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

by

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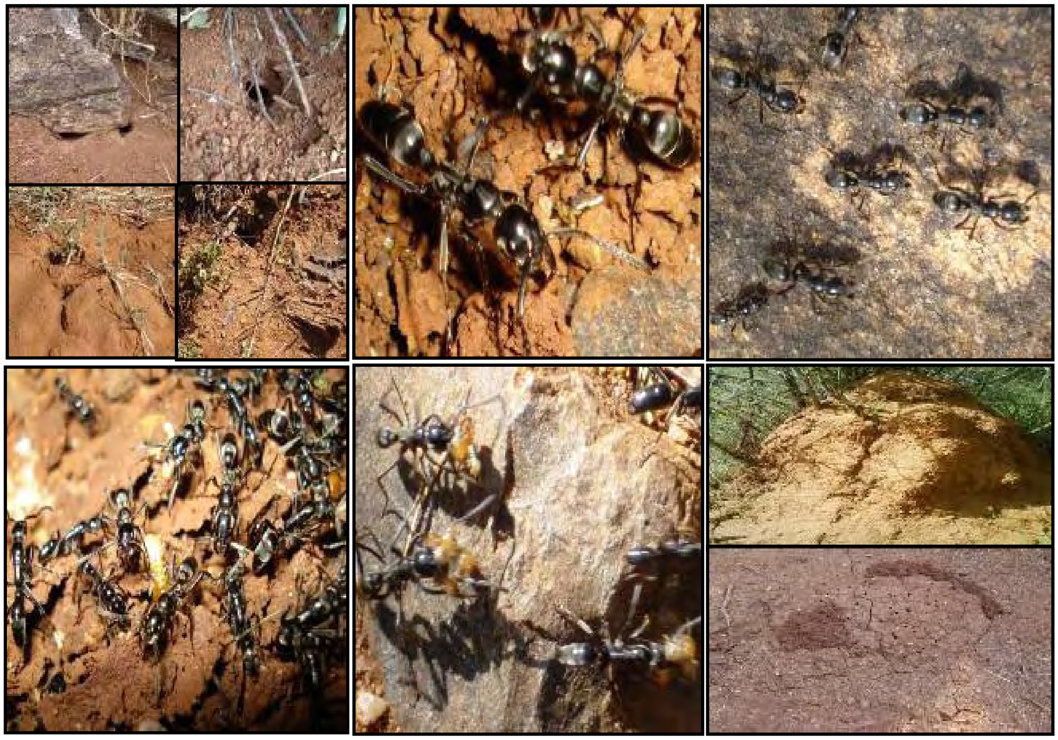
Declaration

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).

© Photos: www.termite-i.com

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).

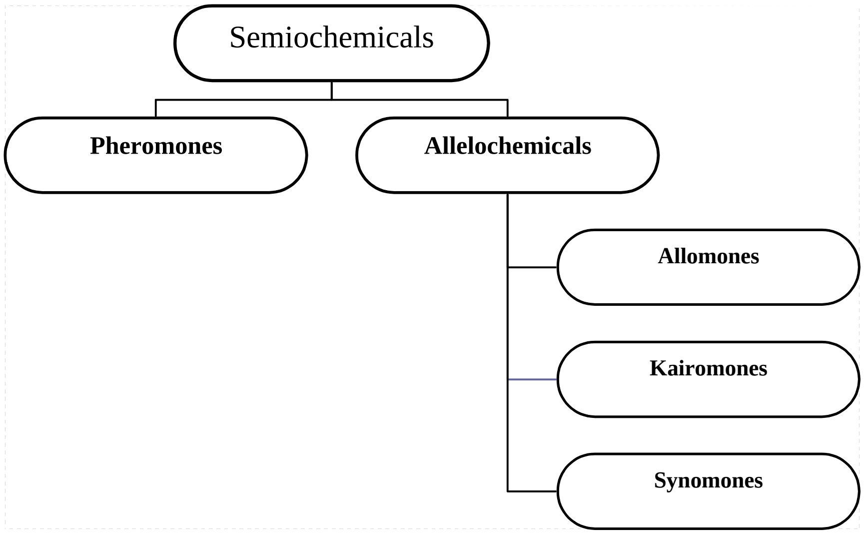

Figure.3 Schematic diagram showing the classification of semiochemicals

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.

