailed raiding behaviour and chemically-mediated communication between *P. analis* and its prey are virtually unknown. The aim of this study was to undertake detailed behavioural studies on termite raiding behaviour of *P. analis*, and to investigate whether *P. analis* uses olfactory cues for intra-specific communication during termite raids, and for detecting its prey.

Termite raiding behaviour of *P. analis* was monitored at Mpala, a Kenyan savannah for six months (April to September, 2007). During this period, raids were found to occur mainly in the mornings and evenings, with late night raids occurring during dry periods. *P. analis* at Mpala mainly nests under rocks and in deserted termite mounds. *Microtermes* and *Odontotermes* were the main preyed termite genera, and ant raiding behaviour was synchronised with termite prey behaviour, and was influenced by foraging costs, prey defences and rewards.

Olfactometric assays showed that *P. analis* workers used olfactory cues in their intra-specific chemical communication, with workers responding more to volatiles of

individuals of the same size class (major to major and minor to minor) than between groups. Major workers discriminated more between the volatiles of the two groups than minor workers. GC-MS analysis of volatiles from major and minor workers revealed a cocktail of 48 compounds, majority of which were hydrocarbons. Volatile compounds were colony specific and quantitative analysis showed that major and minor workers alone released 2.5 fold more volatiles than the mixed stages. This suggests that ants have the innate ability to regulate the levels of the colony odour which they make up for with higher release levels when separated from each other.

Using a Mandible Opening Response (MOR) bioassay, ants were able to distinguish between nestmates and non-nestmates based on cuticular hydrocarbon (CHC) profiles. This suggests that *P. analis* uses CHCs as short range contact recognition cues within the nest in traditional nest protection and during raids on termite species. GC-MS analyses revealed hydrocarbons of chain lengths in the range C_8-C_{31} in the CHC profiles, comprising mainly alkanes, alkenes and methyl-branched alkanes. The CHCs were colony and individual worker specific. Nestmate recognition in *P. analis* may be encoded in the alkenes and methyl-branched alkanes.

Dual choice olfactometric assays revealed that *P. analis* uses olfactory cues in locating potential termite sources with an average of 65% of workers choosing odours against the blank (clean air). When termite odours were offered to both major and minor workers, their choices were biased towards the termite odours, with minor workers attracted more to the odours than were major workers. Although ants responded to odours from the soil obtained for the termite gallery, overall, odours from termites inside their galleries were the most attractive to ants. These results suggest that the combined odours from both the termites and gallery components (in particular soil), serves as an effective nest location cue for the ants. Comparative GC-MS analyses showed that the composition of the volatiles from the gallery soil was richer than that released by the termites. Consistent with previous studies, the volatiles of the gallery soil were found to contain hydrocarbons, naphthalene and derivatives of this compound.

In conclusion, these studies have revealed the rich diversity of chemical communication cues used by this ant species for nestmate recognition and for prey location during raids in search for its food source.

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GENERAL INTRODUCTION

Ants and termites in the terrestrial ecosystem

Ants are cosmopolitan in their distribution, but only occasionally noticed. As active participants in most of the terrestrial habitats in the world acting as premier soil turners, and channelers of energy, they are the most dominant among the insect fauna (Hölldobler and Wilson, 1990). In the Ivory Coast savannah, the density of ants is reported to be 7,000 colonies and 20 million individuals per hectare (Lévieux, 1982). Room (1971) recorded 48 genera and 128 species from 250 square metres in a cocoa farm in Ghana. Ants and termites are in the proportion of 8 million ants to 1 million termites in the Amazonian *terra firme* rain forest (Fittkau and Klinge, 1973). Their impact on the terrestrial environment is great as they are among the leading predators of other insects and small invertebrates (Wilson, 1971; Longhurst *et al.,* 1978; Longhurst and Howse, 1979; Lepage, 1981; Lévieux, 1982; Hölldobler *et al.,* 1994. and Dejean *et al.,* 1999).

Termites are members of the order Isoptera which share almost the same habitats as ants with dual roles in the ecosystem. On the one hand they are nutrient channelers in terrestrial ecosystems and on the other; they constitute the most important of the wood-destroying insects and are responsible for heavy economic losses (Janssen, 2006). Out of more than 2900 species of termites in the world, only a small minority have negative economic effects (Culliney and Grace, 2000) since only 70 - 80 different species cause significant damage to structures (Edwards and Mill, 1986).

The biology of termites, economic importance and pest management strategies

Termites (or white ants') are medium-sized, cellulose-eating social insects that always live in communities with large numbers of individuals reaching several millions in some species (Hickin, 1971). They are placed in the order Isoptera of the class Insecta (Borror *et al.,* 1992; Pearce *et al.,* 1996). Recently DNA analysis has shown that members of the termite family (Isoptera) are closely related to cockroaches (Eggelton, 2001; Inward *et al.,* 2007). Of the known termite species in the world, over 40% have at least one representative species in Africa (Borror *et al.,* 1992; Pearce *et al.,* 1996).

Termites are exceedingly abundant throughout the tropical and sub-tropical regions of the world, and in some areas extend into the temperate regions (Hickin, 1971; Grace *et al.*, 1996; Carr, 2006). Nest densities of termites in the tropics range from 3.8 nest ha^{-1} in Kenya (Lepage, 1981) to 2.2 - 37.5 nest ha^{-1} in Ivory Coast (Lévieux, 1982 and Lepage, 1984).

Termites mostly live in moist subterranean and dry habitats above ground, and some species usually construct earthen tubes between the soil and wood above ground. These tubes are made of soil mixed with a secretion from a pore on the front of the head. In tropical species these nests (termitaria) may reach up to 9 metre in height (Borror *et al.*, 1992).

The food of termites is composed of cellulosic vegetable materials, but they also feed on the cast skins and faeces of other individuals. During their efforts to find cellulose, they also cause extensive damage to a host of other materials. Dry wood termites mostly live only in wood and are able to digest woody materials with the aid of colonies of Protozoa, which secrete cellulase, an enzyme that breaks down cellulose into simpler materials capable of being digested (Borror *et al.*, 1992). Subterranean termites (*Macroterme*s species) gather cellulosic material, chew it up and then allow it to be partially digested by fungi before they consume it (Hickin, 1971).

Economic importance of termites

Economically, termites are seen from two broad perspectives. On the one hand, they are beneficial because they assist in nutrient re-cycling. While on the other hand, they cause huge economic losses worldwide as they forage for cellulose. Termites feed upon and often destroy various wooden portions of buildings, furniture, books, utility poles, fence posts, many fabrics and over 48 species of plants (Figure 1) (Hickin, 1971; Culliney and Grace, 2000). Termites are said to cause damage worldwide amounting to 40 billion USD (Janssen, 2006). An annual estimate of damage to wood and wood products caused by termites in the United States exceeds $750 million, 95% of this is attributed to subterranean termites (Family Rhinotermitidae) (Mauldin, 1986; Culliney and Grace, 2000). Moreover subterranean termites account for 80% of the approximately US$ 2-3 billion spent annually for termite control in the USA alone (Su, 1993 and Carr, 2006).

In Africa, the reputation of termites as pests is coupled with the presence of large mounds in agricultural fields and in forests. The greatest pest potential is mainly within the subfamily Macrotermitinae, which has a symbiotic association with termitomyces fungus. *Macrotermes*, *Odontotermes*, *Pseudacanthotermes*, *Ancistrotermes*, *Allodontotermes*, *Amitermes*, *Trinervitermes*, *Hodotermes* and *Microtermes* (Mitchell, 2002) are the genera of economic importance. Reported damage by *Microtermes subhyalinus* (Silvestri) in a savannah in the Central African Republic was between 5-15% of annual sugarcane production (Mora *et al.* 1996), 18 % in Sudan and 28% in planted sugarcane seedlings in Nigeria (UNEP/FAO, 2000). In Kenya, foraging activities by termites are responsible for the destruction of 800-1500 kg ha^{-1} of pasture annually (Lepage, 1981). While about 18 genera of termites are known to attack crops in Southern Africa with estimated losses of between 3-100% of annual yield with 90% of tree mortality in the forests (Mitchell, 2002). Traditionally built houses and granaries can be destroyed by termite infestation within 3-9 years if care is not taken (Pearce *et al.*, 1996).

Figure 1. Termite damage in (a) wood, (b) maize and (c) palm tree.

Termite pests Management strategies

Termite control measures include: cultural, physical, chemical (conventional termiticides), biological control methods and the possible use of semiochemicals (Hickin, 1971; Culliney and Grace, 2000; Carr, 2006). These, are tailored towards protecting growing crops, both annual and perennial, including tree plantations and structures that are made of vulnerable cellulosic and non cellulosic materials.

The cultural control practices frequently involve variations of standard horticultural practices centred on modifying the relationships between a pest population and its natural environment (Meyer, 2006). These methods are commonly referred to as ecological control methods, and include processes like crop rotation, intercropping, and managed application of water or fertilizer, use of ash and proper sanitation (UNEP/FAO, 2000).

Distinct from that are the physical termite management strategies were commonly practiced in the 1930's and 1940's. These included, stirring up dust to suffocate the termites, use of physical barriers during building pre-construction phase. Some of the most commonly used barriers are stainless-steel wire mesh (TermiMesh ®).

Conventional termiticides comes in different formulations and packaging, some are in granular form (granules), dusts, soluble powders or wettable powders, emulsifiers, aerosols or Ultra low-Volume Concentrates (ULV) containing about eight different compounds in one formulation. Termiticides are used in soil treatment by creating a chemical barrier between termites and the soil (Jones, 2006). Some termiticides in use include bifenthrin (Talsar®), cypemethrin (Demon ®), and permethrin (Dragnet ®) (Jones, 2006).

Biological control of termites constitutes an environmentally acceptable alternative to traditional chemical control measures. When successfully implemented, it can yield permanent, cost-effective (even zero-cost) management of pest populations with minimal environmental disturbance.

Termites have a wide variety of predators, both opportunistic and specialist, ranging from invertebrates to vertebrates (New, 1991; Culliney and Grace, 2000). Ants are seen as the greatest enemies of termites in all regions of the world (Hölldobler and Wilson, 1990); because their habitats overlap (Fujiwara-Tsujii *et al.*, 2006). A large percentage of ant species from the largest genera, *Pheidole spp* and *Componotus spp*, prey opportunistically on termites (Hölldobler and Wilson, 1990). Termite specialist ants are mostly in the subfamilies Ponerinae and Myrmicinae (Lévieux, 1966; Maschwitz and Mühlenberg, 1975; Longhurst *et al.*, 1978; Longhurst *et al.*, 1979; Maschwitz and Schönegge, 1983; Lepage, 1984; Corbara and Dejean, 2000).

Pachycondyla analis an ant noted for its organised raids on termites preyed upon most members of the Macrotermitinae (Longhurst *et al.,* 1978; Lepage, 1981; Bayliss and Fielding, 2000). *P. analis* may make repeated raids on a termite colony until the colony is destroyed (Sheppe, 1970). Recently, studies by Cornelius *et al*., (1995); and Cornelius and Grace (2000), have explored the potential of semiochemicals from ants in repelling termites

The Biology of *Pachycondyla analis*

Pachycondyla analis (Latreille, 1802) is a Hymenopteran in the suborder Apocrita, superfamily Vespoidea, Family Formicidae, Sub-family Ponerinae, and Tribe Ponerini (ITIS, 2007).

P. analis nests underground, often beneath rocks, in deserted termite hills, or under trees. These nests often extend up to 0.7 m below the surface. Each colony uses a single nest where, eggs and larvae are kept together with the queen in one chamber, while the cocoons are often placed in the sun outside the nest entrance (Lepage, 1981; Villet, 1990). Colonies of *P. analis* consist of between 442-1400 members with a polymorphic worker caste (Longhurst and Howse, 1979; Lepage, 1981). Workers have been regarded as dimorphic by taxonomists, but measurements of head width versus head length indicated that rather than being dimorphic, this species exhibit monophasic allometry (Crewe *et al.,* 1984). These termitophagous ponerine ants are widely distributed in Africa (Sheppe, 1970; Longhurst *et al.,* 1978; Lepage, 1984; Bayliss and Fielding 2002, and Taylor, 2006). The size of workers varies between 5-18 mm (Rödel and Braun, 1999), the morphology of the queen is convergent with that of the workers, and like in other social insects the biology of the males is different from the workers (Villet, 1990).

Morphological differentiation is exhibited among workers in terms of size. Larger workers (Figure 2) have a fine pubescence, which is much reduced in smaller workers with an interocular width less than 1.12 mm. Smaller workers are black and shiny. Mated workers are absent in the colonies of *P. analis*, with reproduction being exclusively the role of the queen (Villet, 1990).

Figure 2 Major workers of *Pachycondyla analis*

Termite predation by *P. analis*

Livingstone (1857) was the first to describe the hunting raids of the African Ponerinae ant *P. analis*. Later a great number of entomologists became interested in the hunting behaviour of this species, which is known to feed exclusively on termites, mostly Macrotermitinae, and to forage by group predation. Sheppe (1970) reported *P. analis* as one of the frequent and specialised predators of termites. He further observed that *P. analis* organises two types of raids on termites, i.e. quick raids by hundreds of ants on underground nests or solitary raids by single ants on termites on the surface.

The organised raids as described by Longhurst *et al.*, (1978), start with single major workers (scout ants), emerging from the nest in search for foraging termites. During the searching phase a scout ant moves slowly (ca 3.0 cms^{-1}) (Longhurst and Howse, 1979) actively palpating the litter and any termite soil sheeting with their antennae, searching through litter and under wood. This searching phase can last for an hour and can cover an area of up to 95 metres from the nest. If the scout ant is not successful, it generally returns to the nest by a circuitous route, but its behaviour changes for the return journey if potential termite sources were found. The stimulus for the behavioural change is attributed to chemicals from termites incorporated into the soil sheeting (Longhurst and Howse, 1977). *P. analis* detects termites using kairomones of its prey, which are

believed to act as pheromones (see page 17 for details on semiochemical classes) within the termite colony (New, 1991). After locating the prey the scout ant returns to the nest laying a chemical trail that is used to recruit a column of worker ants to the termites nest/gallery. On the return journey to the colony the scout ant moves more rapidly (ca. 4.5 cms^{-1}) (Longhurst and Howse, 1979) with its gaster bent downwards and the sting partially extruded, the antennae held upwards and away from the soil surface. Return journeys to the nest are more direct than the outward journeys (Longhurst, *et al*, 1978). However, it has been suggested that if termite nests are abundant this process of recruitment by scout ants could be absent (Lévieux, 1966).

If recruitment is successful, a column of ants, comprising of all worker caste, emerges from the nest in about 60-300 seconds (Sheppe, 1970; Longhurst and Howse, 1979) after the return of the scout ant, which leads them, accompanied by 5-12 other major workers. Foraging parties follow the same route used by the scout ant on return from scouting with each ant bending its gaster downwards.

During the raids, the ants spread out and break open the soil sheeting constructed by termites to cover their food and dig into the termite galleries. Sister workers are attracted to points where single ants are digging and assist them even when they are not in direct line-of-sight with the attracted ants, suggesting non-visual cues. When the raid has been completed, worker ants, predominantly majors, pick up a number of termites (2-10) in their mandibles and the column of ants returns to the nest along the same trail usually keeping close formation with a column width of 3-5 ants wide in close ranks (Sheppe, 1970; Longhurst *et al.,* 1978; Longhurst and Howse, 1979). When ants are disturbed either during outward or return journeys, they make audible stridulatory sounds. Hölldobler *et al.,* (1994) showed that stridulation signals made by *P. analis* do not serve any intraspecific communication, but serve as a warning to potential predators such as birds and mammals of the powerful sting a *P. analis* worker can administer.

Raids by *P. analis* are reported to occur twice in a day, during the first two hours after sunrise and the last two hours before sunset (morning and evening, Sheppe, 1970; Longhurst and Howse, 1977; Longhurst *et al.,* 1978; Longhurst and Howse, 1979). Lepage (1981) also reported a third peak of activity in Kajiado a Kenyan semi-arid ecosystem. Morning raids are most frequent between 0700 to 0930hr and the evening

raids between 1630 to 1830hr. The termite species preyed upon by *P. analis* mainly belong to the genus Macrotermitinae (Longhurst *et al.*, 1978) which are of great economic importance mostly in sub-Saharan Africa (see section on the economic importance of termites).

Semiochemicals

A generally accepted terminology has evolved to classify the functional chemical substances in insect communication (see Figure 3 for a schematic of semiochemical groupings). A **semiochemical** or **infochemical** is any chemical compound used in communication, whether between species (as in symbioses) or between members of the same species (Hölldobler and Wilson, 1990; Meyer, 2006). The signals transmitted between individuals of different species are called **allelochemicals**, while those mediating behaviour between individuals of the same species are known as **pheromones**. Pheromones are usually glandular secretions which when released by one individual trigger a behavioural response from other individuals upon tasting or smelling it (Hölldobler and Wilson, 1990; Howse *et al.*, 1998; Torto, 2004).

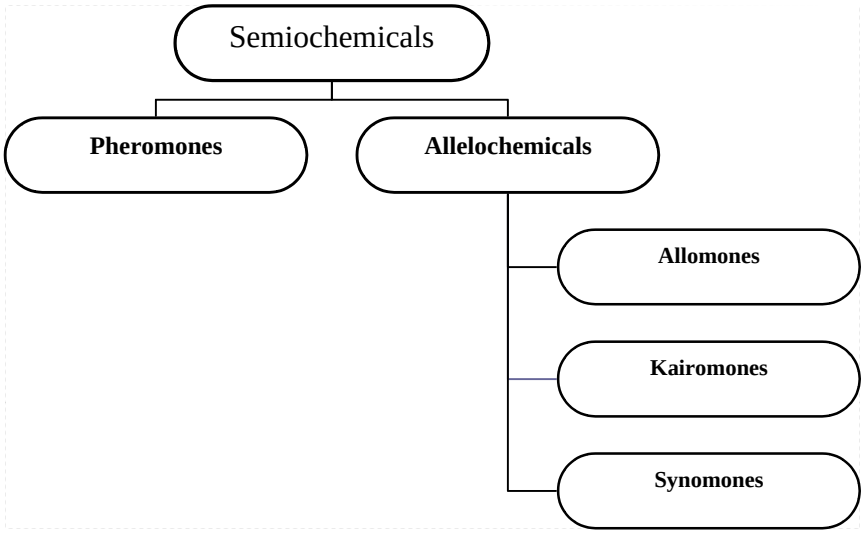

Figure.3 Schematic diagram showing the classification of semiochemicals

Allelochemicals are those chemicals used between species. Allelochemicals are further subdivided into three broad classes: **allomones**, **kairomones** and **synomones** (Nordlund, 1981; Hölldobler and Wilson, 1990; Torto, 2004). Allomones are signals that benefit the emitter while being of negative or no significance to the receiver, e.g. a

lure used by a predator to attract a prey. Kairomones are signals which are of benefit to the receiver by either evoking a behavioural or physiological reaction e.g. chemicals used by insect pests to locate a potential host plant. While synomones benefit both the emitter as well as the receiver, e.g. floral odours from plants which attract pollinators.

Chemical Communication in Ants

Ants communicate in various ways, including; tapping, stridulation, stroking, grasping, nudging, atenations, as well as tasting, puffing or streaking of chemicals that induce responses ranging from recognition to recruitment and alarm (Hölldobler and Wilson, 1990).

Chemicals used in communication by ant societies contain complex mixtures of substances which vary in molecular composition and in their relative proportions (Hölldobler and Wilson, 1990). These multicomponent signals are either produced in single exocrine glands or can be blends composed of secretions from several glands (Hölldobler and Wilson, 1990). Chemical signals can be combined with cues of other sensory origin, such as vibrational or tactile stimuli (Hölldobler, 1995).

The role of odours in behaviour of ants

The ambient atmosphere contains complex mixtures of millions of volatile compounds, that makes it difficult to understand how organisms distinguish and discriminate between certain odours. Olfaction is said to be a universal sense that permits all animals to find food, identify con-specific mating partners, and avoid predators (Keller and Vosshall, 2003; Bruyne and Baker, 2008). Blends of volatile organic chemicals that differ in size, shape, charge, and functional groups are said to make up the stimuli that control olfactory-driven behaviours (Keller and Vosshall, 2003). The puzzle of how the brain processes complex and often contradictory blends of odorant chemicals from the environment into meaningful odour stimuli is still not clearly understood (Keller and Vosshall, 2003). Recently Bruyne and Baker (2008), attributed the detection and encoding of odours in insects to volatile codes encoded in the olfactory receptor neurons (ORN).

According to Malnic *et al*., (2004), the stimulus spectrum which the olfactory system has to deal with is very different from that of other senses i.e. touch and taste. Light and sound stimuli occur (in very limited dimensions) in contrast to odour molecules, which

are multi-dimensional in nature with different chain lengths, chiralities and functional groups. While the visual system functions with a lower number of photoreceptors, the olfactory receptors (ORs) constitute the largest gene family in the human genome, made up of approximately 300 functional genes (Malnic *et al.*, 2004), with 170 Or genes (Robertson and Wanner, 2006) annotated in the honeybee. Yet the human sense of smell is surpassed by that of other animals (Keller and Vosshall, 2003). Insects have a large number of olfactory receptors on a given organ e.g. the antenna, and have developed an extreme sensitivity to certain odours (Keller and Vosshall, 2003). Many insects rely almost exclusively on odour cues in their search for food, nest and mates and in the detection and avoidance of their natural enemies.

What do we know about chemical communication in *P. analis*?

The raiding behaviour of *P. analis* has attracted entomologists and myrmecologists to study it and try to look at the coordinated way in which it is organised. Studies like those of Longhurst *et al.*, (1979) and subsequently those of Hölldobler *et al.*, (1994) and Janssen *et al.*, (1995) did try to understand the use of trail pheromones and their sources in *P. analis*.

