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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