References

Bayliss, J., and Fielding, A. (2002). Termitophagous Foraging by *Pachycondyla analis* (Formicidae, Ponerinae) in a Tanzanian Coastal Dry Forest. *Sociobiology, 39 (1)*:103-121.

Borror, D.J., Triplehorn, C. A., and Johnson, F., N. (1992). *An Introduction to the study of Insects*, 6th ed, Saunders College Publishing, USA.

Bruyne M. de, and Baker T. C. (2008). Odor Detection in Insects: Volatile Codes, *Journal of Chemical Ecology*, *34*: 882-897.

Carr, R. (2006). Review of the Behavioral Ecology of Subterranean Termites (Isoptera: Rhinotermitidae: Coptotermes sp. and Reticulitermes sp.) With Discussion on Applications to Alternative Control Methods [online] http://www.colostate.edu/Depts/Entomology/courses/en507/papers_1999/carr.htm (7th November 2006].

Corbara, R., and Dejean, A. (2000). Adaptive Behavioural flexibility of the ant *Pachycondyla analis* (= *Megaponera foetens*) (Formicidae: Ponerinae) during Prey capture. *Sociobiology 36 (3)*:465-483.

Cornelius, M. L., and Grace, J. K. (2000). Semiochemicals Extracted from a Dolichoderine Ant Affects the Feeding and Tunnelling Behaviour of the Formosan Subterranean Termite (Isoptera: Rhinotermitidae). *Journal of Economic Entomology 87 (3):*705-708.

Cornelius, M. L., Grace, K. J., Ford, P. W., and Davidson, B. S. (1995). Toxicity and Repellency of Semiochemicals Extracted from a Dolichoderine Ant (Hymenoptera: Formicidae) to the Formosan Subterranean Termite (Isoptera: Rhinotermitidae). *Environmental Entomology 24 (5)*:1263-1269.

Crewe, R. M., Peeters, C. P., and Villet, M. (1984). Frequency distribution of worker sizes in *Megaponera foetens* (Fabricius). *South. African. Journal of Zoology. 19(3)*: 247-248.

Culliney, T. W., and Grace, J. K. (2000). Prospects of the Biological control of subterranean termites (Isoptera: Rhinotermitidae), with special reference to *Coptotermes formosanus, Bulletin of Entomological Research 90*:9-21.

Dejean, A., Schatz, B., Orivel, J., Beugnon, G., Lachaud, J. P., and Corbara, B. (1999) Feeding Preferences in African Ponerine Ants: A Cafeteria Experiment (Hymenoptera: Formicidae). *Sociobiology 34 (3):*555-568.

Edwards, R., and Mill, A. E. (1986). *Termites in buildings: their biology and control*. East Grinstead, United Kingdom, Rentokil LTD.

Eggelton, P. (2001). Termites and trees: a review of recent advance in termites phylogenetics. *Insectes Sociaux, 48*:187-193.

Fittkau, E. J., and Klinge, H. (1973). On biomass and tropic structure of the central Amazonian rain forest ecosystem. *Biotropica, 5 (1)*:2-14.

Fujiwara-Tsujii, N., Cheong, C. H., Maryati, M., and Yamaoka, R. (2006). Identification of a potent termite repellent from the Bornean Dolichoderine ant *Dolochoderine sulcaticepus* (Hymenoptera: Formicidae). *Journal of Tropical Biology and Conservation, 2 (1):*71-78.

Grace, J. K., Tome, C. H. M., Shelton, T. G., Oshiro, R.J., and Yates, J. R. III. (1996). Baiting Studies and considerations with *Comptotermes formosanus* (Isoptera: Rhinotermitidae) in Hawaii. *Sociobiology, 28*:511-520.