Longhurst *et al*., (1979) identified the Dufour's and mandibular glands as the sources of trail pheromones in *P. analis*. The active compounds identified in the Dufour's gland were *n*-undecane and *n*-tridecane. While they identified dimethyl disulfide, dimethyltrisulfide from the secretions of the mandibular glands as those responsible for attraction and digging by sister worker ants during raids.

Hölldobler *et al.*, (1994) discovered other sources of trail pheromones in *P. analis*, as the pygidial glands. Janssen *et al.*, (1995) using microreaction techniques with GC-MS identified *N, N*-dimethyluracil, and actinidine as the volatiles in the poison gland. Using the same techniques and approach to identify the chemical components of extracts from the pygidial gland proved futile. They attributed this to the volatility of the components as suggested in Hölldobler *et al.*, (1994).

Thesis organisation

The aim of this study was to undertake detailed behavioural studies on the raiding behaviour of *Pachycondyla analis* in order to determine the ant's ecology, prey

preferences and factors affecting prey choices in the study area (Mpala, Kenya) for comparison with other habitats. Also *P. analis* seemed to be a potential candidate for use in pest control strategies aimed at termites, however, little is known about its intraspecific chemical communication apart from studies on its trail pheromones. On the other hand, there is no literature that clearly shows how or what cues *P. analis* uses in detecting its termite prey. It is also of considerable interest to understand this interaction between ants and termites from the perspective of co-evolutionary arms race since ants are said to have co-evolved with termites for over 100 million years ago (Hölldobler and Wilson, 1990).

Each chapter in this thesis is presented as a research article

Chapter one investigates the detailed behavioural studies of the interactions between *P. analis* and its termite prey in the field. It examines the nesting habits, raiding dynamics, prey preference based on prey capture and abundance of prey species and possible factors determining prey choice by *P. analis*. **Chapter two** asks the question whether olfactory cues are used by *P. analis* to determine the presence of nestmates either inside or outside the nest during raids when workers are not in line of sight. This chapter also analysed and identified chemicals in the volatile emissions of *P. analis* workers within a colony. GC-MS was used to see whether the volatile profiles differed between different worker groups when they were together and when they were separated. **Chapter three** asks the question if *P. analis* uses contact chemical cues in nestmate and non-nestmate discrimination within and outside the colony. This was tested using a mandible opening response bioassay with ant cuticular hydrocarbons as chemical cues. Also, the chemical composition of the cuticular hydrocarbons (CHCs) from different colonies of *P. analis* were identified using GC-MS. **Chapter four** looks at detection of termite prey by *P. analis*, Olfactometric bioassays were conducted to investigate if ants detect potential food sources using olfactory cues from termites. Chemical composition of the volatiles emitted by termite galleries and termites were also identified by GC-MS. The **General conclusion** looked at the findings from all the chapters and proposed ideas for future research.

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CHAPTER ONE

Termite raiding behaviour of *Pachycondyla analis* at Mpala, a Kenyan savannah

Abstract

Predation on termites of the subfamily Macrotermitinae by the ant *Pachycondyla analis* was studied over a period of six months (April-September 2007) at Mpala, a semiarid savannah in central Kenya. A total of 37 nests of *P. analis* were studied and 330 raids were observed and recorded. There were 17.0 nests/ha, with most nests located under rocks. There were two peaks of raiding activity; one in the morning and the other in the evening. Foraging parties travelled a distance ranging from 0.7 m to 39.8 m, with speeds of outward journeys being slower than those of return journeys (6.70 cms^{-1} and 13.03 cms^{-1} respectively). Larger foraging parties spent more time and carried more termites than smaller ones. *Microtermes* spp. was the most frequently preyed upon termite species (66.0%), followed by *Odontotermes* spp. with a prey frequency of 34.0%. Ants spent more time during raids on *Microtermes* than during raids on *Odontotermes*. The results here indicate that prey choice by *P. analis* is not only based on the abundance of prey but also on the costs of foraging, which was influenced by prey defences, size and foraging behaviour.

Introduction

Ants are among the greatest enemies of termites in all regions of the world (Deligne *et al.*, 1981; Hölldobler and Wilson, 1990). They share the same habitats and are extremely abundant in terms of biomass and density (Fujiwara-Tsujii *et al.*, 2006). Predation by ants is thought to have favoured the development of a soldier caste specialised both in chemical and physical defence systems within termite colonies (Waller and LaFage, 1987). According to Hölldobler and Wilson (1990), during their 100 million years of coexistence, ants and termites have engaged in a co-evolutionary arms race, with ants evolving several predatory tactics while termites have responded with defensive strategies. In ants, the most specialised predatory species are concentrated in the morphologically primitive sub-families Ponerinae and Myrmicinae (Lévieux, 1966; Longhurst *et al.*, 1978, Longhurst *et al.*, 1979; Maschwitz and Schönegge, 1983; Lepage 1981; Corbara and Dejean, 2000).

Group raiding strategies are considered to be more advanced than solitary foraging because they involve cooperative behaviours among workers and enable the capture of large prey and hence energy saving (Corbara and Dejean, 2000).

The African poneromorp ant, *Pachycondyla analis* (Latreille) (formerly *Megaponera foetens* Fabr.), is widespread and relatively common throughout most of sub-Saharan Africa. The recorded average number of workers in a colony ranges from 400 in one locality (Lévieux, 1966) to 1475 in another (Lepage, 1981). Group predatory behaviour of the species was first described by Livingstone (1857) and became the focus of several subsequent studies (Wheeler 1936, Hölldobler and Wilson, 1990). The ant feeds exclusively on termites, mainly Macrotermitinae. Scout ants that detect a termite source lay scent trails directly back to their nests (Longhurst *et al.*, 1978; Longhurst *et al.*, 1979; Hölldobler *et al.*, 1994; Janssen *et al.*, 1995). Once in the nest, a scout recruits between 22 and 840 nestmates and guides the column back to the prey by following the trail. Both major and minor workers of *P. analis* take part in the raids (Crewe *et al.*, 1984; Villet, 1990). On arrival at a termite source, major worker ants break open termite galleries and the minor workers invade the galleries (Figure 1.1). Those that capture termites, sting them, carry them out of the galleries and place them near the gallery entrance, and then return to continue hunting. Piles of paralysed termites grow at the

entrance. After about 13-20 minutes, the workers stop hunting and return to the pile of paralysed termites. Major workers grasp 1-7 termites between their mandibles, while minors grasp 1-3 termites. Some do not carry any termites, but lead columns of ants loaded with prey back to their nest (Longhurst *et al.*, 1978).

Figure 1.1 *Pachycondyla analis* during a raid on a termite source at Mpala, Kenya.

The raiding patterns and dynamics of *P. analis* as well as the effects of termite defences, prey size (reward) and abundance of termites on the raiding behaviour of *P. analis* at Mpala, Kenya is reported here. This was aimed at obtaining background information that will be used in understanding the chemical ecological interactions between *P. analis* and its termite preys.

Materials and methods

Study site

This study was conducted at Mpala (0°17'N, 37°52'E), on the 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 northern two-thirds of Mpala is underlain by dissected Archean terrain with

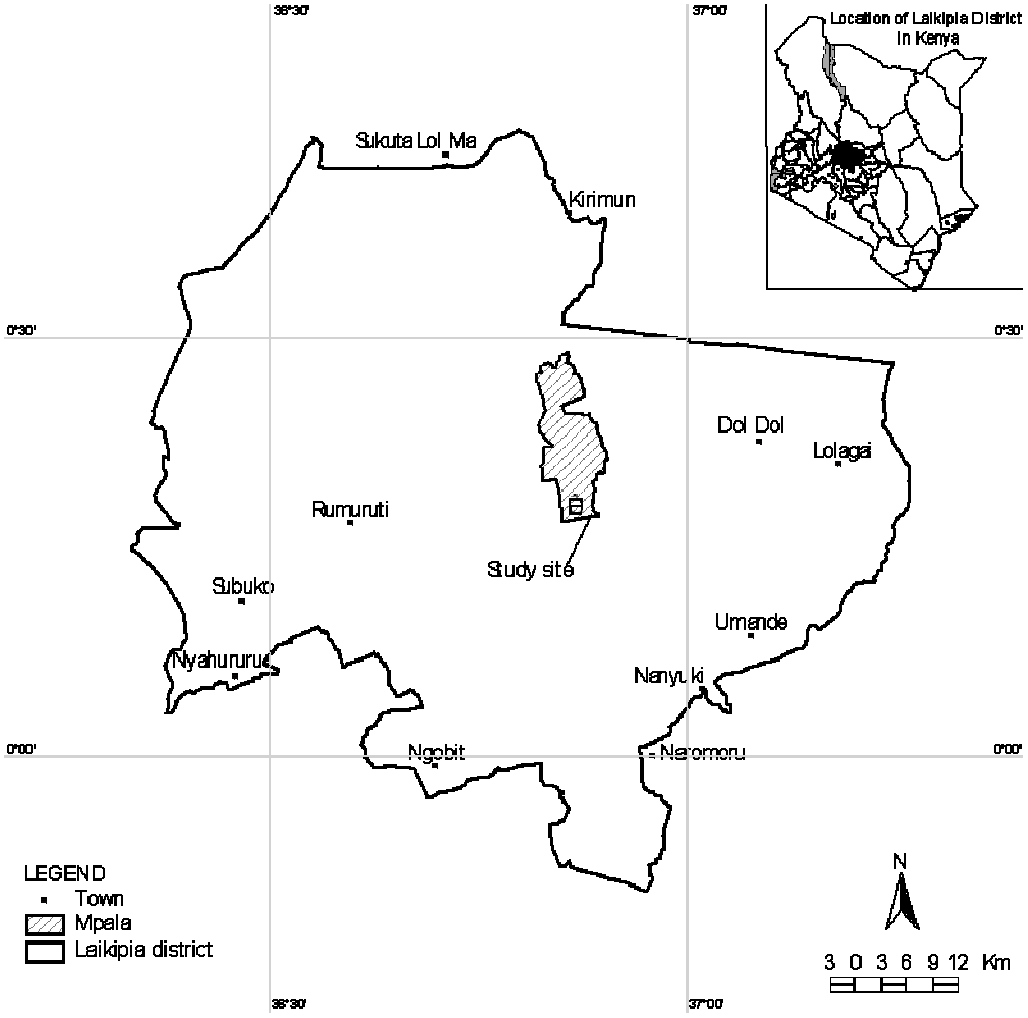

Figure 1.2 Map of Laikipia showing the study site (site) at Mpala research centre. Insert Map showing the location of Laikipia in Kenya).

thin dark red sandy loams (latosols). The southwestern one-third of Mpala is characterised by a 100m high phonolite lava flow. Soils developed on the flow consist of a catena of black clay vertisols with impeded drainage, with brown calcareous loams (chestnut soils) on the higher elevations and steeper slopes. Warm days and cool nights are predominant at Mpala, with very low humidity in the driest season (January-April),

and moderate humidity at other times. Rainfall is weakly trimodal, with peaks in April-May, July-August, and October-November, and a distinct dry season in January-February.

Nest location, distribution and density

Nests of *P. analis* were located using three different approaches (Longhurst *et al.*, 1978; Bayliss and Fielding, 2002; Marcus Stüben; personal communication). These included following ants returning from raids, following scout ants, and looking for pupal cases, termite body fragments, or dead ants near likely nest sites. Nest density was calculated using standard methods (Lepage 1984; Bayliss and Fielding 2002; Diehl *et al.*, 2005). Eight quadrats (50 m x 50 m each) were set randomly within the study area, and the number of nests were counted and expressed as nests per hectare.

Foraging parties

To monitor morning (07.00-11.00hrs) and evening (16.00-19.30hrs) raids, nests were selected opportunistically from each of the eight quadrats. Ant colonies were also monitored for late evening raids between 2200-2400hrs. The monitoring periods were based on prior observations for 14 days of raiding behaviour. Raiding behaviour was monitored daily throughout the study period (April – September).

The number of ants in a raiding column, those carrying termites, and, number of termites carried per ant in a random sample of 10 ants from each raiding column were counted. Ants setting out on a raid were counted one after another if there were less than about 200 in the group. Where there were more than 200 ants, the number was estimated in groups of 10. The number of ants carrying termites was also recorded in the same way. The number of termites carried per ant was determined by carefully grabbing the ants by the thorax using entomological forceps. That triggered the ant to attack the forceps and release the termites. Termites were identified to the generic level by their caste information as outlined in Pearce *et al*. (1996). The number of injured ants during raids was also determined for each raid by counting the numbers of injured ants carried back to the nest by nestmates. Duration of the different phases in a raid (outward journey to termite source, time spent at termite source and time spent on return journeys to nest) was determined for each raid. Foraging distance (m) for each raid was measured. Ground temperatures at departure from ant nests for raids were recorded for

each raiding column. Mean rainfall was also recorded for each month during the study period.

Foraging velocity

The foraging velocity was calculated using the time an ant took to travel a distance of 50 cm. This was repeated fourteen times on the outward and return journeys for randomly selected foraging parties with ants selected at random. Measurements were taken from ants travelling in the main body of the column as the activities of ants on the periphery showed a high level of variation. If an ant stopped during the timing period the record was discarded. These measurements were carried out on open ground to eliminate the effect of variable amounts of litter.

Predation

Predation rate was calculated for each month based on the number of termites taken per nest per day using the equation of Lepage (1981) as modified by Bayliss and Fielding (2002):

$$P = F \times T \times R \times N \times t$$

Where;

P= Termites predated per nest per day

F= Average number of ants carrying termites of that species per raid

T= Average number of termites per ant per calendar month

R= Average number of raids per day per nest per calendar month

N= Number of ant nests per hectare

t= Average duration of raid per calendar month

Predation was also analysed in relation to the abundance of termite genus in the field by comparing the frequency of raids on a given termite species in relation to their abundance within the study quadrats. Termite abundance was estimated in September using a modification of the Jones *et al.* (2003) method. Transects were set up within the same quadrats used to study *P. analis* raids on termites. Each termite transect was 50 m long × 2 m wide, and divided into 10 contiguous numbered sections (each 5 m × 2 m). Two collectors spent 30 minutes each per section. In each section the collectors searched the following microhabitats which are common sites for termites: surface soil

to 5 cm depth; accumulations of litter and humus at the base of trees; the inside of dead logs, tree stumps, branches and twigs; the soil within and beneath very rotten logs; all subterranean nests, mounds, carton sheeting and runways on vegetation, arboreal nests up to a height of 2 m above-ground level and animal dung. The protocol was designed to offer a flexible approach to sampling, whereby the collectors used their experience and judgment to search for, locate, and sample as many species of termites in each section as time allowed. Specimens from each termite population encountered were collected, identified to generic level and sent to the National Museum of Kenya (NMK) Nairobi for confirmation. Voucher specimens were also deposited at the NMK. All castes were collected if present, but priority was given to soldiers and workers. Termites were placed in vials labelled with the section number and identified afterwards. This transect sampling method provided a semi-quantitative measure of the relative abundance of termites based on the number of encounters or 'hits' with each species within a transect (Jones *et al*., 2003).

Statistical analysis

The Mann Whitney U (MWU) test and Student's t-test were used to test for differences between morning and evening activities. Additionally Kruskal Wallis ANOVA (KWA) was used to test for differences in the raiding behaviour (duration of preying at the food source, those of outward and return journeys to and from food source) with respect to the prey species and time of the day. MWU tests were performed for pairwise comparisons. MWU statistics were also applied to test for differences in the efficiency of the ants preying on different termite species, with prey species being the independent variable. The ratio of ants carrying termites to the total raid size, number of termites carried per ant, number of termites per meter distance travelled, number of termites per minute travelled, or the number of injured ant workers carried back was the dependent variables. Only raids in which the prey was fully identified were used in the analyses. Spearman rank correlation was used to test the correlation between raiding party size, rainfall, number of termites taken, and months of study. Chi square (χ^2) tests were performed to compare the predation rates on the two genera of termites during the different months and to the data on prey abundance. All statistical analyses were performed using SAS (9.1) statistical package and at an α level of 0.05.

Results

During the study period in 2007, the month of June which was characterised by low rainfall in previous years, had the highest rainfall (mm): (Mpala Station Records: April, 86; May, 36.4; June, 152.2, July, 80.4; August, 34.9; September, 98.4).

Nest location, distribution and density

A total of 37 nests were located within the sampled quadrats. With regard to the location of the 37 nests, 43% were under rocks, 30% were in old termite mounds, 16% were under trees and 11% were in an open field (Figure 1.3, and 1.4). The distance between the nests ranged from 1.7 m to 29.7 m (Figure 1.5). Mean number of nests per quadrat was 4.25 ± 2.71 which is equivalent to ~17 nests per hectare.

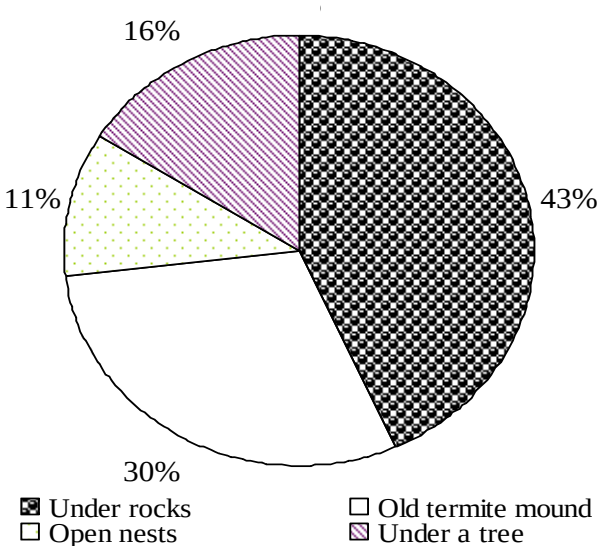

Figure 1.3 Distribution of *Pachycondyla analis* nests in the study area.

Foraging parties

There were 330 raids recorded from the 37 nests. Of these 56% occurred in the morning (0700-1100hrs), 42% in the evening (1600-1930hrs), and 2% were nocturnal (2000-2400hrs). The temperature at which raiding parties of ants departed from the nest to a

Figure 1.4.Nesting habitats of *Pachycondyla analis* at Mpala, Kenya. (A) nest under a rock, (B) nest under a tree, (C) nest in a deserted termite mound and, (D) an open field nest.

termite source was 23.1°C ± 0.21 (range: 17.8°C – 36.4°C). Ants took the same foraging path and direction regularly as long as the supply of termites was abundant. However, with diminishing supply of prey; the ants either changed the foraging direction or divided themselves into smaller foraging parties going in different directions.

The mean number of ants in a raiding party, the number carrying termites, and the total number of termites taken per raiding party varied between months (Figure 1.6). The size of the raiding parties increased significantly during the observation period (Spearman Rank correlation: r = 0.315, $P < 0.01$). The total number of termites taken per raiding party was not significantly different among the different months. However the percentage of ants carrying termites within a raiding party was significantly different and was highest from June to August (Kruskal Wallis ANOVA, $\chi^2 = 23.03$, $df = 5$, $P < 0.001$, Figure 1.7). The number of termites carried per ant was also significantly different (overall mean 2.69 ± 1.21; KWA $\chi^2 = 47.77$ $df = 5$, $P < 0.001$), peaking in July

and falling to April levels by September. The raiding behaviour seems not to be affected by the amount of rainfall, because the number of termites taken (Spearman Rank correlation: r = 0.105, n.s.) and the size of the raiding party (Spearman Rank correlation: r = 0.107, n.s.) did not correlate significantly with rainfall.

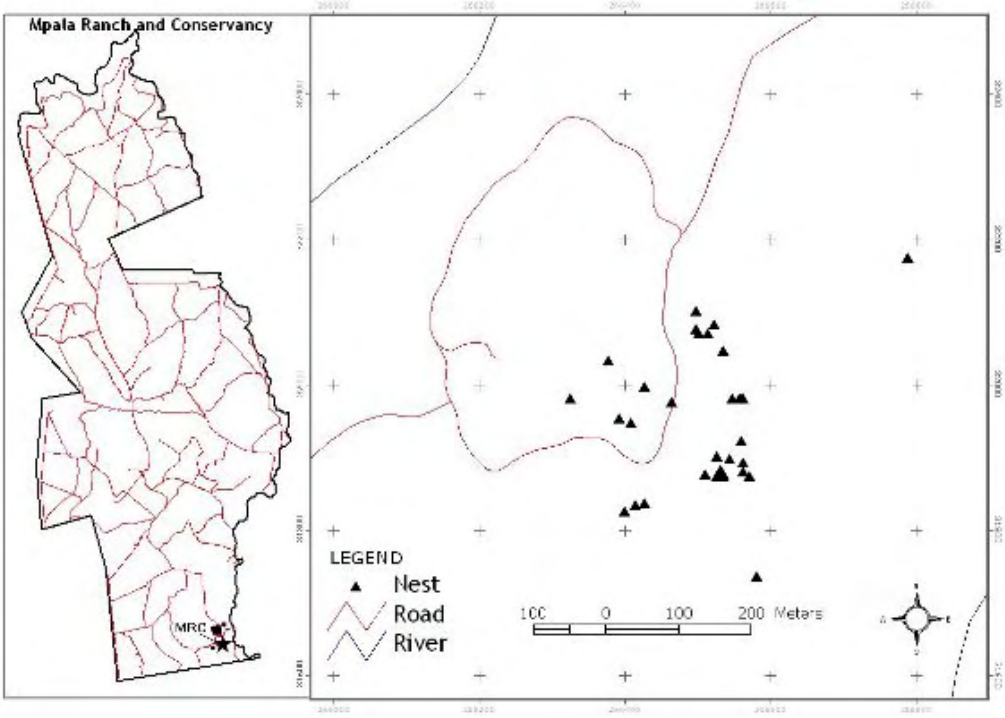

Figure 1.5 Map of Mpala Ranch and Conservancy showing the location of *P. analis* nests within the study area (Mpala Research Centre).

