An effective method for maintaining the African termite-raiding ant *Pachycondyla analis* in the laboratory

A.A. Yusuf[1,2*], R.M. Crewe[1] & C.W.W. Pirk[1]

[1]*Social Insects Research Group, Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield, Pretoria, 0028 South Africa*
[2]*International Centre for Insect Physiology and Ecology, P.O. Box 30775-00100 GPO, Nairobi, Kenya*

Pachycondyla analis Latreille (Hymenoptera: Formicidae) is a common African Ponerine ant that organizes group raids on termites considered a huge burden to agriculture. This ant has been the subject of various entomological and natural history studies aimed at understanding their group raiding behaviour and impact on the ecosystem as well as the roles they play in regulating field termite populations. However, colony maintenance under laboratory conditions for long-term research purposes has largely been unsuccessful. Herein, we report an effective method for maintaining *P. analis* in the laboratory for long-term studies that may include behavioural, life history and chemical ecology. Using a simple set-up made up of a Perspex foraging arena and an aluminum nest box in the laboratory, queen right colonies were successfully maintained for an average of 27.0 ± 6.0 weeks and a maximum of 34 weeks before declining. High ant mortality (6–48 %) was observed in the first week of captivity in the laboratory. This declined to a weekly mortality of 4.0 ± 3.6 % (24 ± 22.5 ants per colony) after the ants had settled in their new laboratory nest. Therefore, using our laboratory rearing set-up, and keeping laboratory conditions similar to those in the field, as well as feeding *P. analis* on its usual diet of termites, could increase colony survival time up to 4.5 times longer than previously reported rearing protocols.

Key words: African ponerine, Formicidae, Hymenoptera, Macrotermitinae, Matabele ant, *Megaponera foetens*, termitophagus, rearing protocols.

INTRODUCTION

The African termite raiding ant *Pachycondyla analis* Latreille (Hymenoptera: Formicidae) has been an insect of great interest to many entomologists and naturalists (Livingston 1857; Wheeler 1936; Lévieux 1966; Longhurst *et al.* 1978; Lepage 1981; Yusuf *et al.* 2010). This is because of its unique feeding habits that include organizing group raids, feeding solely on termites (Dejean *et al.* 1999) and the prospects such a specialized feeding behaviour hold for the biological control of field populations of termites (Bayliss & Fielding 2002). Workers of *P. analis* have been regarded as dimorphic by taxonomists, but measurements of head width *vs* head length indicated that rather than being dimorphic, this species exhibits monophasic allometry (Crewe *et al.* 1984). Morphological differentiation is exhibited among workers in terms of size, with the larger (major) workers possessing a fine pubescence while the smaller (minor) workers are black and shiny (Villet 1990). This termitophagous ant organizes group raids mostly on termite species belonging to the subfamily Macrotermitinae which are of economic importance especially in agriculture (Longhurst *et al.* 1978; Lepage 1981). These raids are initiated when a scout ant detects a potential food source and then returns to the colony where it recruits nest mates using trail pheromones (Longhurst & Howse 1979). Upon arrival at the food source, the ants spread out, break open and invade the termite galleries to seek th termites. The termites are captured through stinging which results in paralysis, after which the invading ants carry them out to a place near the gallery entrance to continue the hunting. After gathering enough termites they stop hunting, re-group in columns and begin the return journey to their nest (Longhurst *et al.* 1978). A major worker can grasp up to seven termites between its mandibles, while a minor worker can grasp up to three termites. Some do not carry any termites but lead the columns of nest mates on the return journey to the nest (Longhurst *et al.* 1978; Yusuf 2010). The raids last between 4 and 50 min depending on the foraging distance and

*Author for correspondence.
E-mail: aayusuf@zoology.up.ac.za / ayusuf@daad-alumni.de

the termite species being raided (Yusuf 2010). Studies from different habitats in sub-Saharan Africa where *P. analis* is found, have shown that the ant has a wide range of termite prey, consuming available and abundant termite species within its immediate habitat (Longhurst *et al.* 1978; Bayliss & Fielding 2002; Yusuf 2010). Recent studies revealed that *P. analis* uses contact chemical cues in nestmate recognition and ultimately in communication (Yusuf *et al.* 2010).

The vast amount of knowledge generated on insects can in part be attributed to the availability of long-term rearing protocols which have permitted their maintenance over a generation. Such rearing protocols are usually based on natural and artificial diets. While suitable artificial diets are increasingly becoming available for rearing various insect species (Anderson & Leppla 1992), *P. analis* rearing continues to be a challenge. Moreover, rearing insects on artificial diets is an expensive process especially in developing countries where funds for research are not easily accessible (Abbasi *et al.* 2007). It is therefore important to develop economically viable rearing and maintenance methods for various insects that suit the needs and resources of resource-poor researchers. However, despite the importance of *P. analis*, detailed information on its maintenance in the laboratory is lacking. This study was premised on the assumption that, if *P. analis* is maintained in the laboratory on its normal diet of termites under similar field conditions, such as temperature and humidity, their survival rate would increase thereby leading to a successful captive maintenance of these ants in the laboratory. This method would be useful in both short- and long-term experiments aimed at studying various aspects of the biology of *P. analis* and exploring its potential in termite biological control programmes.