Hickin, N. E. (1971). *Termites a World Problem*, Hutchinson and Co. Publishers LTD London, UK.

Howse, P., Stevens, I., and Jones, O. (1998), *Insect Pheromones and their use in Pest Management*, Chapman and Hill, London.

Hölldobler, B., and Wilson, E. O. (1990). *The Ants*, Harvard University Press, Cambridge, Massachusetts, USA.

Hölldobler, B., Braun, U., Gronenberg, W, Kirchner, W. H., and Peeters, C. (1994) Trail Communication in the Ant *Megaponera foetens* (Fabr.) (Formicidae, Ponerinae), *Journal of Insect Physiology*. *40(7)*:585-593.

Hölldobler, B. (1995). The Chemistry of Social Regulation: Multicomponent Signals in Ant Societies. *Proceedings of the National Academy of Science, 92 (1)*:19-22.

Inward, D., Beccalon, G., and Eggleton, P. (2007). Death of an order: a comprehensive molecular phylogenetic study confirms that termites are eusocial cockroaches. *Biology Letters*. *3*:327–330.

ITIS. (2007). Integrated taxonomy information system. Taxonomic serial no. 583667. Tuesday April 10, 2007.

Janssen, E., Bestman, H. J., Hölldobler, B., and Kern, F. (1995). N, N-Dimethyluracil and Actinidine, Two Pheromones of the Ponerine ant *Megaponera foetens* (Fab.) (Hymoneptera: Formicidae), *Journal of Chemical Ecology, 21(12)*:1947-1955.

Janssen, P**.** (2006) Termite Control [online] URL:http://www.janssenpharmaceutica.be/pmp/Pages/termite.htm (17th Nov., 2006).

Jones, S. C**.** (2006). *Termite Control Extension FactSheet*, *Entomology*, The Ohio State University, Extension, USA.

Keller, A., and Vosshall, L. B. (2003). Decoding olfaction in *Drosophila*. *Current Opinion in Neurobiology, 13:*103-110.

Lepage, M. (1981). Ětude de la Predation de *Megaponera foetens* (F.) Sur lés Populations Recoltantes de Macrotermitinae dans un Ecosysteme Semi-aridé (Kajiado- Kenya). *Insectes Sociaux, 28(3)*:247-262.

Lepage, M. (1984). Distribution, Density and Evolution of *Macrotermes bellicosus* Nests (Isoptera: Macrotemitinae) in the North-East of Ivory Coast, *Journal of Animal Ecology, 53*:107-117.

Lévieux, J. (1966). Noté préliminaire sur les colonnes de chasse de *Megaponera foetens* F. (Hymenoptera: Formicidae). *Insectes Sociaux, 13*:117-126.

Lévieux, J. (1982). A comparison of the ground dwelling ant populations between a Guinea savannah and an evergreen rain forest of the Ivory Coast. In Breed, M.D. *et al*., The Biology of Insects (Proceedings of the Ninth congress of the International Union for the study of social insects, Boulder Colorado, 1982), Westview Press, Boulder, pp 48-53.

Livingstone, D. (1857). *Missionary travels and researches in South Africa*. John Murray, London.

Longhurst, C., and Howse, P. E. (1977) Predatory behaviour of *Megaponera foetens* (Fabr.) (Hymoneptera: Formicidae) in Nigerian. *Procedure VIIIth Int. Congr. I.U.S.S.I.,* Wageningen.

Longhurst, C., Johnson, R. A., and Wood, T. G. (1978). Predation by *Megaponera foetens* (Fabr.) (Hymoneptera: Formicidae) on Termites in the Nigerian Guinea Savannah. *Oceologia (Berl) 32:*101-107.

Longhurst, C., and Howse, P. E. (1979). Foraging, Recruitment and Emigration in *Megaponera foetens* (Fab.) (Hymoneptera: Formicidae) from the Nigerian Guinea Savannah. *Insectes Sociaux, 26(3)*:204-215.