The mean number of ants per raid was significantly higher in the morning than in the evening (t = 2.48, *P* = 0.01, *df* = 323, Figure 1.8). The mean number of ants carrying termites during morning and evening raids was not significantly different (*t* = 1.88, *P* = 0.06, *df* = 309, Figure 1.8) nor was the number of termites carried per raiding party (t = 1.04, *P* = 0.29, *df* = 293) (Figure 1.8).

The foraging distances were longer in the morning than in evening raids, in May, August and September, but the reverse was the case for April, June and July (Table 1.1). The number of termites taken and the size of the raiding party were positively correlated

with distance (Spearman rank correlation: party size, r = 0.38, $P < 0.001$, termites taken: r = 0.4, $P < 0.001$).

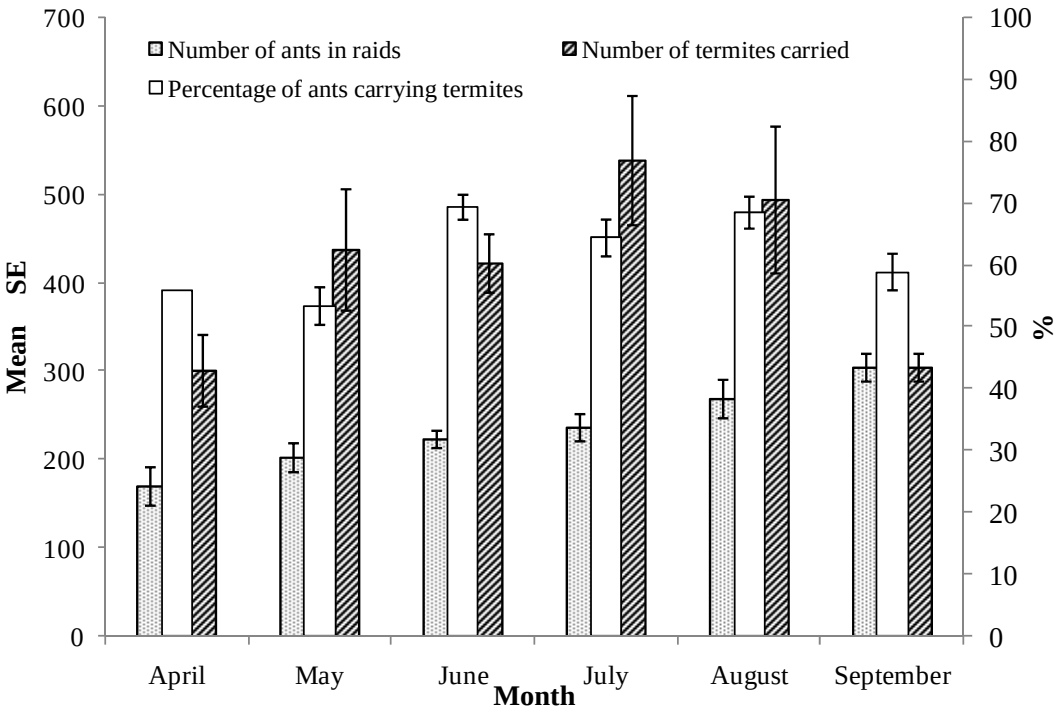

Figure 1.6 Mean numbers (± SE) of ants in a raiding group, percentage of ants carrying termites and number of termites carried.

Duration of raids

The total time spent by foraging parties on a raid ranged between 4 to 99 minutes and the duration of outward journeys (journey to termite sources) was as short as 1 minute and could last up to 35 minutes. Actual time spent in attacking termites at their nest (duration at termite colony) ranged from 2 to 69 minutes and the return journeys took between 1 to 29 minutes. The number of termites taken positively correlated with the time spent at the termite source; the longer the ants stayed at the termite source, the greater was the number of prey captured (spearman rank correlations: r = 0.252 P = 0.001).

There were no significant differences in duration of the outgoing or return journey between termite genera raided (KWA: outgoing: $\chi^2 = 3.8$, n.s.; return: $\chi^2 = 6.7$, n.s. Figure 1.8), but there was a significant difference in the length of time spent at the food source (KWA: staying: $\chi^2 = 3.8$, $P < 0.05$). The ants spent significantly more time at the *Microtermes* sites than at the *Odontotermes* ones (MWU: Z = -2.2, $P < 0.03$, Figure 1.8).

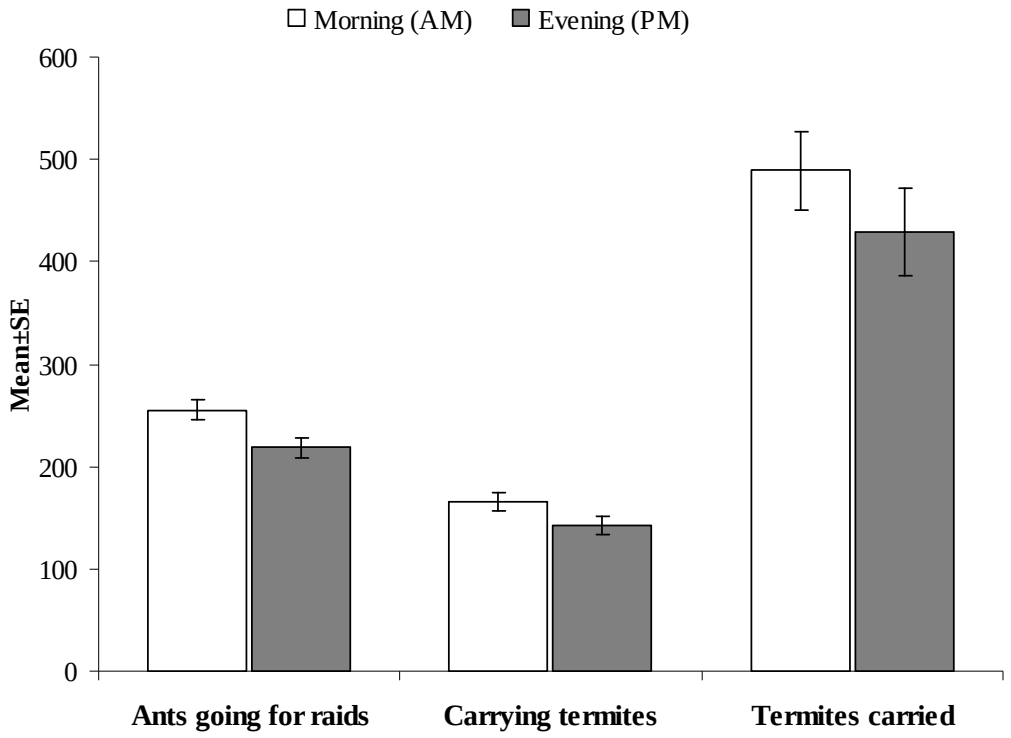

Figure 1.7 Mean numbers (± SE) of ants going on raids, carrying termites and termites carried in morning and evening raids at Mpala. White bars = morning, grey bars = evening

Foraging velocity

Speeds for outward journeys from ants nests were between 3.21 cms^{-1} and 11.06 cms^{-1} (mean = 6.70 ± 2.3 cms^{-1}). Return journeys (mean = 13.03 ± 6.4 cms^{-1}) were significantly faster than outward journeys (MWU: Z = -2.9, $P < 0.003$, n = 28), with a minimum of 3.72 cms^{-1} and a maximum of 22.83 cms^{-1}.

Table 1.1 Number of raids (N), minimum, maximum and mean (± SD) of foraging distances of *P. analis* raiding parties at Mpala between months and time of raids (mornings and evenings). Means were separated using Student-Newman-Keuls (SNK). Means in the same column followed by same letters are not significantly different (α = 95%).

		Foraging distance (m)			
Month	**Period of the day**	***N***	**Min**	**Max**	**Mean ± SD**
April	Morning	3	3.7	10.7	6.6 ± 2.2a
	Evening	15	2.4	29.9	11.1 ± 1.8b
May	Morning	27	2.5	25.6	10.9 ± 1.2a
	Evening	35	1.9	29.6	10.1 ± 0.9b
June	Morning	40	1.5	26.5	9.3 ± 0.6a
	Evening	20	3.4	26.3	11.1 ± 1.1b
July	Morning	49	1.6	39.8	11.4 ± 2.7a
	Evening	18	4.2	31.0	13.6 ± 0.8b
August	Morning	43	0.7	38.2	13.4 ± 1.4a
	Evening	19	1.1	17.3	9.5 ± 1.0b
September	Morning	38	4.5	39.7	12.1 ± 1.0a
	Evening	18	5.2	32.9	11.5 ± 1.6b

Note: Five late night (nocturnal) raids observed in April were not included in the table because it was difficult collecting data from those raids when they occurred.

Predation

P. analis was only observed to prey on two genera of termites at Mpala during the study period. Of the 330 raids, it was only possible to identify prey for 237 raids. *Microtermes* was targeted on average in 66% of these raids and *Odontotermes* in 34%. The latter was more preyed on in April and August (75% and 59%), while *Microtermes* was the main prey in subsequent months (69%, 74%, 75% and 70% respectively).

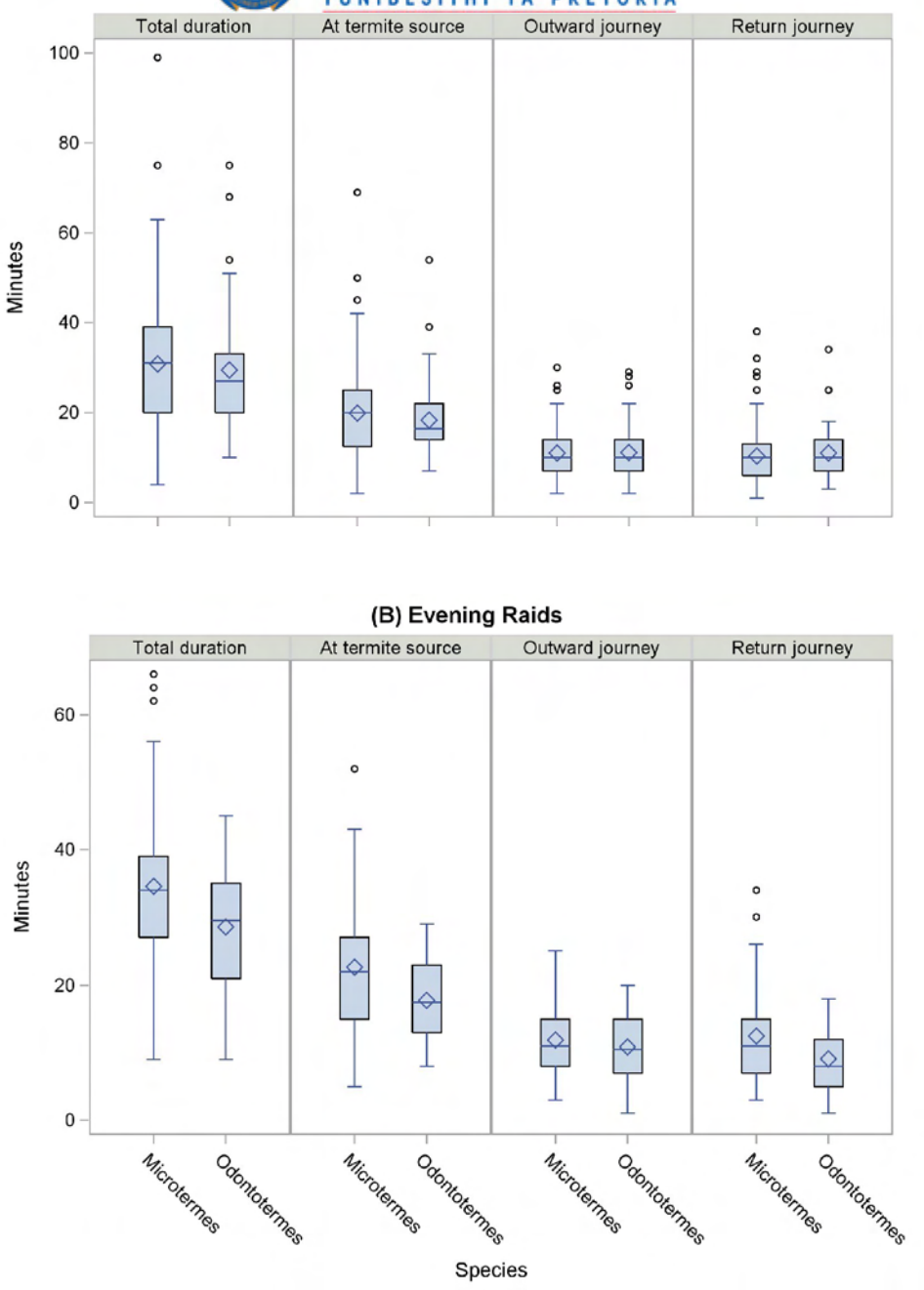

Figure 1.8 Total duration of raids, outward journey, time spent at termite colonies, return journey from raids for (A) morning and (B) evening raids for the two termite genera preyed upon by *P. analis* at Mpala. Circles represent outliers, squares represents the median, middle lines represent the mean, while lower and upper lines represents the 1st and 3rd quartiles respectively.

The average abundance (81%) of *Microtermes* was higher, based on transects surveys in the field compared to the observed prey frequency by *P. analis* (70%) in September (n = 21). However, when comparing the predation rate on the two species for each month relative to their abundance, the ants were preying significantly more often on *Microtermes* in June (χ^2 = 4.9, P < 0.05) and more on *Odontotermes* in August (χ^2 = 8.14, P < 0.01; Figure 1.9).

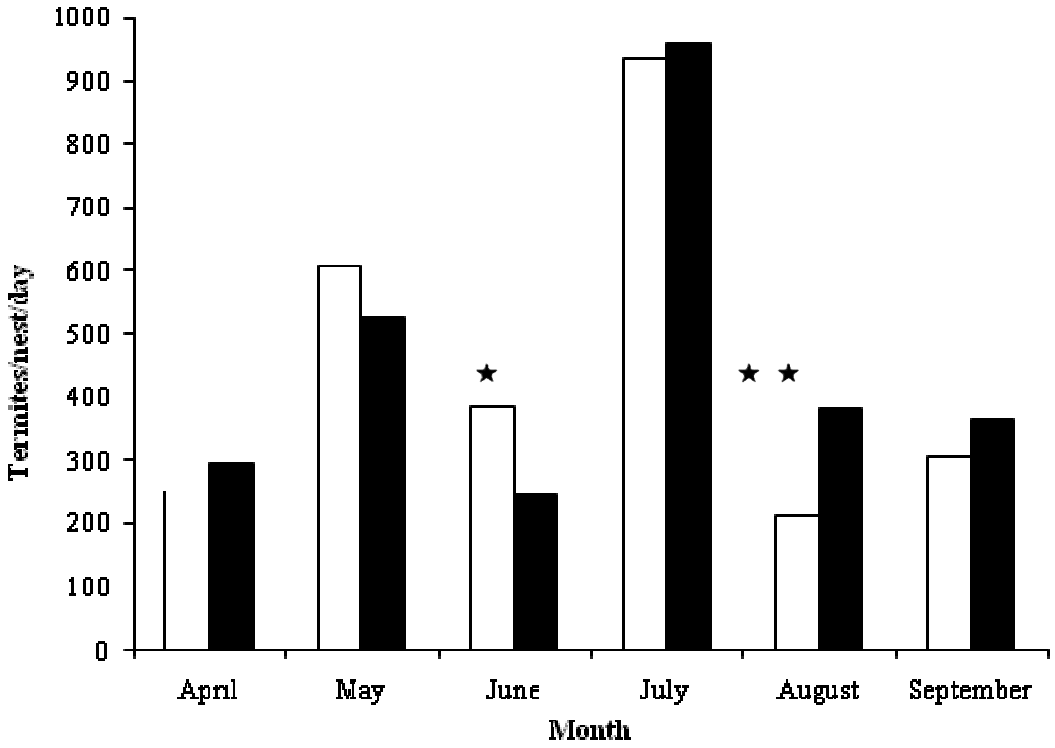

Figure 1.9 Monthly predation rates by *P. analis* based on termite genera prey captures. White bars represents predation rates on *Microtermes* and black bars represent predation rates on *Odontotermes*. χ^2- test, * significant at P < 0.05 and ** significant at P < 0.01.

Overall, the percentage of ants carrying termites (64.62 ± 22.03 for *Microtermes*; 64.25 ± 20.08 for *Odontotermes*) and the total number of ants in the raiding party (259 ± 138.25 for *Microtermes*; 260.60 ± 142.29 for *Odontotermes*) was not significantly different between prey species (MWU: Z = -0.16, n.s.). Similarly, no significant differences were recorded when comparing the termites carried per ant (2.59 ± 1.29 for

Microtermes; 2.89 ± 1.16 for *Odontotermes*) (MWU: Z = -0.89, n.s.) and the termites carried per meter (43.30 ± 35.02 *Microtermes;* 52.16 ± 54.26 for *Odontotermes*) or per minute (25.39 ± 24.74 for *Microtermes*; 34.02 ± 44.09 for *Odontotermes*) (MWU: per meter: Z = -0.55, n.s., per min: Z = -1.79, n.s.) as measures of predator efficiency. However, significantly more injured workers (see Appendix I-ii) were carried back when preying on *Odontotermes* spp. (2.25 ± 2.71) compared to (1.63 ± 2.18) *Microtermes* spp. (MWU: Z = -2.2, $P < 0.03$).

Discussion

The thirty seven nests of *P. analis* encountered in this study were found in various nesting places. Most nests were under rocks and in old abandoned termite mounds. Studies in other localities have indicated a preference for sites that are provided by the particular habitat. Thus *P. analis* at Mpala exhibits a different nesting pattern than observed in the Tanzanian coastal dry forest (Bayliss and Fielding, 2002) and the Nigerian savannah region (Longhurst and Howse 1979). These nesting patterns at Mpala could have advantages in the regulation of the nest temperature and provide protection against rains. A relatively high nest density per hectare of *P. analis* in a savannah habitat with 17.0 nest/ha was observed in this study, compared to 3.8 nests/ha at Kajiado in Kenya (Lepage, 1981), 9.0 nest/ha in a Nigerian guinea savannah (Longhurst *et al.,* 1978) and Tanzanian coastal dry forest (16.0 nests/ha, Bayliss and Fielding, 2002). However, the observed density was lower than reported in a gallery forest in the Ivory Coast (Lévieux 1966, 20.0 nests/ha). Although savannah woodlands and secondary costal dry forests are normally richer in biodiversity than semiarid savannah, other habitat factors may favour the abundance of *P. analis* in Mpala.

The main raiding activities of *P. analis* were in the mornings (0700 - 1100hrs) and evenings (1600 - 1930hrs), similar to the behaviour reported in other habitats. Longhurst *et al*., (1978) recorded two raids daily per nest in a Nigerian savannah between 0700 - 0930h and 1630 - 1830hrs. Bayliss and Fielding (2002) also reported two similar peaks of activity per day in a Tanzanian coastal dry forest. However, observations revealed a minor raiding activity in the early part of the night (nocturnal) between 2000 - 2400hrs during the month of April, which represented about 2% of the total raids observed. Lepage (1981) recorded three peaks of activities during the rainy season and two peaks

of activities in the dry season at Kajiado, a Kenyan savannah. Nocturnal raids at Mpala were only observed in the drier month of April rather than in wet months (June, July and September) similar to earlier results (Lepage 1981). This may reflect the need to avoid high daytime temperatures and low humidity and reduce the risk of losing the trail pheromone laid by scouts and workers during foraging and raids in the morning. Alternatively, but not mutually exclusive the foraging activity of the prey (termites) might be increased at that time resulting in an increase in the raiding behaviour by *P. analis* at night or the evening. Indeed, Asian *Odontotermes* spp. show increased foraging activity in the pre-monsoon season (Rajagopal, 1990) and April 2007 constituted the end of the dry season with the long rainy season commencing in June. This is because soil texture, environmental conditions, especially ground temperatures, and relative humidity favours the foraging activity of termites especially *Microtermes* spp. and *Odontotermes* spp. (Sattur *et al*. 2007; Badawi *et al.*, 1984). This suggests that the change in foraging activity of the ants might be as a result of the changes in termites foraging activity.

Ground temperatures for departing *P. analis* foraging parties at Mpala fell in the range of 19 - 34 °C (average 23°C) similar to that reported by Bayliss and Fielding (2002). The average temperature is lower than that reported by Inara and Paulo (1995) for raids of the Neotropical termite-hunting ant *Pachycondyla marginata* in a semi-deciduous forest in Brazil during rainy (30°C) and dry (28°C) seasons. No foraging party was observed leaving the nest at midday, presumably because of heat stress and perhaps the volatility of their trail pheromone. On one occasion, ants coming back from a raid were disoriented when it rained making it difficult for them to detect the trail laid on their way out.

At Mpala, the average number of ants in raiding columns was smaller than those previously reported by other workers from a Nigerian savannah (Longhurst *et al.* 1978), in Kajiado Kenya (Lepage, 1981), and a Tanzanian coastal dry forest (Bayliss and Fielding, 2002). This suggests that *P. analis* colonies at Mpala may be smaller than those previously reported elsewhere if the number of ants per foraging party as suggested by Bayliss and Fielding (2002) reflects the size of the colony. Indeed, nests excavated for chemical ecological analysis had 270 - 425 individuals per colony (n =

11), which is fewer than the average of 400 individuals (Lévieux, 1966) and 1475 (Lepage, 1981) from Ivory Coast and Kajiado in Kenya respectively.

The maximum foraging distance (39.8 m) was higher than 35.4 m reported by Lepage (1981), but shorter than 44.0 m from a Tanzanian coastal forest (Bayliss and Fielding, 2002). It was considerably shorter than the 95.0 m reported by Longhurst *et al.* (1979) in a Nigerian savannah and 97.0 m for *P. marginata* (Inara and Paulo, 1995) in a Brazilian semi-deciduous forest. In general, mean foraging distances at Mpala were shorter than those reported previously by Longhurst and Howse (1979), Lepage (1981) and Bayliss and Fielding (2002). Longhurst and Howse (1979) observed shorter foraging distances at the beginning of the rainy season in June, which then increased in July, August and September. This foraging pattern is influenced by the behaviour of termites during rainy seasons when the colony increases in numbers with reproductive alates flying away to form new nests (Pearce *et al.*, 1996), thus increasing the abundance of prey items for *P. analis*. Therefore the observed shorter foraging distances might be a result of a higher abundance of food in closer proximity to the ant nests.

The duration of raids at Mpala for both morning and evening was shorter than those in a Tanzanian coastal dry forest (Bayliss and Fielding, 2002), and at Kajiado Kenya (Lepage, 1981). The longest raids lasted for just over 1.5hrs. This is much shorter than raids of *P. marginata* which usually last up to 24hrs covering distances of about 38 m (Inara and Paulo, 1995). Raids in *P. marginata* occur approximately after every 2-3 weeks, whereas in *P. analis* they occur more frequently with at least one raid per week. The length of the stay at the termite source was longer than in previous studies (e.g. Longhurst and Howse, 1979; Lepage, 1981; Bayliss and Fielding, 2002) which might be related to differences in the prey species. In the previous studies (Longhurst and Howse, 1979; Lepage, 1981; Bayliss and Fielding, 2002) *Macrotermes* was the main prey which has a formidable soldier caste which when recruited to a site of attack may reduce the duration of foraging by the ants. The time spent at a termite source is strongly correlated with the number of termites taken thus supporting some of the earlier findings (Bayliss and Fielding, 2002). Furthermore, the ants spent significantly more time in raids on *Microtermes* than *Odontotermes*. This might be due to defence by the soldier caste of *Odontotermes* that resulted in significantly more ants workers being injured when raiding *Odontotermes.*

Foraging velocities for return journeys were faster than those of outward journeys. This is in agreement with reports by Longhurst and Howse (1979), but contrary to findings in a coastal dry forest (Bayliss and Fielding, 2002). The foraging velocity of outward journeys is likely to be lower because all the ants in the foraging party rely on the scout ant who leads in following the original trail laid by the scout. In a situation where the scout was unable to locate the trail, the foraging columns went back to the nest. This may partly explain why group-raiding ants prepare to raid at lower temperatures when trail pheromones are likely to persist for longer (B. Torto, personal communication). Return journeys from raids may be faster because of a better defined and more concentrated trail laid collectively during the outward journey.

The predation rates per nest per day by *P. analis* on *Odontotermes* spp. and *Microtermes* spp. at Mpala were higher (Figure. 1.9) than those reported by Longhurst and Howse (1979) and Lepage (1981). This reflects a higher prey capture in this study that may be due to the higher relative abundance of prey.

The two genera of termite prey of *P. analis* at Mpala were also reported in Nigerian savannah (Longhurst *et al.,* 1978). Lepage (1981) found *Macrotermes* spp. and *Odontotermes* spp. to be the main prey of *P. analis* at Kajiado Kenya with a few predating on *Synacantothermes* spp. The most frequently raided genus of termites at Mpala during the study period was *Microtermes* which was taken more in the months of June through to September. Although *Trinervitermes* was encountered in lower proportions (~5%) during termite sampling, *P. analis* were not seen preying on these termites. Longhurst *et al.* (1978) suggested that several factors might influence prey selection. These include relative abundance of termite species, seasonal foraging patterns, foraging behaviour, size, and the interactions between the ants and termites. At Mpala, *Microtermes* were most frequently preyed upon (66%); however, this was significantly lower than their relative abundance (81%) within the study site. This may be attributed to the relative body mass between the two termite genera. *Odontotermes* being bigger than *Microtermes* are a more rewarding food source for the ants (Longhurst *et al.,* 1978) and fewer individuals may be taken as a result. Moreover, foraging behaviour of a termite species can also influence its predation by *P. analis*. *Odontotermes* spp. forages principally on wood, grass or litter on the surface, covering their food with a thin layer of soil and feed beneath this protective cover. Foraging ants

usually break open these soil sheathings and remove the termites. Other species such as *Microtermes* forage on the surface only occasionally. They principally forage within their food sources that include roots, grasses and wood litter, entering it at points of contact with the soil that are perhaps more difficult for *P. analis* workers to enter. *Microtermes* forage less at the surface during the dry season, whereas *Odontotermes* forage mainly during the dry season (Bayliss and Fielding, 2002). This results show likelihood that abundance of *Microtermes* contributed to its preference by *P. analis* as the ants had about a six-fold greater chance of encountering *Microtermes* compared with *Odontotermes* (see termite abundance results in Appendix I). In addition, physical interactions between *P. analis* and its termite prey may also play a role in the choice of prey, since preying on *Odontotermes* resulted in more injured ant workers (Appendix I-ii). Although *Odontotermes* spp. is more defensive the greater weight per termite provides a greater reward. Our results indicate that prey choice by *P. analis* may not only be based on prey abundance, but also on the costs involved in foraging.

Acknowledgement

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APPENDIX I-i

Termite abundance within sampling quadrats at Mpala

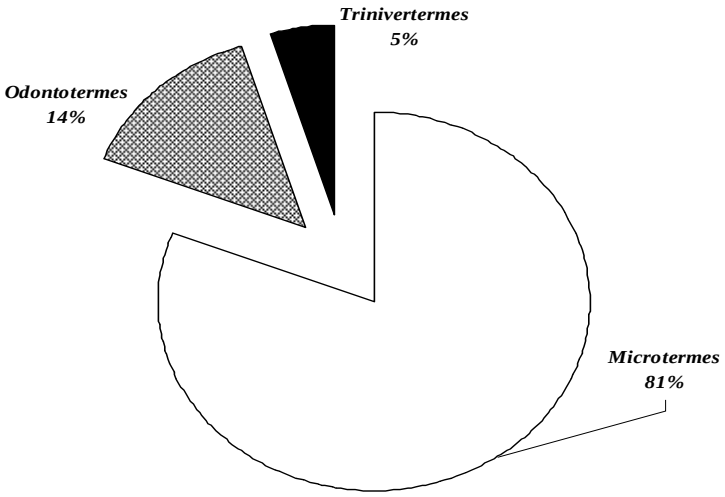

Figure 1. Relative abundance of termite genera within sampling quadrants at Mpala.

Appendix I-ii

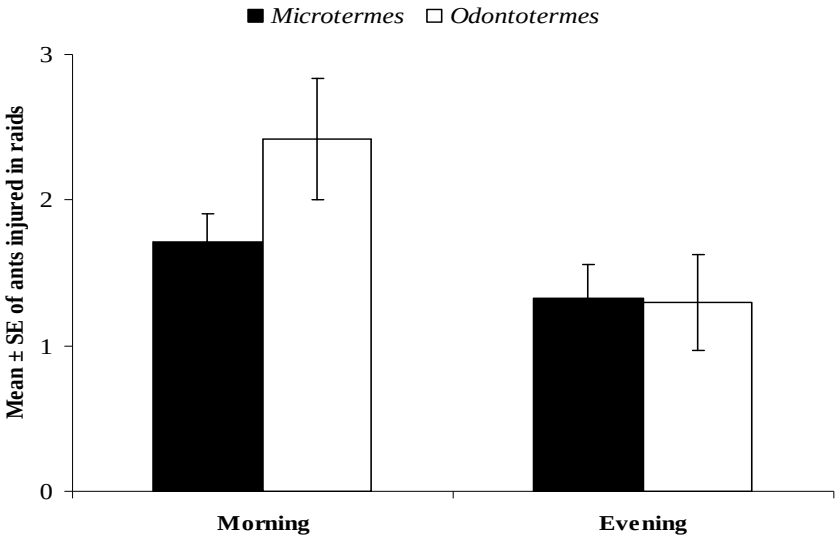

Figure 2. Number of ants injured calculated from the number of ants carried by con-specifics when returning from a raid. Black and white bars represents injured ants from *Microtermes and Odontotermes* raids respectively.

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.

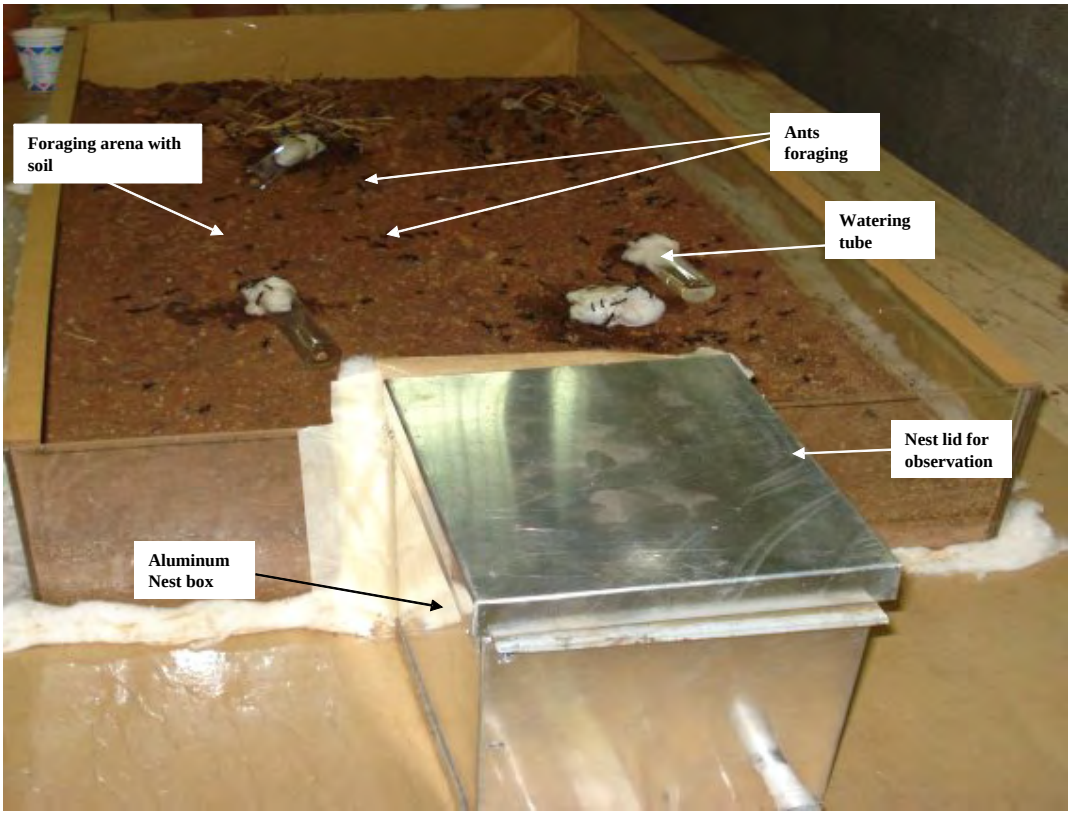

Figure 2.2 Ant rearing set-up in the laboratory, showing the ants nesting box, foraging arena, watering point and nest observation lid.

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).

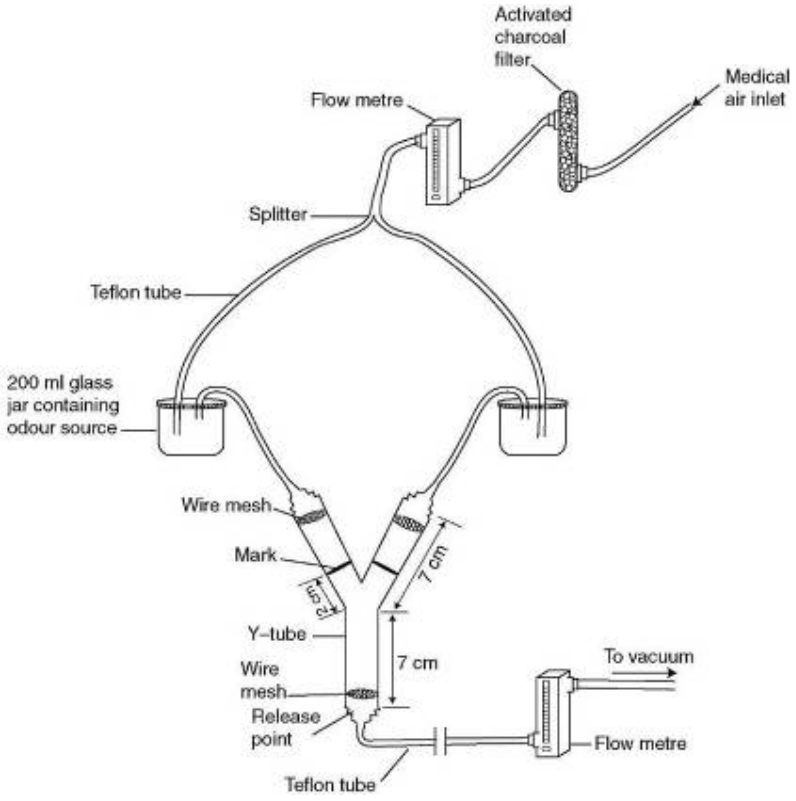

Figure 2.3. Schematic diagram of the Y- tube olfactometer bioassay set-up (not drawn to scale) used in the olfactometric bioassays of conspecific volatiles of *P. analis*.

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.

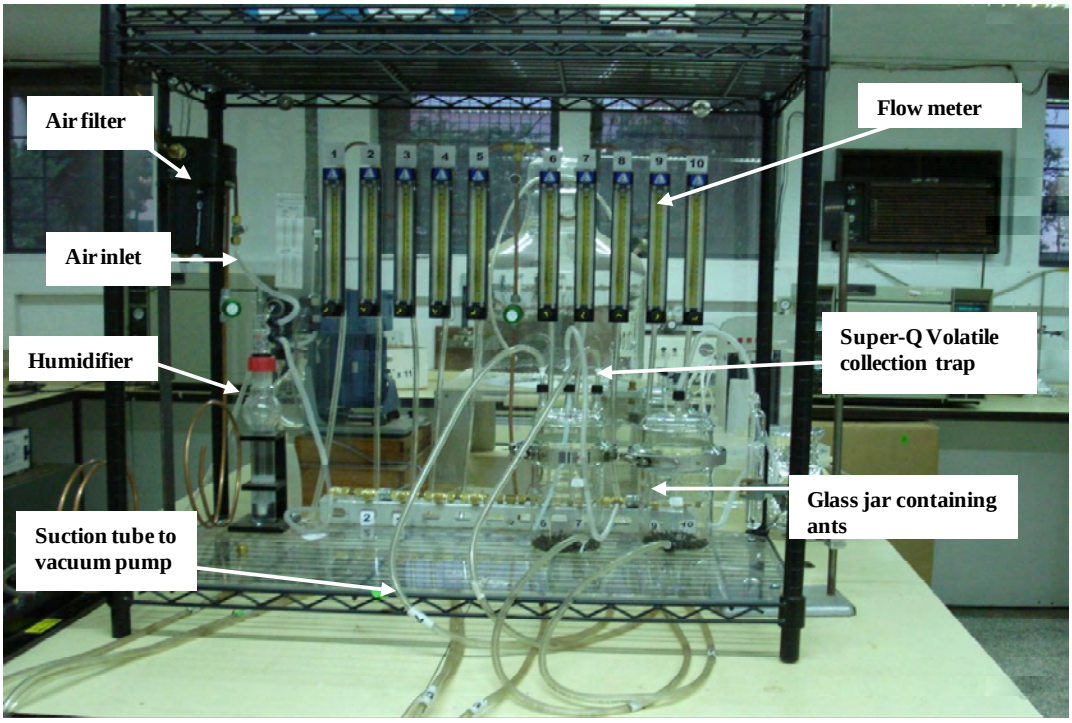

Figure 2.4 A push-pull volatile trapping collection system used to trap chemical volatiles from ants.

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).

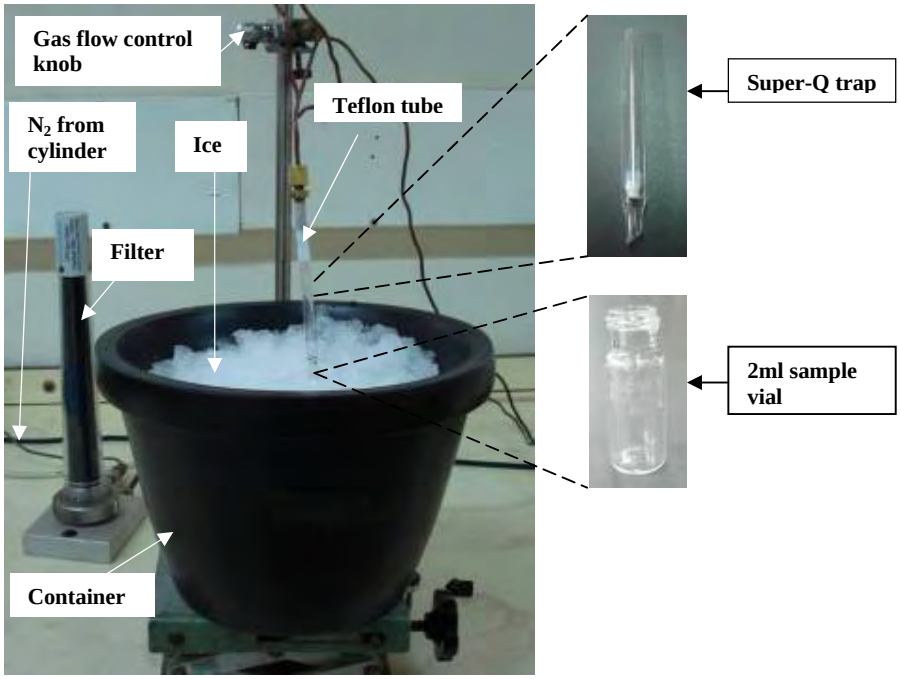

Figure 2.5 Set-up for eluting volatiles from Super-Q traps in the laboratory after collecting volatiles from ants.

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).

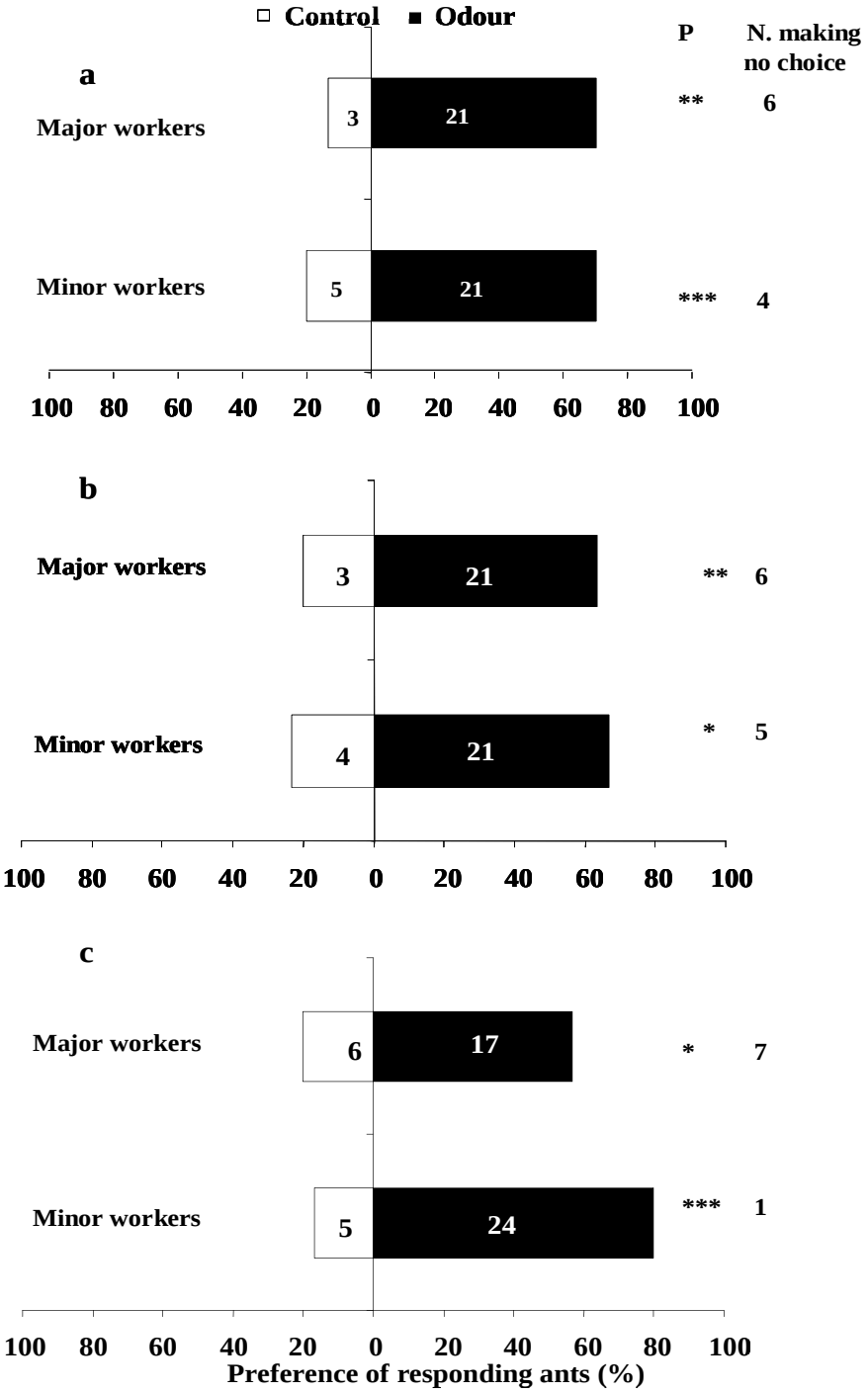

Figure 2.6 Preference of major and minor workers *P. analis* to odors of (a) Mixed ants (major and minor), (b) Major and (c) Minor workers respectively. Numbers within bars refer to the number of ants making a choice, while numbers outside bars refer to ants that made no choice. (*N*= 30 each for major 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).