Longhurst, C., Baker, R., and Howse, P. E. (1979). Termite predation by *Megaponera foetens* (Fab.) (Hymenoptera: Formicidae) coordination of Raids by Glandular Secretions. *Journal of Chemical Ecology, 5(5):*703-719.

Malnic, B., Godfrey, P. A., and Bulk, L. B. (2004). The Human olfactory receptor gene family. *Proceedings of the National Academy of Science America, 101:*2584-2589.

Maschwitz, U., and Mühlenberg, M. (1975). Zur Jagdstrategie einger orientalischer *Leptogenys*-Artens (Formicidae). *Oecologia, 20:*65-83.

Maschwitz, U., and Schönegge, P. (1983). Forage communication, nest moving recruitment, and prey specialisation in the oriental ponerine *Leptogenys chinensis*. *Oecologia, 57*:175-182.

Mauldin, J. K. (1986). Economic importance and control of termites in the United States, in Vinson, S.B. (Ed) *Economic impact and control of social insects.* Praeger Publishers, New York, 130-143.

Meyer, J. R. (2006). *General Entomology textbook* [online] http://www.cals.ncsu.edu/course/ent425/index.html, [10th Sept 2006].

Mitchell, J. D. (2002). Termites as pests of crops, forestry, rangeland and structures in Southern Africa and their control. *Sociobiology 40*: 47-69.

Mora, P., Rouland, C., and Renoux, J. (1996) Foraging, nesting and damage caused by Microtermes subhyalinus (Isoptera: Termitidae) in a sugarcane plantation in the Central African Republic. *Bulletin of Entomological Research*, ***86****(4)*, pp. 387-395New, T. R., (1991). *Insects as Predators*, South Wales University Press, Australia.

Nordlund, D. A. (1981). Semiochemicals a review of the terminology, in D. A. Nordlund, R. L. Jones, and W. J. Lewis, eds *Semiochemicals,* John Wiley, New York.

Pearce, M. J., Bacchus, S., and Logan, J. W. (1996). *What Termite? A guide to Identification of Termite Pest Genera in Africa*, Natural Resources Institute, Overseas Development Administration, UK.

Robertson, H. M., and Wanner, W. (2006). The chemoreceptor superfamily in the honeybee, *Apis mellifera*: Expansion of the odorant, but not gustatory receptor family. *Genome Research, 16:* 1395-1403.

Room, P.M. (1971) in Hölldobler, B., and Wilson, E., O (1990). *The Ants*, Harvard University Press, Cambridge, Massachusetts, USA.

Rödel, M-O., and Braun, U., (1999). Association between Anurans and Ants in a West African Savanna (Anura: Microhylidae, Hyperoliidae, and Hymenoptera: Formicidae). *Biotropica, 31 (1)*:178-183.

Sheppe, W. (1970). Invertebrate predation on termites of the African savanna. *Insectes Sociaux 17*:205-218.

Su, N. Y**.** (1993). Managing Subterranean Termites populations, in Wildey, K.B. and Robinson, W.H. (eds) *Proceedings of the 1st International Conference on Insect Pests in Urban Environment, Cambridge*, 30 June- 3 July, 1993:45-50.

Taylor, B. (2006). The Ants of Africa Distribution Card[online] http://antbase.org/ants/africa/distribution_cards/notes.htm (9th November, 2006).

Torto B. (2004). Chemical Signals as Attractants, Repellents and Aggregation Stimulants. In *Encyclopedia of Life Support Systems (EOLSS),* developed under the auspices of the UNESCO. Eolss Publishers, Oxford, UK (http://www.eolss.net).

UNEP/FAO (2000). Finding Alternatives to Persistent Organic Pollutants for Termite Management, UNEP/FAO/Global IPM Facility Expert Group on Termite Biology and Management, United Nations.

Villet, M. H. (1990). Division of labour in the Matebele ant *Megaponera foetens* (Fabr.) (Hymenoptera Formicidae). *Ethology Ecology and Evolution, 2 (4)*:397-417.

Wilson, E. O. (1971). *The Insect Societies*. Belknap press of Harvard University press, Cambridge, Mass, U.S. A.