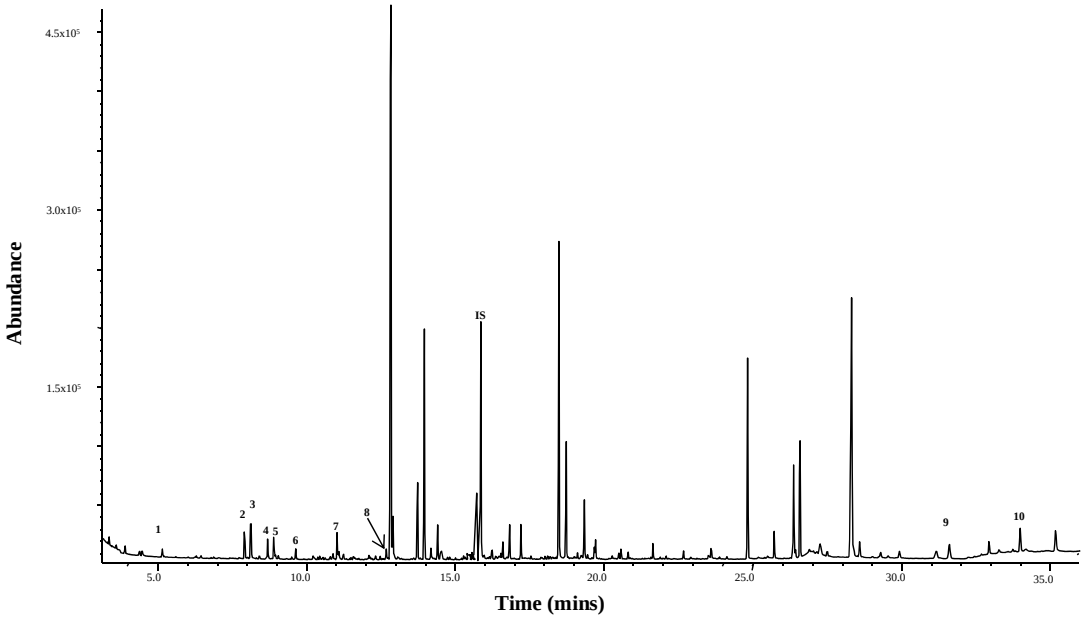

Figure 2.7 Representative GC-MS profile of odours collected from mixed (100 major and 100 minors) worker ants of *P. analis* by trapping for 14 hours overnight. Numbered peaks are compounds unique to volatiles of mixed workers. 1) Ethyl propanoate, 2) ethylbenzene, 3) p-xylene, 4) o-xylene, 5) Nonane, 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.

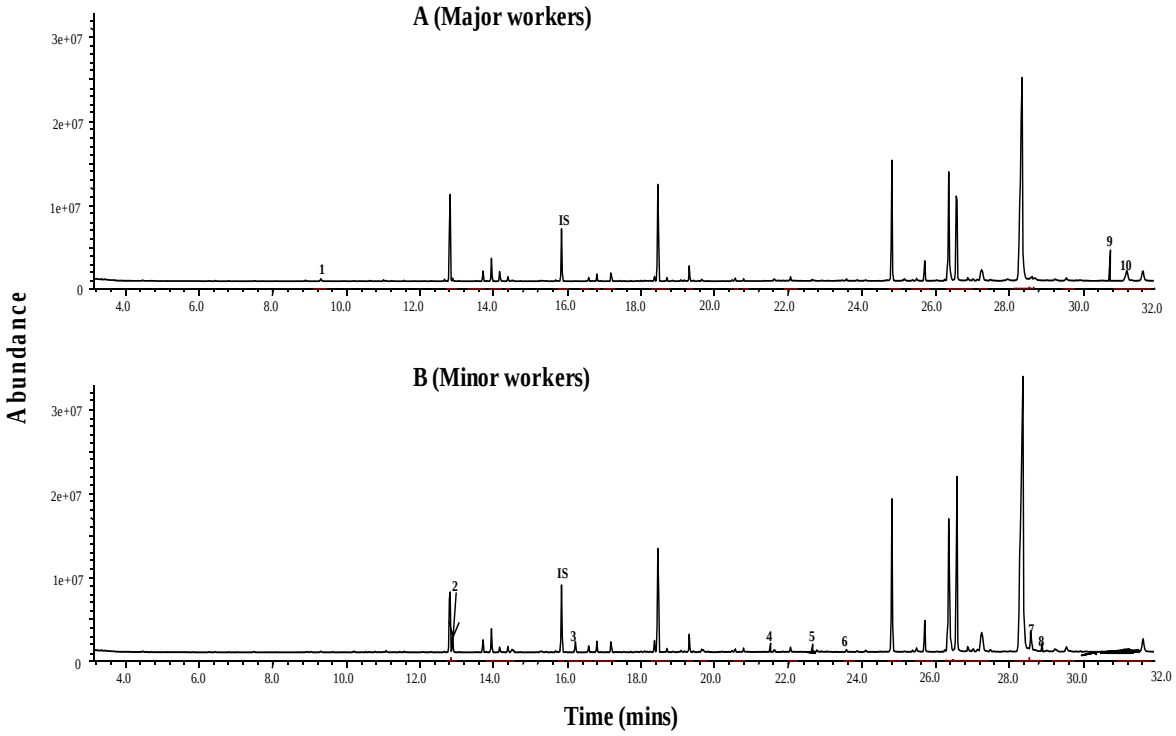

Figure 2.8 Representative GC-MS profiles of odours collected from 200 ants (a) major workers and (b) minor workers of *P. analis* by trapping for 14 hours overnight. Numbered peaks are compounds unique to each. Peaks numbered are 1) p-benzoquinone, 2)9-hexacosene, 3) 1-heptacosanol, 4) 1-hexacosene 5) *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.

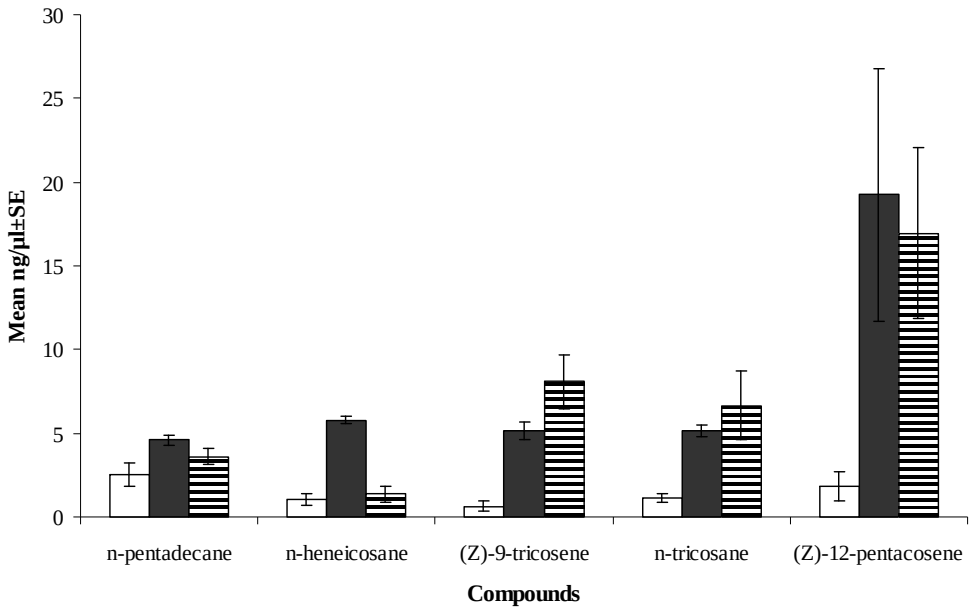

Figure 2.9 Mean concentrations in µg/µl ± SE of the most abundant components emitted in the volatiles of *P. analis* workers. Compounds are represented in ascending order with starting from the least to the most abundant,.□= mixed workers, ■=Major workers and ▤ minor workers.

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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CHAPTER THREE

Nestmate recognition and the role of cuticular hydrocarbons in the African termite raiding ant *Pachycondyla analis*

Abstract

Cuticular hydrocarbons (CHCs) are used as a means of chemical communication among nestmates in many ant species and they may play a role in the discrimination of nestmates and non-nestmates. Using the mandible opening response (MOR) bioassay, the response of the African termite raiding ant *Pachycondyla analis* to CHC extracts of nestmates and non-nestmates was explored. The ants were able to distinguish between chemical cues from controls, nestmates and non-nestmates. Based on a CHC recognition threshold, aggression was demonstrated between non-nestmates. Gas chromatography (GC) and GC-mass spectrometric analyses showed that the CHC components of the different ant colonies had chain lengths ranging from C_8 to C_{31}, comprising mainly *n*-alkanes, alkenes and methyl branched alkanes, with the *n*-alkanes occurring in the same proportions among all colonies. The ants were successfully grouped according to their colonies of origin using discriminant analysis. Using the MOR, nestmate recognition was demonstrated in *P. analis* and, it appeared that some of the cues involved in nestmate recognition could be encoded in the alkenes and methyl-branched alkanes.

Introduction

Among social insects, nest recognition enables integration within a colony and prevents non-colony members both conspecifics and heterospecifics from exploiting the colony's resources (Crozier and Pamilo, 1996). The presence of non-nestmates (intruders) usually elicits active defensive behaviours (Hölldobler and Wilson, 1990; Vander Meer and Morel, 1998). Nestmate recognition in social insects can be adaptive because workers obtain benefits from aiding nestmates and discriminating against non-nestmates, provided that the nestmates are more closely related to each other than to members of other conspecific colonies (Hölldobler, 1995). The cues involved can be of genetic or environmental origin and can differ between populations (Pirk *et al*., 2001)and season even in species which form super-colonies like *Formica exsecta* (Katzerke *et al.,* 2006). The primary cues of communication in most insects are chemical in nature (Wyatt, 2003), which are perceived by olfaction or contact chemoreception (Breed, 1998).

Ants are among the dominant social insects in the world and they employ complex forms of chemical communication. Over 100 exocrine glands have been described in social insects with more than half of these found in ants (Billen, 2004). An array of signals and information on an individual's species, sex, age, caste, status and relatedness as well as alarm and trail pheromones are encoded in the secretions from these glands (Howard and Blomquist, 2005).

Fielde (1901) proposed that nestmate discrimination signals were encoded in cuticular lipids, particularly those hydrocarbons that coat all insects. Since then, the role of cuticular hydrocarbons (CHCs) has been a subject of much debate and various studies have attempted to determine their functions in chemical communication of insects. Examples of these roles include: as cues for recognition at various levels such as the individual (e.g. D'Ettore and Heinze, 2005), nestmate (e.g. Wagner *et al*., 2000; Akino *et al*., 2004, Martin *et al*., 2008a and Martin *et al.,* 2008b), and species (e.g. Neems and Butlin, 1995; Dapporto, 2007), kin (Arnold *et al*., 1996); and as cues for reproduction and division of labour (e.g. Dietemann *et al*., 2003; Martin and Drijfhout, 2009). Most recently CHCs have been found to be responsible for enforcing altruism in ants (Smith *et al*., 2009). In adult insects CHCs are synthesised internally in the oenocytes

(Blomquist and Dilwith, 1985), and hence are under strong genetic influence reflecting an insect's genetic makeup (Lockey, 1991). After synthesis they are transferred to the cuticle by lipophorin (Schal *et al*., 2001). CHCs are made up of a homologous series of long straight chain saturated alkanes which could be modified by addition of methyl groups or the introduction of double bonds (Jackson and Morgan, 1993).

The ant *Pachycondyla analis* is a specialised termite predator, which is widely distributed in sub-Saharan Africa (Lévieux, 1966). This ant species, commonly referred to as 'Matabele ants', organises group raids on termite species that mainly belong to the sub-family Macrotermitinae (Longhurst *et al*., 1978). There is no information on CHCs of *P. analis* and the role they play in nestmate recognition. This chapter presents results from the study of CHCs of different colonies of *P. analis* and the role they may play in nestmate recognition.

Materials and methods

Ants

Colonies of *P. analis* were excavated from Mpala Research Centre (0°17'N, 37°52'E) Central Kenya, 250 Km north of Nairobi (Figure 1.2), as described in Chapter 1 and transported to the Animal Rearing and Containment Unit (ARCU) located on the *icipe* Duduville campus Nairobi, Kenya.

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 ant's soil (which served as nesting site). The box was attached to a 1.0 × 1.5 m foraging arena made of Perspex also partially filled with soil which was previously washed with water and sterilised by drying in an oven overnight (Figure 2.2). Ants were fed on live termites (mainly from the subfamily Macrotermitinae) twice daily that were collected around the Duduville campus of *icipe* in Nairobi, Kenya.

Extraction of CHCs for mandible opening response (MOR) bioassay

Cuticular hydrocarbons from five (2 major and 3 minor) ants per colony were extracted for use as sources of chemical stimuli in the mandible opening response (MOR)

bioassay. Ants previously in contact with their own colony odour were selected for extraction of CHCs. The ants were first killed by placing them on ice for 15 min and CHC extracted by washing them in 500 µl of pentane for 10 min. The solvent was evaporated under a gentle stream of nitrogen, and the residue dissolved in 50 µl of pentane and stored at - 20°C until required for analysis. Twenty four extracts were prepared from each of the three colonies making a total of 72 extracts. A solvent control (pentane) was also subjected to the same extraction procedure. An average quantity corresponding to the extract of one ant (10µl) was poured unto the tip of a Pasteur pipette using a Hamilton syringe. The pipette tip was held downwards until the solvent evaporated from the tip, leaving the residue of the extract around the lower and outer part of the pipette.

Mandible Opening Response (MOR) Bioassay

Ants were removed from their colonies and transferred into 20 ml glass vials and were then immobilised by placing them on ice. The ants were then harnessed using methods previously described (Guerrieri and d'Ettorre, 2008). The ants were kept undisturbed in a room for 2 hours to recover and to habituate to the harness.

Aggressive responses were quantified by presenting four different types of stimuli, viz., a) solvent extract only (CTRL); b) extract from colony 1 (C1); c) extract from colony 2 (C2); d) extract from colony 3 (C3) to the test ants from colonies 1, 2 and 3. For a test ant, extracts from individuals of its own colony served as nestmate stimuli, while extracts from individuals of other colonies served as non-nestmate stimuli. All ants were tested with all the extracts and the control.

In each trial, one stimulus was presented to a previously harnessed individual ant. A test individual was removed from its resting place and allowed to habituate for 2 min prior to presenting it with the test stimulus. After habituation its antennae were gently touched for 5 sec with the tip of the stimulating pipette (Figure 3.1 a). When it opened its mandibles continuously i.e. displacing them from the resting position, it was recorded as aggression (score = 1) (Figure 3.1b). If the individual did not open its mandibles and instead antennate continuously the response was recorded as non-aggressive (score = 0) (Figure 3.1 c) following the protocol of Guerrieri and d'Ettorre (2008). After presenting a stimulus, the test ant was returned to its resting place. Stimuli

were presented at random to the individual ants after an interval of 20 min to allow for the recovery of antennal receptors. From each of the three colonies studied, 24 ants chosen at random were tested, with each of the four stimuli, thus a total of 72 ants were tested.

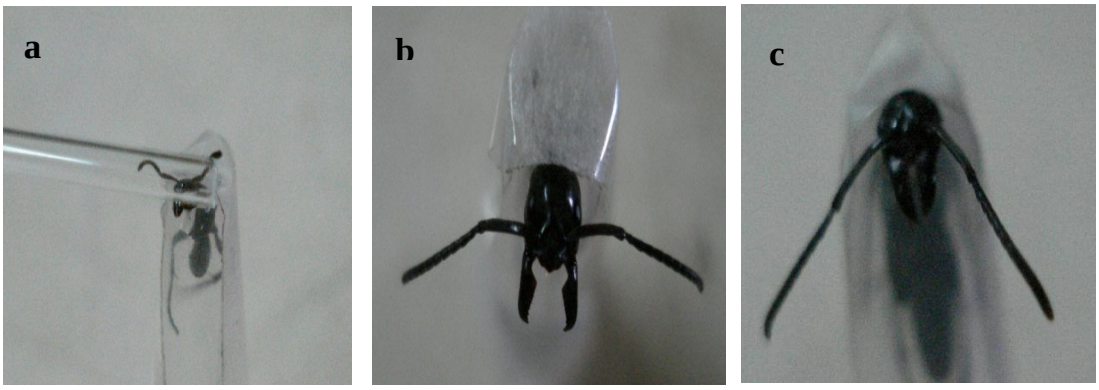

Figure 3.1 Immobilised ants showing (a) stimulation of ant antenna (b) mandible opening (aggression) and (c) mandibles closed with continuous antenation (non-aggression).

Extraction of Cuticular Hydrocarbons (CHC) for Chemical Analyses

Cuticular hydrocarbons were extracted in a similar way to those used for MOR, but this time each ant was extracted in 1 ml of solvent (pentane). Ants were also grouped based on their colonies of origin and worker size (major and minor). Another colony (colony 4) was also added for comparison purposes. The solvent was evaporated under a gentle stream of nitrogen, and the residue re-suspended in 100 µl of pentane and stored at -20 °C until required for analysis. Six extracts were prepared from each of the four colonies making a total of 24 extracts. A solvent control (pentane) was also subjected to the same extraction procedure.

Chemical analysis

Gas chromatographic (GC) analysis was carried out on a HP 5890 series II gas chromatograph equipped with a flame ionisation detector (FID) and a HP-5 column (30 m × 0.25mm ID × 0.25µm film thickness). Nitrogen was used as a carrier gas with a column pressure of 46 psi and injection temperature of 250°C. One µl of sample was

injected in the splitless mode, with the oven temperature programmed at 60°C for 5 min and at 10°C/min to 280°C, and held at this temperature for 13 min. GC-MS analysis was carried on an Agilent Technologies 7890A gas chromatograph equipped with a capillary column HP-5 MS (30 m × 0.25mm ID × 0.25µm film thickness) and coupled to 5795A mass spectrometer. One µl of sample was injected in the splitless mode, and helium was used as the carrier gas at 1.0 ml min^{-1}. The oven temperature was 35°C held for 5 min, increased to 280°C at 10°C min^{-1}, and then held at this temperature for 15 min. The analysis was carried out at 70eV in the electron impact ionization mode. All the *n*-alkanes, 2-methylheptadecane, 1-heptadecene, (*Z*)-9-tricosene and squalene were identified by GC-MS co-injection and comparison of mass spectral data with those of authentic standards. The other methyl-branched alkanes and alkenes were tentatively identified using EI diagnostic ions (El-Sayed, 2009).

Chemicals. *n*-Undecane, *n*-Dodecane, *n*-Tridecane, *n*-Tetradecane, *n*-Pentadecane, *n*-Hexadecane, and *n*-Heptadecane with the purity of > 99% were obtained from Aldrich, Gillingham, Dorset, UK. *n*-Octadecane, *n*-Nonadecane, *n*-Eicosane, *n*-Heneicosane, *n*-Docosane, *n*-Tricosane, *n*-Tetracosane, *n*-Pentacosane, *n*-Hexacosane, *n*-Heptacosane, *n*-Nonacosane, and *n*-Hentriacontane were provided by Dr. Peter Teal, USDA/ARS-CMAVE, Florida, USA. 1-Heptadecene, (*Z*)-9-tricosene, Squalene and 2-methylheptadecane were provided by Dr. Antony Hooper, Rothamsted Research, Harpenden, UK

Statistical analyses

Logistic regression was performed on the dichotomous data (1 vs. 0) of the aggressive response of ants. Differences in aggression response of ants to the control, nestmate and non-nestmate extracts were tested. The levels of aggression between colonies were tested using *Kruskal Wallis* ANOVA. The relative areas of the peaks of the individual compounds in the CHC profile for each ant were standardised to 100%. The standardised peak areas were then transformed following the method proposed by Aitchinson (1986):

$$Z_{ij} = \ln[Y_{ij}/g(Y_j)]$$

where Z_{ij} is the standardised peak area *i* for individual ant *j*, Y*ij* is the observed peak area *i* for individual ant *j*, and g(Y*j*) is the geometric mean of all peak areas for ant *j* included in the analyses. A stepwise discriminant function analysis (stepwise DA) was performed on the transformed variables followed by canonical discriminant analysis on the selected peaks to determine whether the colonies, major and minor workers could be separated on the basis of their CHC profiles. Pairwise generalised square distances between colonies and classification error rates were also calculated. All statistical analyses were carried out using SAS 9.1. Statistical software at an α level of 0.05.

Results

Mandible opening response bioassay (MOR)

The number of ants that opened their mandibles when presented with the control (solvent) was significantly lower when compared with an extract (*Wald's* χ^2= 58.34, *df* = 1, *P* < 0.0001). There was significantly less mandible opening when ants were presented with a nestmate extract compared with an extract from a non-nestmate (*Wald's* χ^2= 101.24, *df* = 6, *P* < 0.0001). In general, the levels of aggression (MOR) increased when an ant was presented with a non-nestmate stimulus compared to the nestmate extract and a control (*Wald's* χ^2 = 132.19, *df* = 2, *P* < 0.0001, Figure 3.2). Ants from colony 1 were slightly more aggressive than those from colonies 2 and 3 (Figure 3.2a), although aggression between colonies were not significantly different (*Kruskal Wallis ANOVA*, χ^2 = 5.08, *df* = 2, *P* = 0.0815).

CHC profiles of ***P. analis***

GC-MS analysis revealed that the CHCs of *P. analis* were a complex mixture of alkanes, alkenes, and methyl branched alkanes ranging from C_8-C_{31} (Figure 3.3 and Table 3.1). The major components varied between colonies, with (*Z*)-9-tricosene being present in varying proportions in all the colonies. The proportions of alkanes in the extracts remained constant while there was variation in the proportions of the alkenes and the methyl-branched alkanes between the colonies (Figure 3.4).

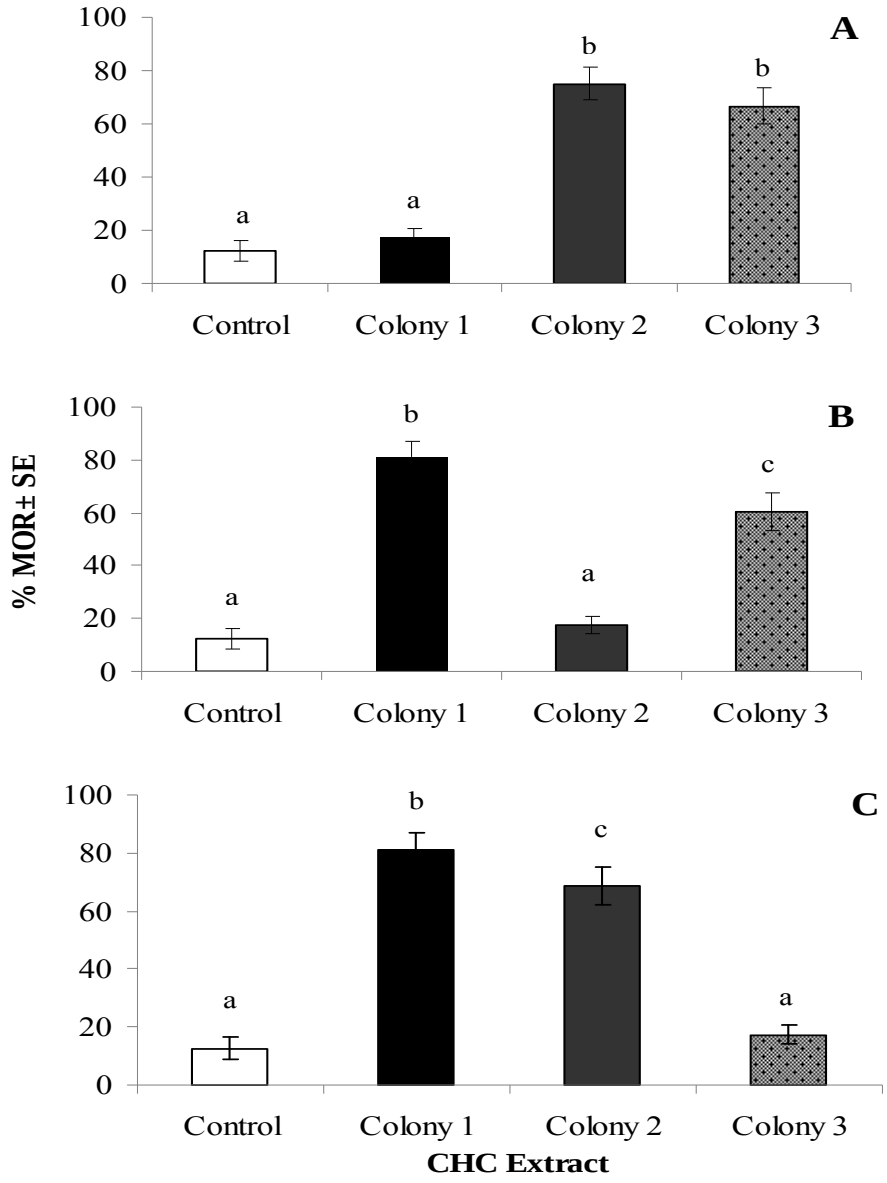

Figure 3.2 Mandible opening response (MOR) ±se for, (A) ants from colony 1, (B) ants from colony 2 and (C) ants from colony 3 to the presented extracts. □ = Control solvent (pentane), ■ = CHC extract from colony 1, ■ = CHC extract from colony 2 and ▩ = CHC extract from colony 3. Ants responded significantly differently to test stimulus and control (*Wald's* χ^2 = 58.34, $P < 0.0001$). Response of ants to the extract of nestmate and those of non-nestmate also differed significantly (*Wald's* χ^2 = 101.24, $df = 6$, $P < 0.0001$). Same letters on bars represent means that are not significantly different.

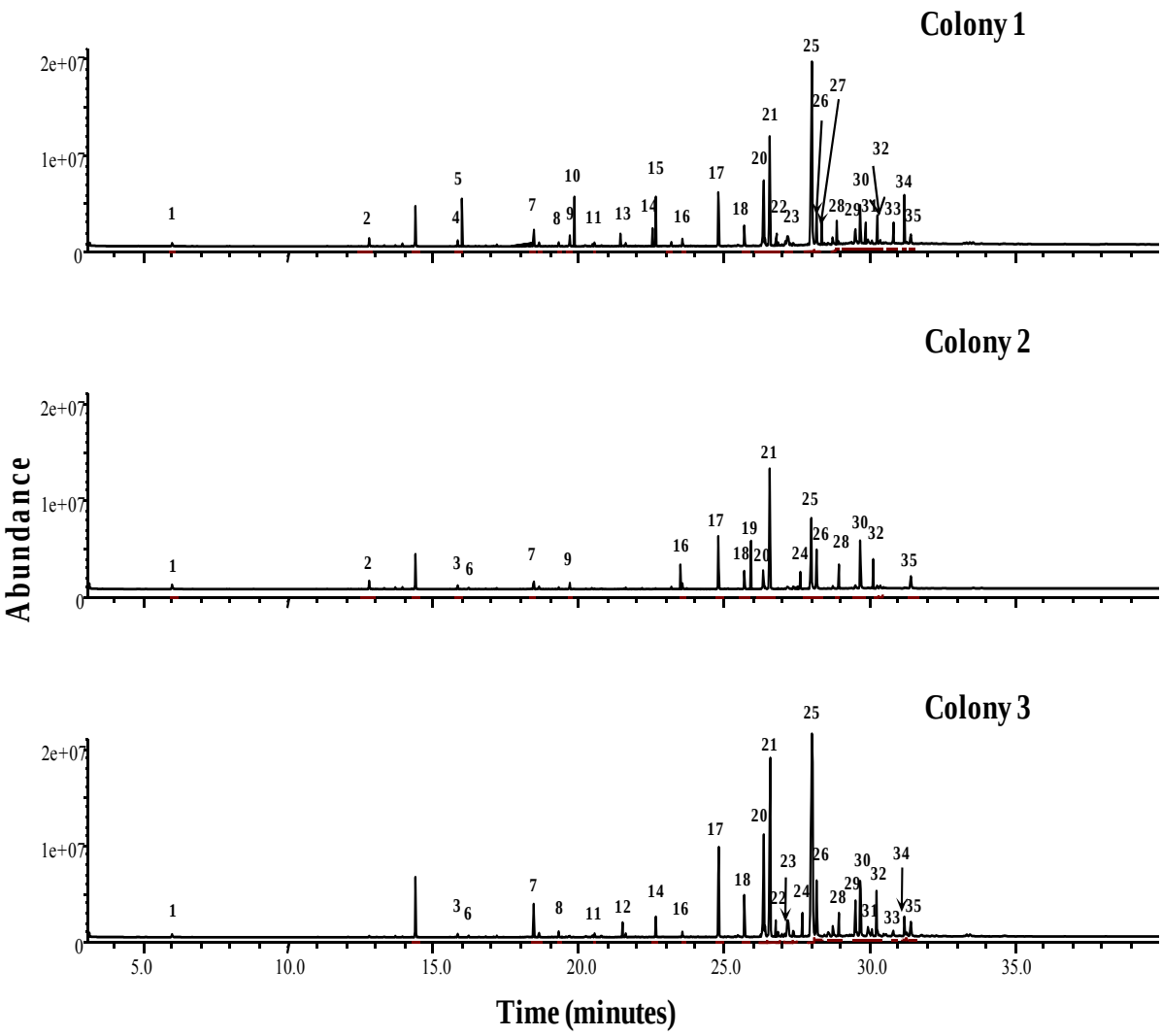

Figure 3.3 Total ion chromatograms for the cuticular hydrocarbons of ants from the colonies studied. Colony 1= CHC extracts from colony 1 ants, Colony 2 = CHC extracts from colony 2 ants, Colony 3 = CHC extracts from colony 3 ants. (see Table 3.1 for list of compounds).

CHC differentiation among colonies

Ants from the different colonies could be distinguished using the transformed peak areas of the 35 identified compounds (Figure 3.3, and Table 3.1) that differed among the colonies. Using the stepwise DA, 17 compounds clustered the ants according to their colonies of origin (*Wilk's* λ = 0.0007, *df* = 34, 10, $P < 0.0001$). Discriminating compounds selected by the stepwise DA were: *n*-undecane, 3-methylundecane, 3,6-

dimethylundecane, 3,8-dimethyl decane, pentadecane, heptadecane, 3-methylheptadecane, 2-methylheptadecane, octadecane, nonadecane, heneicosane, tricosane, 1-nonadecene, 9-nonadecene, 9-methyl nonadecane, squalene and hentriacontane. Using these 17 compounds, ants were grouped into their colony of origin (*Wilk's* $\lambda = 0.0000$, *df.* = 34, 10, $P < 0.0001$) with function 1 explaining 89.5 % of the variation separating colony 3 from both colonies 1 and 2, and function 2 explaining 7.3 % further separating colony 3 from 2 and 1 and colony 4 from 1, 2 and 3 (Figure 3.5). All the ants were grouped into their colonies correctly based on their CHC profiles. Generalised square distances between colonies showed that colony 1 was much closer to colony 2 than colony 3, and colony 2 much closer to colony 1 than colony 3. Colony 4 was also closer to 1 and 2 than it is to colony 3.

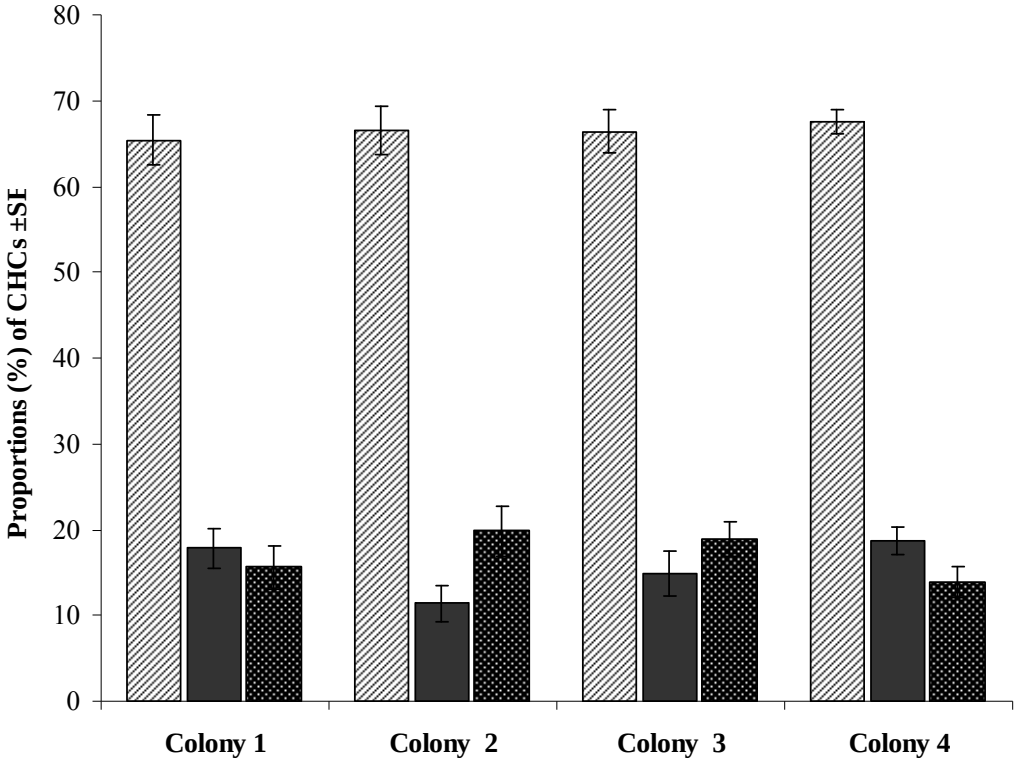

Figure 3.4 Proportions ± SE of the different groups of hydrocarbons (▨ = *n*-Alkanes, ■ = Alkenes and ▩ = Methyl branched alkanes) in the cuticular hydrocarbon profiles of *P. analis* ants from colonies 1, 2 , 3 and 4, error bars represent mean proportions ± SE

Table 3.1 Compounds identified from the cuticular hydrocarbon profiles of *Pachycondyla analis,* along with retention indices and diagnostic ions [#].

Peak No	***Compound***	***Retention index***	***Diagnostic ions***
1	*n*-Octane	800	114
2	*n*-Undecane	1100	156
3	5-methylundecane	1154	43, 57, 71, 85, 99, 112
4	3-methylundecane	1169	43, 57, 71, 85, 99, 112, 141, 170
5	3,8-dimethyldecane	1063	57, 71, 85, 99, 113, 141, 155, 170
6	*n*-Tridecane	1300	184
7	*n*-Pentadecane	1500	212
8	3-methylpentadecane	1572	43, 57, 71, 85, 99, 113, 127, 141, 155, 168, 197, 226
9	2-methylheptadecane	1765	43, 57, 71,85, 99, 113, 127, 141, 155, 169, 183, 195, 211, 239, 254
10	1-Heptadecene	1679	83,97,111,125,196, 210,239
11	8-Heptadecene	1679	41, 55, 69, 83, 97, 111, 125, 140, 238
12	5-Octadecene	1789	43, 55, 69, 83, 97, 111, 125, 139, 166, 180, 195, 224, 252
13	*n*-Octadecane	1800	254
14	9-Nonadecene	1875	43, 55, 69, 83, 97, 111, 125, 139, 153, 167, 238, 266
15	*n*-Nonadecane	1900	268
16	*n*-Eicosane	2000	282
17	*n*- Heneicosane	2100	296
18	1-Docosene	2195	43, 57, 69, 83, 97, 111, 125, 280,308
19	*n*-Docosane	2200	310
20	(Z)-9-Tricosene	2270	43, 55, 69, 83, 97, 111, 125, 139, 153, 223, 237, 294, 322
21	*n*-Tricosane	2300	324
22	Unidentified		
23	1-Tetracosene	2396	43, 57, 69, 85, 97, 113, 309, 338
24	*n*-Tetracosane	2400	338
25	Cyclotetracosane	2445	43, 57, 69, 83, 97, 111, 125, 139, 153, 207, 392
26	9-Pentacosene	2465	43, 57, 69, 85, 97, 113, 141, 169, 197, 326, 350
27	*n*-Pentacosane	2500	352
28	(Z)-12-Pentacosene	2496	43, 57, 69, 83, 97, 125, 236, 257, 290, 322, 350
29	1-Hexacosene	2593	43, 57, 69, 83, 97, 111, 125, 139, 336, 364
30	*n*-Hexacosane	2600	366
31	*n*-Heptacosane	2700	380
32	*n*-Octacosane	2800	394
33	Squalene	2663	41, 55, 69, 81, 95, 109, 121, 136, 148, 341, 367, 410
34	*n*-Nonacosene	2900	408
35	*n*-Hentriacontane	3100	436

[#] Only compounds that were represented by at least 0.5 % peak area in the Total Ion Chromatogram are represented on the table.

CHC differentiation among major and minor workers

Major and minor workers from the four different colonies could be distinguished using the transformed peak areas of the identified compounds (Figure 3. 6) that differ among the colonies. Using the stepwise DA, 12 variables clustered the ants according to their sizes and colonies of origin (*Wilk's* λ= 0.000, *df* = 31, 7, $P < 0.0001$). Discriminating compounds selected by the stepwise DA were: octane, undecane, 3,8-dimethyl decane, pentadecane, 3-methylpentadecane, nonadecane, eicosane, (Z)-9-tricosene, cyclotetracosane, heptacosane, nonacosane and 17-pentatriacontene.

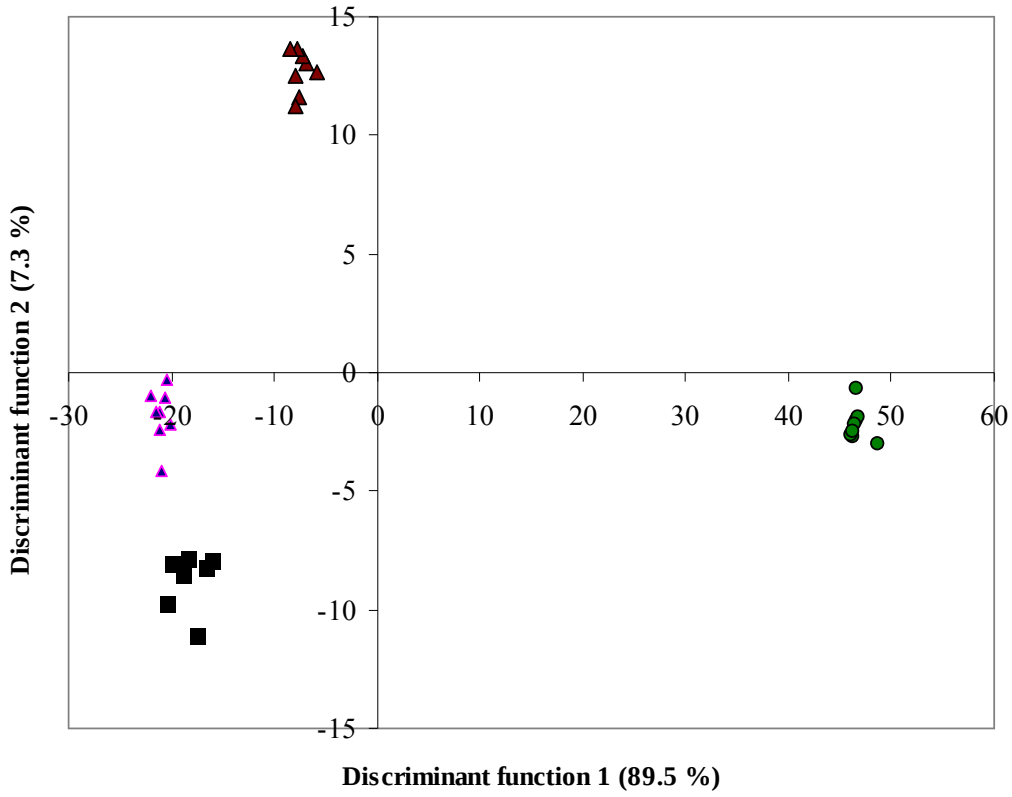

Figure 3.5 Discriminant function analysis of ants from the four colonies of *P. analis* based on relative proportions of 17 cuticular hydrocarbons determined in stepwise fashion□ = Colony 1,▲ = Colony 2, ● = Colony 3 and▲ = Colony 4. All individuals were clearly grouped in to their respective colonies based on their CHC profiles.

Using these 12 compounds, major and minor worker ants were successfully grouped into their sizes and colony of origin (*Wilk's* λ= 0.0000, *df*.= 31, $P < 0.0001$) with function 1 explaining 82.3 % of the variation separating colony 3 from both colonies 1, 2 and 4, and function 2 explaining 17.7 % further separating colony 4 from 2, 1 and 3. (Figure 3.6). All the ants were grouped into their colonies correctly based on their CHC profiles.

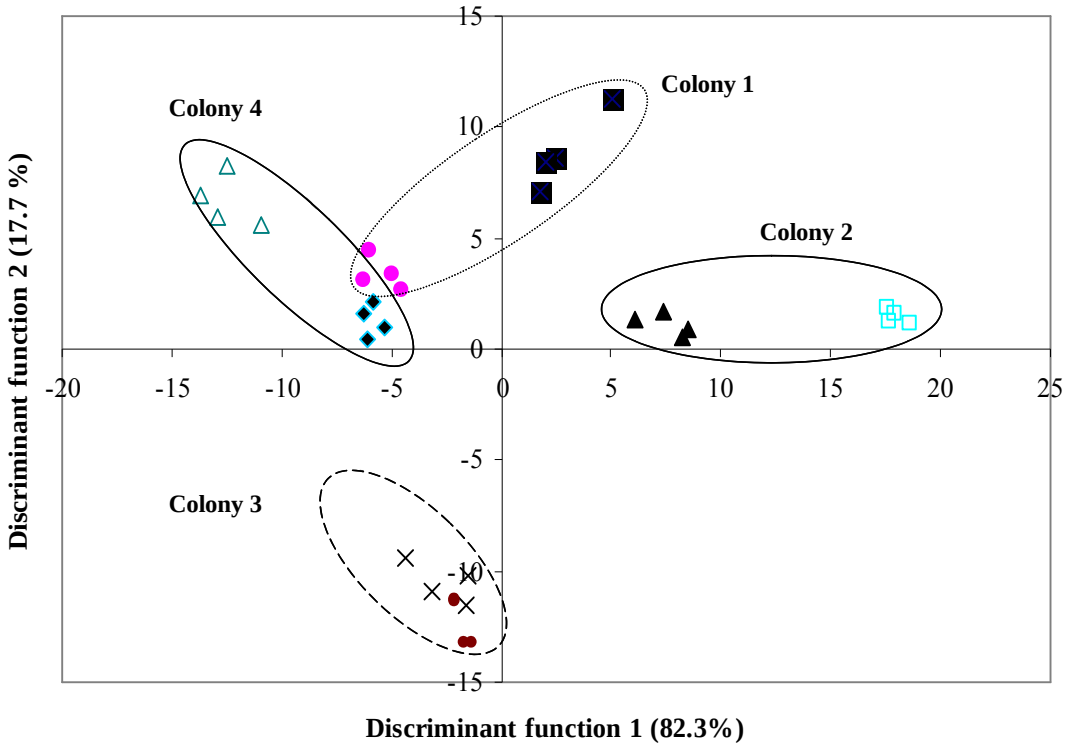

Figure 3.6 Discriminant function analysis of major and minor ants from the four colonies of *P. analis* based on relative proportions of 12 cuticular hydrocarbons determined in stepwise fashion ■ = major worker colony 1, ● = minor worker colony 1, ▲ = major worker colony 2, □ = minor worker colony 2, × = major worker colony 3, ● = minor worker colony 3, △ = major worker colony 4 and ■ =.minor worker colony 4. All individuals were clearly grouped in to their respective colonies based on their CHC profiles, except for an overlap between minors from colony one and majors from colony 4.

Discussion

The use of a 'yes or no' aggression bioassay was demonstrated, using mandible opening as a measure of aggression/acceptance between different colonies of *P. analis*. These

results show that *P. analis* discriminate between nestmates and non-nestmates since they were significantly more aggressive to extracts from non-nestmates (Figure 3.2). The colonies were indeed discrete in such a way that non-nestmates received different aggression levels. Results here are in agreement with those previously reported for queen adoption in the invasive Argentine ant (*Linepithema humile* (Mayr.)) that responded strongly in similar assays (Vásquez *et al.*, 2008). Thus, confirming that the MOR is a sensitive assay that can be used effectively to set recognition or aggression thresholds in ants. Recognition thresholds are usually based on a template odour that is characteristic of a given colony, with ants deciding to accept or reject an individual when it smells greater than a minimum similarity threshold or below a dissimilarity threshold (Reeve, 1989).

Aggression towards nestmates of similar CHC profile could be either due to errors arising while reacting to recognition cues as demonstrated in the invasive Argentine ant (*L. humile*) by Vásquez et al. (2008) or due to lower threshold to avoid false-positive identification. In the present study, the MOR bioassay was successfully used to demonstrate and measure inter-colony aggression at the colony level in *P. analis*.

Using GC-MS, thirty five different compounds were identified in the CHCs of different colonies of *P. analis*. These were mainly alkanes, alkenes, and methyl-branched alkanes, as previously reported for other ant species (Dietemann *et al*., 2003; Lucas *et al*., 2005; Martin *et al*., 2008b), with (*Z*)-9-tricosene occurring in variable proportions between colonies. In *P. analis n*-alkanes occurred roughly in the same proportion in all colonies, with the alkenes and methyl-branched alkanes present in different proportions between the colonies (Figure. 3.4) unlike in *Formica* ants where *n*-alkanes varied between colonies (Akino *et al*., 2004; Martin *et al.*, 2008b). In the genus *Pachycondyla,* species like *P. villosa* (Lucas *et al*., 2004) and *P. apicalis* (Soroker *et al*., 1998) have been shown to produce varying amounts of *n*-alkanes and alkenes. These differential amounts may be influenced by environmental conditions including temperature and relative humidity, as reported in a previous study on the desert harvester ant (*Pogonomyrmex barbatus*) (Wagner *et al*., 2001).

Nestmate recognition cues in *P. analis* could be encoded in the alkenes and methyl-branched alkanes in the CHCs. (*Z*)-9-Alkenes have been reported as nestmate

recognition and as aggression cues in *Formica* ants (Akino *et al*., 2004; Martin *et al*., 2008a and b). They also serve as recognition cues in the desert ant *Cataglyphis niger* (Lahav *et al*., 2001). Nestmate recognition cues for *P. analis* identified in this study might serve two purposes, colony defence which is the traditional role of nestmate recognition and group raiding behaviour which may require recognition of nestmates when foraging as well as, for task allocation in and outside the nest and to prevent attacking the wrong individuals (nestmates instead of termites). The roles played by the alkenes and methyl-branched alkanes in nestmate recognition and aggression in *P. analis* need to be further investigated by manipulating the CHC profiles of ants using synthetic compounds and using them in bioassays to see whether ants respond differently to the manipulated nestmate or non-nestmate CHCs.

The results from the discriminant function analysis clearly showed that clear cut differences existed in the CHC profiles between colonies of *P. analis*. The chosen compounds (*n*-undecane, 3-methylundecane, 3,6-dimethylundecane, 3,8-dimethyl decane, pentadecane, heptadecane, 3-methylheptadecane, 2-methylheptadecane, octadecane, nonadecane, heneicosane, tricosane, 1-nonadecene, 9-nonadecene, 9-methyl nonadecane, squalene and hentriacontane) can effectively be used to group the ants into their respective colonies correctly. The colony specific nature of CHCs in *P. analis* confirmed findings in other ant species (e.g. Lahav *et al*., 2001; Akino *et al*., 2004; Lucas *et al*., 2004; Denis *et al*., 2006; Martin *et al*., 2008a; b). These clear groupings, based on CHC profiles, can explain the degree of aggression between different colonies. Colony 1 was further away from colonies 2 and 3, hence the high aggression, likewise colony 2 and 3 were closer together than colony one. These clear differences could explain the differential acceptance of workers from different colonies competing for the same resources. Intruders or encroachers are usually killed upon encounter. By contrast the invasive Argentine ant (*L. humile*) displays minimal nestmate discrimination and individuals which are non-nestmates are often integrated into an alien colony (Vásquez *et al*., 2008).

Colony odour recognition cues in ants are phenotypic and are derived either from the environment (diet, nesting sources) or produced endogenously (genetically determined or both) (Vander Meer and Morel, 1998) and the relative importance can vary between populations (Pirk *et al*., 2001). Whatever the source is, it is predicted that each colony

will display a uniform odour that constitutes a *gestalt*. However, in some studies, it has been shown that this is not always the case because different castes within a colony may possess different CHC profiles which could code different information within the colony (Dietemann *et al*., 2003; Martin and Drijfhout, 2009). In the present study, CHCs in *P. analis* are colony specific. A further investigation of the different groups of workers in a colony based on their body sizes (major and minor) revealed differences in their CHC profiles (Figure 3.6). This is a clear indication that CHCs are not only colony specific but also worker group specific. This finding further strengthens the assertion that CHCs are also involved in task allocation within and outside the nest during raids.

In summary, the MOR bioassay was successfully used to measure responses of *P. analis* workers that show differences based on colony of origin. Aggression was found to be associated with colony odour, mainly in the CHCs. As in other ant species, CHCs in *P. analis* comprise three main groups; *n*-alkanes, alkenes, and methyl-branched alkanes. The *n*-alkanes were consistent between colonies with the alkenes and methyl alkanes serving as possible nestmate recognition cues. Also CHCs are worker size specific, with majors having an odour profile which is different from those of minor workers.

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CHAPTER FOUR

Behavioural evidence of olfactory detection of prey by the termite raiding ant *Pachycondyla analis* (Hymenoptera: Formicidae)

Abstract

There exists a co-evolutionary arms race between termites and predatory ants, both of which occupy and share the same habitats. Over time, these two different groups of insects developed several predatory and counter predatory strategies against each other to enable them survive in these shared habitats. The African termite raiding ant *Pachycondyla analis* organises raids on colonies of Macrotemitinae which are responsible for a certain degree of losses in agricultural production in sub-Saharan Africa. Here we asked whether ants chemically detect their potential termite prey before and during raids in order to reveal the possible cues involved. Using Y-tube olfactometric assays, we tested the responses of ants to odours emitted from termites alone, termite gallery soil and termites inside their galleries. We demonstrate for the first time that *P. analis* detects odours of both termites and those of their galleries; but odours from termites inside their galleries were more attractive to both minor and major workers. GC-MS analysis was used to identify the composition of the volatiles. While the volatiles from termite gallery soils were compositionally richer, those from the termites alone were quantitatively richer, releasing about six times more volatiles than gallery soil. Most of the compounds in the volatiles were identified are hydrocarbons. Naphthalene previously identified as an insect repellent was also identified as a component of the volatiles of the gallery soil. In conclusion, these results suggest that odours play an important role in prey detection by *P. analis*.

Introduction

Termites are economically important from two different perspectives. On the one hand, they assist in nutrient re-cycling (beneficial), while on the other hand, they destroy cellulose containing materials (destructive) in their quest to acquire cellulose (Culliney and Grace, 2000). As a result of this behaviour, termites cause damage estimated at many billions of dollars worldwide, and in Africa alone they account for between 15-100 % loss of crops (both on farm and in storage) and in tree production (Janssen, 2006).

Ants (Hymenoptera) are said to be the greatest predators of termites (Isoptera) worldwide (Hölldobler and Wilson, 1990). They both share the same habitats and are abundant in terms of biomass and density (Fujiwara-Tsujii *et al*., 2006). During their 100 million years of coexistence, ants and termites are said to have engaged in a co-evolutionary arms race, with ants on the one hand developing several predatory tactics and termites on the other hand responding with several defensive strategies (Deligne *et al*, 1981; Mill, 1983; Hölldobler and Wilson, 1990;). Despite these counteracting strategies from termites, most ant-termite interactions are antagonistic with the well armed ants wining the battles against the soft bodied termites. Among ants there exist specialists as well as opportunistic predators of termites. Ants that prey opportunistically on termites are members belonging to two of the largest genera *Pheidole spp* and *Componotus spp* (Hölldobler and Wilson, 1990). The most specialised predatory ant species are concentrated in the sub-families Ponerinae and Myrmicinae (Lévieux, 1966; Maschwitz and Mühlenberg, 1975; Longhurst *et al*., 1978; Longhurst *et al*., 1979; Maschwitz and Schönegge, 1983; Lepage, 1984; Corbara and Dejean, 2000).

A lot of energy and time is used by virtually all animals in search for food regardless of their social organisation (Bell, 1991). For a colony of ants, foraging is energetically expensive but in the long run it is beneficial by an increase in the probability of retrieving more food items (Lighton *et al*., 1987). In ants, scouts have the ability to learn and recognise prey characteristics such as their spatial distribution and availability (Hölldobler and Wilson, 1990; Schatz *et al.*, 1999) or their specific odours or kairomones (Durou *et al.*, 2000); this has the potential to increase their foraging efficiency. Since termite availability is determined by their complex spatial and temporal presence (foraging and nesting habits), their exploitation as prey requires some

synchronisation on the part of the predators as observed in the raiding behaviour of *P. analis* (AAY, unpublished data).

Pachycondyla analis (Latreille) is a specialised termite predator, which is 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 it then recruits nest mates using trail pheromones (Longhurst *et al.,* 1979). Upon arrival at the food source, the ants spread out, break open the termite galleries and then invade them to raid the termites. *P. analis* capture termites by stinging, which paralyses them after which they carry them out of the galleries to a place near 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 can grasp up to seven termites between its mandibles, while a minor can grasp up to three termites (AAY personal observation). Some do not carry any termites but lead the columns of nest mates on the return journey back to the nest (Longhurst *et al.*, 1978). The raids last between 4-50 min depending upon the foraging distance and the termite species being raided.

However, the cues involved in the detection of prey by *P. analis* have not been well studied. Previous studies by Longhurst and Howse (1978) reported that *P. analis* scouts either use chemical or mechanical cues of termite origin to detect potential termite prey. However, New (1991) was of a different opinion; that *P. analis* detects termites using termite pheromones, which serve as kairomones for *P. analis*. These suggestions have not been supported by any experimental evidence that documented the cues used by *P. analis* while foraging for termites.

These gaps in our knowledge of this specific interaction prompted us to investigate whether olfactory cues mediate the detection of prey by *P. analis*. To investigate this, we tested the responses of worker ants to the odours of termites, termite galleries and termites inside their galleries. We also compared the composition of the volatiles from these odour sources by GC-MS.

Materials and methods

Study insects

Colonies of *P. analis* with all representative individuals (queen, workers, males, brood and eggs) were excavated from Mpala Research Centre (0°17'N, 37°52'E) Central Kenya, 250 Km north of Nairobi. The ant colonies were kept in artificial nests boxes (20 cm × 20 cm × 20 cm) made of aluminium that were connected to foraging arenas (1.5 × 1.0 m) made of Perspex. The nests were maintained at 25 ± 1 °C, with about 50-60% relative humidity and a 12L: 12D photoperiod. Ants were fed on live termites (mainly from the subfamily Macrotermitinae) collected twice daily around the Duduville campus of *icipe* in Nairobi, Kenya.

Termites (*Odontotermes sp*) and gallery soils were obtained from termite foraging galleries in and around the Duduville campus of *icipe* in Kasarani Nairobi, Kenya.

Bioassays

The olfactory responses of major and minor workers of *P. analis* to odours were tested in a Y-tube olfactometer. The odour source consisted of: (a) 40 workers and 10 soldiers (termites only), (b), 250g gallery soil (termite gallery soil only), (c) a combination of (a) and (b) offered as choices. The bioassays were conducted at room temperature (24 ±1°C) and 50-60 % RH. In order to simulate ants foraging and raiding behaviour as observed in the field, all bioassays were carried out in the mornings (0700-1000 hr) and evenings (1600-1730 hr) local time over a number of days using ants from different colonies.

Y-tube olfactometer set-up

The olfactometer set-up consisted of a glass Y-tube (base 7.5 cm long; Y-arms 7.5 cm long; internal tube diameter 10 mm), as described in Chapter two Figure 2.3. Each arm was extended by fitting it with a small piece of stiff Teflon tube which was connected to a long flexible Teflon hose that entered directly into the odour source. The base tube was also extended with a piece of stiff Teflon tube to a further 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 exiting 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) when comparing odours with blank. When comparing odours with each other, each arm was provided with a different odour source. The odours were extracted through the base arm at 500 ml/min by a vacuum pump 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.

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 the 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 stayed for at least 1 min in an arm, or when it frequently visited an arm. No-choice was recorded when the ant remained in the base arm for more than 5 min. Each test was terminated after 10 min from the introduction of the ant into the Y-tube. Sixty ants were used for each treatment (30 minor and 30 major workers). To avoid positional bias, odour chambers were rotated for every replicate. A clean Y-tube was used for each ant test in order to avoid carryover of odours. 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 odours or contaminants. Glassware was cleaned with Teepol® laboratory detergent, 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.

Extraction of compounds and chemical analyses

Termite galleries: ~2g of termite gallery soil was weighed into a clean 2 ml glass vial, and to this 1 ml of *n*-pentane was added. The sample was vortexed for about 10 min, and then extracted for 2 hrs at room temperature, after which the supernatant was filtered through solvent-cleaned glass wool and concentrated under charcoal-purified

nitrogen to about 100 µl. If samples were not analysed immediately, they were stored in the freezer at -20°C until used.

Ten whole bodied termites which were previously killed on ice were extracted in 1 ml of *n*-pentane kept in ice for 2 hrs. After extraction the extracts were filtered through glass wool and the filtrate concentrated under nitrogen to 100µl. Extracts were either analysed immediately or stored at -20°C until used.

GC analysis

Gas chromatographic (GC) analysis was carried out on a HP 5890 Series II gas chromatograph equipped with a flame ionisation detector (FID) and a HP-5 column (30 m × 0.25mm ID × 0.25µm film thickness). Nitrogen was used as a carrier gas with a column pressure of 46 psi and injection temperature of 250°C. One µl of sample was injected in the splitless mode, with the oven temperature programmed at 60°C for 5 min and at 10°C/min to 250°C, and held at this temperature for 13 min. GC-MS analysis was carried on an Agilent Technologies 7890A gas chromatograph equipped with a capillary column HP-5 MS (30 m × 0.25mm ID × 0.25µm film thickness) and coupled to a 5795C mass spectrometer. One µl of each sample was injected in the splitless mode, and helium was used as the carrier gas at 1.0 ml min^{-1}. The oven temperature was 35°C held for 5 min, increased to 250°C at 10°C min^{-1}, and then held at this temperature for 15 min. The analysis was carried out at 70eV in the electron impact ionization mode. Compounds were identified tentatively based on a comparison of their mass spectra with published MS spectra and retention indices.

Statistical analyses: Data analysis was carried out using SAS version 9.2 (SAS Institute, 2002). Data obtained using the Y-tube olfactometer assays were analysed using a chi-square test to test whether odours were more attractive to ants than the control (blank). Ants that did not make a choice were not included in the analysis. Since the olfactometer assays were performed under the same conditions, individual assays were pooled to evaluate the differences in attractiveness of the three odour sources by the major and minor workers. A logistic regression model was fitted to the data using PROC GENMOD (SAS Institute Inc., 2008).

Results

Bioassays: In general, significantly more ants (65%) responded to the treatment odours than to the control (clean air). Responses of both major and minor workers to termite gallery soil were significantly higher than the control (Figure 4 1A). In particular, 21 majors, representing 81%, responded to the gallery soil odour as compared to only 19%

Figure 4.1 Preferences of *Pachycondyla analis* major and minor workers for odours from (A) *Odontotermes sp.* gallery soil, (B) *Odontotermes sp.* workers and soldiers and (C) *Odontotermes sp.* and gallery soil when presented alongside clean air. Black bars represent response to odours, while white bars represent response to the control. 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 test, **= significant at $P < 0.05$ and ***= significant at $P < 0.001$).

response to the control ($\chi^2 = 11.56$, $P < 0.001$, n = 26). In the case of minors, 85% chose the gallery soil compared to 15% to the control ($\chi^2 = 12.46$, $P < 0.001$, n = 26). Minors (82% vs. 18% controls; $\chi^2 = 8.90$, $P < 0.01$, n = 22) were more responsive to termite odours than majors (67% treatment vs. 33% control; $\chi^2 = 4.33$, $P < 0.05$, n = 21) (Figure 4.1B). Both majors (95% vs. 5% control $\chi^2 = 18.18$, $P < 0.001$, n = 22) and minors (91% vs. 9%; $\chi^2 = 14.72$, $P < 0.001$, n = 22) were highly attracted to the odours from termite galleries, (Figure 4 1C).

Given a choice between odours from termite only and termites in the gallery soil, the difference in the response of both major and minor workers to these odours were not statistically significant (Majors, $\chi^2 = 0.33$, $P = 0.54$, n = 25, Minors, $\chi^2 = 11.56$, $P = 0.67$, n = 27) (Figure 4.2).

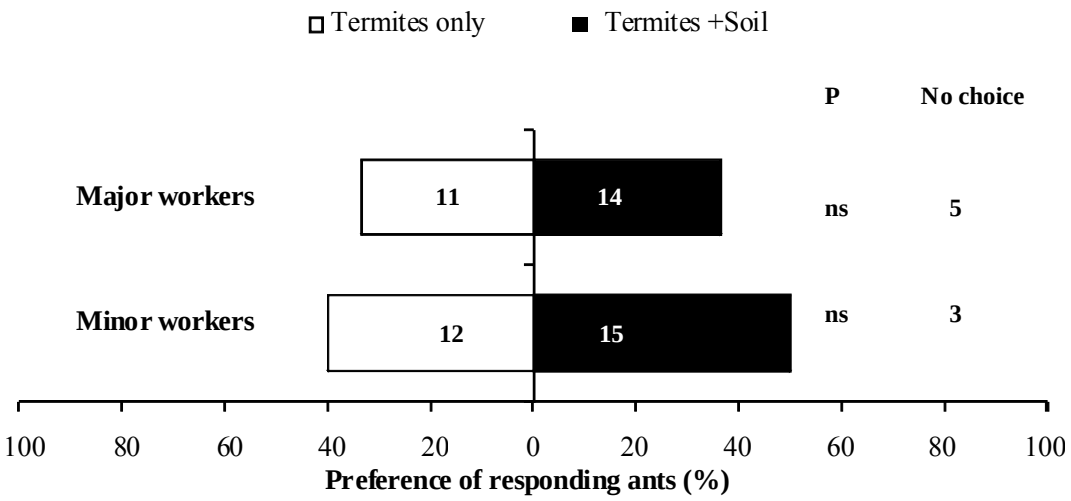

Figure 4.2 Preferences of *Pachycondyla analis* major and minor workers to odours from *Odontotermes sp*. (workers and soldiers), against odours from *Odontotermes sp.* in gallery soil. Black bars represent response to odours from termites in gallery soil, while white bars represent response to odours from termites only. 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, ns= Not statistically significant at $\alpha = 0.05$.

When the responses of both major and minor workers were pooled for all the odours tested, the response of workers to the odours from the gallery soil and those from the gallery with termites inside was not significantly different ($P = 0.54$). However,

responses of the ants to odours from termites and to the combined odours from termites and gallery were significantly different (P = 0.04), with no differences between the responses of major and minor workers, respectively (P = 0.84).

Identification of chemicals

Using GC-MS a total of seventeen components were tentatively from the odours of the termite gallery soil and the termites only (Figure 4.3). These components were mainly hydrocarbons and esters. The volatile from the termite gallery was compositionally richer, with thirteen components compared to nine in the volatile profile of termites (Figure 4.3).

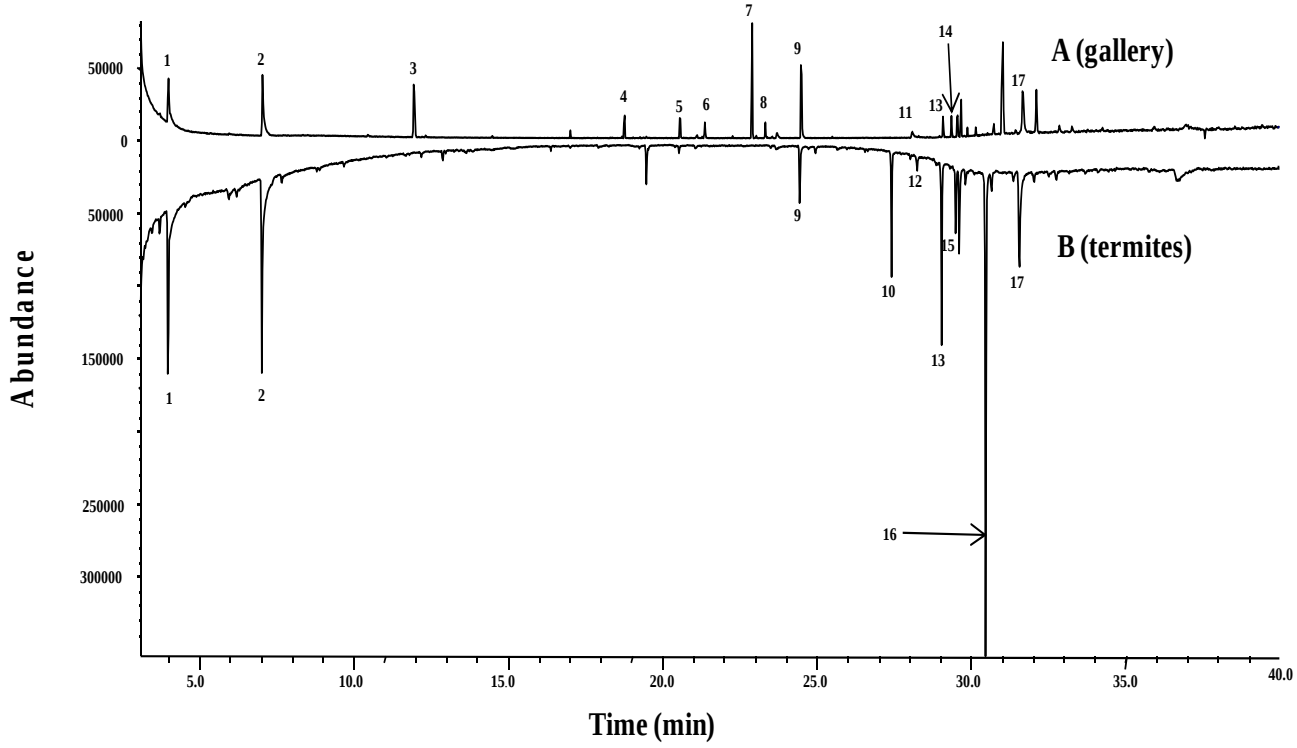

Figure 4.3 GC-MS trace of chemical compounds extracted from (A) ~2g of *Odontotermes* sp. gallery soil and (B) 10 *Odontotermes* sp. workers. Labelled peaks are: 1) *n*-heptane; 2) *n*-octane; 3) α-Phallendrene; 4) naphthalene; 5) Butanoic acid-tridecyl-ester; 6) 2-Napthalenemethanol; 7) Methyl-carbinol; 8) *n*-heptadecane; 9) *n*-eicosane; 10) *n*-tetracosane; 11) *n*-pentacosane; 12) hexylpentadecane; 13) 13-undecylpentacosane,; 14) *n*-octacosane; 15) 1-Nonadecene; 16) Oxalic acid, hexyl pentadecyl ester; 17) squalene.

Discussion

The results from the Y-tube olfactometer bioassays showed that workers of *P. analis* use olfactory cues associated with both termite and termite galleries in prey location. The combined odours from termites plus galleries were most attractive to both major and minor workers (Figure 4.1C). It appeared that, major workers were more sensitive to detecting these odours than minors. This differential sensitivity may be associated with the fact that the majors are frequently engaged in scouting for food and possibly an innate ability to also detect these food sources for the colony. Their specialisation in locating potential food sources accurately was observed in the field during raids. Out of total 330 raids observed in the field, only 5 were unsuccessful.

Longhurst and Howse (1978), performed behavioural assays with scout ants (major workers) in the field and found that they responded more to dry soil sheeting containing live termites (suggesting response to mechanical cues i.e. vibrations from termites) than soils without termites. However, in bioassays involving extracts of fresh soil sheeting, old soil sheeting, top soil and extract from head and thorax of termites, extract from whole termites and fresh soil sheeting Longhurst and Howse (1978) discovered that ants responded differently to these extracts. In the present study, field observations showed no raids by ants on fresh termite soil. As such this type of soil was not included in the study, and furthermore, in preliminary assays ants did not discriminate between odours from wet soil sheeting and the blank. The results from these studies reveal that *P. analis* workers use olfactory cues to locate termites prey accurately in the absence of visual or mechanical cues, which adds a new dimension to host location by *P. analis*.

Comparatively, minor workers were more responsive to odours from termite and termite galleries only than major workers (Figure 4.1A and B). These slight differences were not apparent when odours of the termites in the galleries were offered to different worker groups against a blank. These differential responses may be associated to the type of task minor workers undertake most especially during raids. During raids, the smaller body size of minor workers, unlike that of majors, allow them to enter deep inside the termite galleries to seek, paralyse and carry prey from the galleries. We observed a similar sensitivity to olfactory cues of chemical origin by minor workers in assays we carried out on volatile cues from conspecific *P. analis* workers.

In choice tests with termite odours only and those from termites in galleries, both worker ants responded more to the termites in galleries odours than to odours from termites alone (Figure 4.2). Although the results were not statistically significant, behaviourally it showed that ants could use cues from the galleries as long range cues or as first guides to potential termite sources. The ability of *P. analis* workers to detect odour cues coming from termites only can be used by workers to detect the presence of termite species like *Hodotermes mossambicus* which does not utilise soil sheeting (Longhurst and Howse, 1979). However, for a scout to make a decision to label that source as a potential food source; the scout has to detect the presence of termite prey inside by the use of chemical or mechanical cues from termites.

Although this is the first time olfactory cues in detecting prey by *P. analis* has been demonstrated, a previous study had reported similar detection mechanism in Myrmicinae ant *Crematogaster scutellaris* that uses olfactory cues to detect its fig wasp prey (Schatz *et al.,* 2003). The use of allomones in detecting termites has also been described for the larva of *Lomamyia latipennis* (Johnson and Hagen, 1981).

In the present study, chemical profiles of gallery soils and those of *Odontotermes sp* (termites) were found to be different. Except for 5 hydrocarbons (*n*-heptane, *n*-octane, *n*-eicosane, *n*-pentacosane and squalene) common to the profiles of these two ant attractive sources (Figure 4.3). The composition of the gallery soil was qualitatively richer than that of the termites. However, with respect to quantity, about six-fold more volatiles were released by the termites (Figure 4.3) with a major component of the latter being oxalic acid (not present in the gallery soil). To the best of our knowledge, this is the first time the potential chemical cues from both termites and their galleries had been identified in relation to the raiding behaviour of *P. analis*. The presence of naphthalene and its derivative 2-naphthalene-methanol in the volatiles of the galleries, which are known insect repellents, has previously been reported for subterranean termites (McLaughin, 2004). Termites use naphthalene and related compounds as a repellent to other insects, especially against ant predators. Naphthalene and a naphthalene derivative of plant origin (2-acetonaphthone) have also been reported as repellents for termites (Henderson *et al*., 2007). The chemicals in the termite soil galleries are believed to come from intestinal secretions of workers who mix them with soil particles and their saliva (Briunsma and Leuthold, 1977). These chemical components of termite origin

embedded in the galleries are used by *P. analis* scouts as an indicator of the presence of termites before detecting other cues associated with the termites themselves. The ability of *P. analis* to be attracted to odours from the galleries that include the presence of known ant repellents shows that this ant could have adopted the use of naphthalene and its derivatives of termite origin as possible kairomones along with other components from the cues.

To conclude, this study tested the roles of olfactory cues in the detection of termite prey by the termite-specific ant *P. analis*. Ant scouts initially detect chemical cues from termite galleries and then use a combination of the cues of termite origin and those of the galleries to identify a potential food source in order to initiate a raid. Both major and minor workers of *P. analis* detect these chemical cues. However, there is the need to identify the behaviourally active components, either singly or in a blend contributing to the detection of the termite food source.

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GENERAL CONCLUSION

Preceding chapters in this thesis had described the raiding behaviour, chemical communication and cues used in detecting prey by the termite specialist ant *Pachycondyla analis*. The raiding dynamics and behaviour of *P. analis* at Mpala a Kenyan savannah were described using data obtained from raiding behavioural studies over a period of six months. In order to determine if prey preferences exist and factors which may influence prey selection and preferences in *P. analis*, the composition of termite species preyed upon, the predation rate, and possible factors like reward, prey behaviour and defences were evaluated. For the first time, the use of olfactory cues as a means of communication within and outside the nest during raids by *P. analis* workers was studied using olfactometer bioassays using possible olfactory cues from different combinations of worker ants. The quantitative and qualitative differences in the volatiles released by *P. analis* used in the bioassays were also analysed using coupled Gas Chromatography-Mass Spectrometer (GC-MS) techniques. To determine contact chemical cues in *P. analis* and access their roles either as traditional nestmate recognition, cues used during raids or as task allocation cues within and outside the nest; cuticular hydrocarbons (CHCs) were extracted and used in series of mandible opening response bioassays (MOR). The compositions of CHCs between colonies and between major and minor workers in these colonies were also determined for the first time. In order to see if CHCs are colony and worker specific, discriminant analyses were used to group ants using CHC profiles into their colonies of origin and into either major or minor worker. Using the same approach as one used for the volatiles, the use of olfactory cues in finding potential termite sources in *P. analis* was also studied using termite and their galleries as odour sources. Odours from these sources were analysed qualitatively and quantitatively on the GC-MS.

This chapter summarises the key findings from each of the preceding chapters by bringing together the behaviour and chemical ecology of *P. analis* and highlighting areas for future research into both the behaviour, chemical ecology and the potentials to use the knowledge obtained from the chemical ecology of *P. analis* to develop a potential termiticide.

Nesting habits, termite raiding behaviour and some factors influencing prey preference of *Pachycondyla analis* at Mpala

In this study the ecology of *P. analis* in relation to its termite raiding behaviour was studied at Mpala a semiarid savannah in central Kenya. Findings here showed that the nesting behaviours of *P. analis* at Mpala were different from those in a Nigerian Guinea savannah (Longhurst *et al*., 1978) and Tanzanian coastal dry forest (Bayliss and Fielding, 2002). At Mpala most nests of *P. analis* were beneath rocks which seem to be a survival strategy to adapt to terrain like that at Mpala where the high altitude may mean that temperatures could be high during days and low at night. Rocks can assist ants in maintaining favourable temperatures within the nest during all times of the day. This is because rocks can store heat energy during the day to release it at night when temperatures are low. The phenomenon of regulating temperatures and thermal homeostasis is common in eusocial insects like bees (Seeley, 1985) and it has also been demonstrated in the red wood ant *Formica rufa* (Rosegren *et al.,* 1987). For the first time in the studies of raiding behaviour in *P. analis*, this study analysed some of the factors which may influence prey choice by *P. analis*. Factors looked at were prey abundance, foraging behaviour, reward to ants, costs in terms of raiding efficiency and direct cost to the ants in relation to termite physical defence mechanisms (casualties on the part of the ants after raids). These were some of the factors hypothesised by previous workers like Longhurst *et al*., (1978); Lepage, (1981) and Bayliss and Fielding, (2002) as having direct influences in prey choice by *P. analis*. Considering rewards as a factor in prey choice, raids were more frequent on *Microtermes* (66 %) that gives lower food rewards due to their size than on *Odontotermes* (34%) which could yield more food rewards. From this study, raiding behaviour of *P. analis* at Mpala was based more on prey abundance (*Microtermes* being more abundant with 81% representation against 14% for *Odontotermes* within study quadrats) than on the rewards from individual prey items. Raiding behaviour was synchronised with the foraging behaviour of the two termites genera preyed on by *P. analis* at Mpala. During the drier periods (months of May and June) ants preyed more on *Odontotermes*, while during wet periods (from the months of July, August and September) the prey choices were more for *Microtermes*. Ants invest more time raiding *Microtermes* than they do during raids on *Odontotermes,* although in terms of cost as a result of prey defences, ants get injured more frequently

when raiding *Odontotermes*. Based on the findings from this study, there is therefore a need for more studies on the raiding behaviours in *P. analis*. Such studies should focus on the same variables studied here, but observations and monitoring of raids be extended for at least two consecutive seasons or years as the case may be. While doing these seasonal studies, all nests should be monitored throughout the period or alternatively few nests be selected and their detailed raiding dynamics studied. Such a study will give more insight into any behavioural changes of *P. analis* with seasons or changes in prey habits. A better understanding of seasonal influences and the effects of temperature and rainfall will be important in the context of climate change. Outcomes from such a study can also be used to model the raiding behaviour of *P. analis* as it changes with factors influencing its prey. Such models can also be used to evaluate the impact of ant raiding behaviour on termites and the ecosystem in general. On prey choices and preferences, techniques like stable isotope and elemental analyses (e.g. Smith and Tillberg, 2009) could be employed with the aim of determining the feeding ecology of *P. analis*. This can be used to trace the dietary composition of *P. analis* to a specific termite species within a given period of time and related to prey behaviour.

Chemical ecology and communication of *Pachycondyla analis*

Experiments which explored the chemical ecology and chemical communication of *P. analis,* based on olfactory guided behavioural bioassays were conducted using volatile odour sources and, olfactory based contact bioassays using cuticular hydrocarbons from ants as source of stimulus. This is the first time a study on *P. analis* has focused on its intra-colony communications between nestmates within the nest and during raids on termites. In the past, studies focused mainly on the trail laying signals in *P. analis* and their sources e.g. Longhurst *et al.*, (1979); Hölldobler *et al.*, (1994a) and Janssen *et al.*, (1995), and queen pheromone and its source (Hölldobler *et al.,*1994b). A reason for taking the approach of using chemical volatiles from live ants as odour sources in this study was to test compounds that come from live ants in contrast to extracting glandular components. Using this approach one is able to get compounds released in nature by live ants and at similar rates used to trigger behavioural responses from receiving insects. One of the findings here is that ant workers use olfactory cues from conspecifics for communication within the nest and possibly outside the nest during raids, most especially when ants are not in visual contact with one another. There is also an

indication that ants could use olfactory signals in signalling the start of a raid upon arriving at a termite source after which ants spread, or finish of a raid for ants to regroup for the return journey back to their nest. Both major and minor workers responded to volatile signals from mixed workers (major and minor) and from either minor or major workers. Minor workers showed more sensitivity towards volatiles from all the sources tested, which can be understood because they undertake variety of tasks within and outside the nest (Villet, 1990) except foraging, thus being in contact with all the different types of odours within and outside the nest. Responses to conspecific volatiles in ants were earlier reported for the larvae of imported fire ants (Glancey and Dickens, 1988) and for queens of *Pachycondyla villossa* (D'Ettorre and Heinze, 2005). Analyses of the chemical compositions of volatiles showed that: the odours contained mainly hydrocarbons, with groups of mixed, major, and minor workers sharing several components, although qualitative differences existed. Differences in composition of volatiles between major and minor workers, supports the classification of workers based on morphological traits like interocular distances and scape lengths reported by Crewe *et al.*, (1984). Interestingly, the quantities of volatiles released by workers when placed in mixed groups were two and half times lower than when workers were separated into majors and minors. This is an indication that in the colony with all workers present the colony odour is maintained with little efforts from all workers, but when workers are separated based on their sizes, each group could be in a struggle to keep the colony odour. As a result they use more energy thus producing more in an effort to maintain the colony odour. The general conclusion here is that *P. analis* uses olfaction in its communication between nestmates within the nest or outside during raids and the cues originate from nestmates of the same size or the other or from the colony as a signature. Also the compositions of these chemical cues are different depending on their source and ants are able to decode information from them irrespective of the source.

Further studies on the individual chemical components in the odours of *P. analis* with the aim of identifying the behaviourally active compounds in these profiles will shed more light on the roles they play in the chemical communication of *P. analis*. A possible way to do this is to evaluate synthetic chemical standards of these components in behavioural assays. Studies to look at the roles of nest volatiles in nestmate recognition in order to ascertain if volatiles of nest origin play a role in the

communication system of *P. analis* using similar approaches to those used by Katzav-Gozansky *et al.*, (2008) for *Camponotus fellah* will add to the findings presented here.

Since Fielde in 1901 concluded that cuticular lipids, particularly those that cover insects contained nestmate discrimination signals; the roles of hydrocarbons in insect chemical communication has received attention. This study showed that in *P. analis,* cuticular hydrocarbons (CHCs) play a significant role as nestmate and non-nestmate recognition cues similar to those exhibited by *Formica ants* (Akino *et al.,* 2004; Martin *et al*., 2008). CHCs in *P. analis*, apart from serving as traditional nestmate recognition cues may also play vital roles in communication during raids and as task allocation cues as in the red bull ant *Myrmecia gulosa* (Dietemann *et al*., 2003). CHCs profiles in *P. analis* contained three groups of hydrocarbons (*n*-alkanes, alkenes and methyl-branched alkanes) with chain lengths of C_8 to C_{31}. They varied between colonies with the proportions of *n*-alkanes similar between colonies and the alkenes and methyl-branched alkanes differing in their proportions. Worker groups within the colonies studied also contained varied components within the same colony or in comparison to other colonies. This provides more evidence of chemical cues used in task allocation by workers.

Areas for future research on the CHCs of *P. analis*, include separating the three different groups of hydrocarbons i.e. *n*-alkanes, alkenes, and methyl-branched alkanes using silver nitrate chromatography and 5-Å molecular sieve. These fractions can then be used in the mandible opening response bioassay to determine which fraction is actively involved in nestmate recognition. It will also be useful to manipulate the CHC profiles of ant colonies by supplementing them using synthetic standards, and test the response of nestmates and non-nestmates to the adjusted chemical profiles (as in D'Ettore *et al*.,2004 for *P. villosa*, and Guerrieri *et al*., 2009 for *Camponotus herculeanus*). Such an approach will help to identify the active group of compounds responsible for nestmate recognition in *P. analis*. CHC extracts of *P. analis* can also be evaluated as potential termite repellents aimed towards the development of bio-friendly termiticides. These extracts could possess termite repellent properties. Previous studies that evaluated the potential of semiochemicals of ant origin as termite repellents yielded positive results. For example, semiochemicals extracted from a Dolichoderine ant had toxic and repellent effects on subterranean termites in Hawaii (Cornelius *et al*., 1995).

Recently, a potent termite repellent was isolated from the Bornean Dolichoderine ant *Dolichoderus sulcaticepus* in Malaysia (Fujiwara-Tsuji, *et al*., 2006). Note that these ants are not termite specialists; therefore semiochemicals from *P. analis* (a termite specialist) has greater potential as a candidate termiticide.

With recent trends and advances in molecular biology, there are prospects of applying molecular techniques such as DNA isolation and use of DNA primers (e.g. Gadau, 2009) to trace the origin of ants from different colonies. This will further confirm if colonies are genetically related and could explain the variations in aggression towards non-nestmates between colonies.

Detection of termite prey by *P. analis*

On the question of how *P. analis* detects its prey and the cues it uses to locate prey with precision, this study explored olfactory cues from termites and their galleries and described how *P. analis* detects these cues in olfactometric bioassays. This is the first time the roles of olfactory cues were tested exclusively in relation to prey detection by *P. analis*. Studies in the past laid more emphasis on the use of mechanical and chemical cues by the ant to detect its prey but did not identify the chemical cues responsible (e.g. Longhurst and Howse, 1978). Longhurst and Howse (1978) tested both mechanical and chemical cues (extracts) in the presence of visual cues, and concentrated more on scout ants. This study successfully tested olfactory cues from termites, termite galleries and from termites in their galleries. The results showed that in *P. analis* workers (both major and minor) olfaction is very important in the detection of their termites prey. Cues from both termites and from the gallery soils are both detected, but cues from both (termite and their galleries) have to be present together as signals to a scout ant that prey are present inside their galleries prior to initiating a raid. Accuracy of raids in *P. analis* reveals a specialisation on detecting cues by scout ants, as these scout ants lead nestmates to successful raids with a level of accuracy of about 98% during raids observations in the field at Mpala. Detection of termite odours also by *P. analis* indicates that it has a specialised ability to detect termites, and ants are even able to detect termite species like *Hodotermes mossabicus* (Hagen) which is observed not to build galleries or use soil sheeting while foraging (Longhurst and Howse, 1978).

Chemical analysis of extracts from termites and termite gallery soil revealed that their chemical composition contained mainly hydrocarbons. Gallery soils contained more chemical cues than termites alone and are richer in terms of quantities of the chemical components. Naphthalene and its derivatives were present in gallery soil which were absent in the termites. These are potent insect repellents (Henderson *et al.*, 2007) used by termites against insect predators and are a component of the regurgitant used in nest building. Another intriguing finding is the ability of *P. analis* to be attracted more to gallery soils despite the presence of insect repellents in them. This indicates a possible adaptation to insect repellents of termite origin by *P. analis* as part of the arms race between them and termites.

Findings here on the detection of termite prey by *P. analis* using olfactory cues need to be further investigated using several other termite species. Results from such a study will reveal whether *P. analis* demonstrate a preference for particular termite species which they prey and whether there are any semiochemicals that are diagnostic of different termite species. If preference is observed, this can further be proved by looking at factors like difference in the chemistry of the termite odour and those of their galleries. Screening for behavioural active compounds can also be conducted on the identified compounds to access the ones responsible for either attraction or repelling ants and termites.

In conclusion, these studies have revealed the nesting habits of *P. analis* in a Kenyan semiarid savannah and its foraging behaviour which is synchronised with that of its prey. *P. analis* appears to forage optimally balancing the cost of foraging with rewards. In doing this, factors like prey size, relative abundance, and defences play a great roles. The rich diversity of chemical communication cues used by this ant species for nestmate recognition and for prey location during raids, in search for its food source was also revealed, with ants showing the ability to communicate using odours released by nestmates and those released by prey. These studies have added to our knowledge of the chemical ecology of *P. analis* and serves as a starting point for the exploration of possible termiticides from *P. analis*. Moreover, it shows how efficient and cost effective the raiding behaviour is expressed in this ant and the importance of chemical communication in predator-prey interactions.

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