MATERIAL AND METHODS

Six colonies of *P. analis* with representatives of both sexes (workers and males), eggs, larvae and cocoons containing between 88 and 1191 individuals, were excavated at Mpala (0°17′N 37°52′E), 250 km north of Nairobi, on the research facility of Mpala Wild Life Foundation, Central Kenya, in March, April, May and October 2008, and February and July 2009. Excavations were carried out either in the mornings or late in the evenings by carefully digging around the perimeter of the nest using a pick

Fig. 1. Ant-carrying box used for collecting and transporting ant colonies to the laboratory.

after blocking all openings to the nest to prevent ants from escaping. Ants were carefully collected using a soft paintbrush and placed into an ant-carrying box made of a 21 cm × 15 cm × 40 cm plastic food storage container (Fig. 1), which was partially filled with soil from the excavated nests to serve as temporary nesting material for the ants during transportation. The ant-carrying boxes were then transported to the Animal Rearing and Containment Unit (ARCU) located on the Duduville campus of the International Centre for Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya. All the colonies excavated were queen right except for one collected in July 2009 which was queenless. Within the rearing facility, the ant colonies were provided with aluminium nesting boxes (20 cm × 20 cm × 20 cm), with a lid that could be opened to observe ants' activities inside the nest (Fig. 2). The base of the nesting boxes were also partially filled with soil from the excavated ant's nest to serve as a permanent nesting material. The nest boxes were each attached through a 7 cm opening at the base to a Perspex foraging arena (1.0 m × 1.5 m) that was also partially filled with sterilized soil (Fig. 2). This soil was sterilized by washing with double-distilled water and oven-dried overnight at 160 °C to prevent possible contamination from microbes. Ants were fed twice;

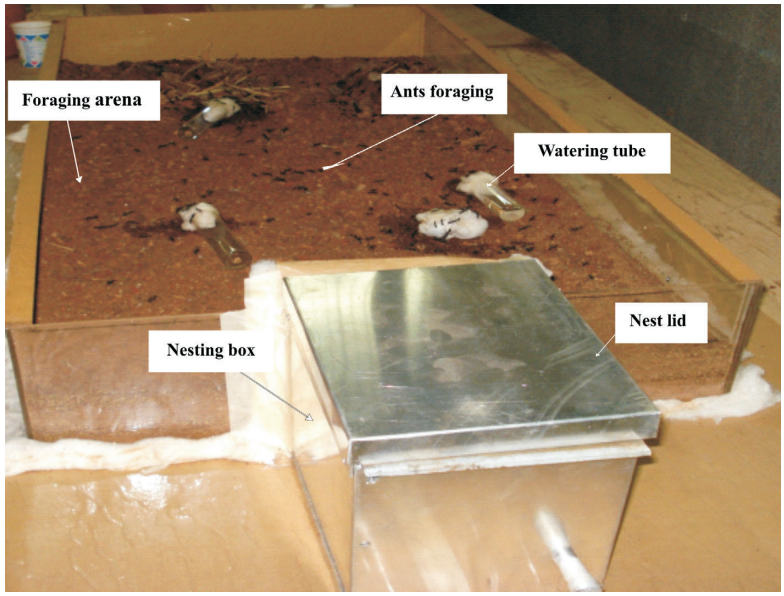

Fig. 2. Ant rearing set-up in the laboratory, showing the ant nesting box, foraging arena (1.0 × 1.5 m), watering point and nest observation lid.

in the mornings (07:00 to 11:00) and late afternoon/early evening (16:00 to 19:00) daily on live termites belonging to the genera *Odontotermes* and *Microtermes* collected from mounds or foraging galleries around the ICIPE Duduville campus. The choice of these termite genera was informed by observations on raiding behaviours of *P. analis* in the field at Mpala. Feeding was achieved by placing the termites at the end of the foraging arena further away from the nest box. This stimulates scouting, foraging and raiding behaviours in the ants since they have to search, find and paralyse the termites prior to capturing and transporting them to the nest boxes. Test tubes filled with water and plugged with cotton wool (Fig. 2) that releases the water gently into the soil in the foraging arena served as a source of moisture for the ants. Conditions in the rearing room were kept between 50 and 60 % RH, and 24–29 °C under a natural photoperiodic cycle (12L:12D) to mimic field conditions.

Weekly ant mortalities were determined by counting the number of dead ants (which are usually removed from the nesting box and placed by the ants in a corner of the foraging arena) daily in the morning and evenings throughout the life of that particular colony. This was used to express the percentage mortality based on the entire colony population.

RESULTS

Six colonies of *P. analis* were successfully maintained in captivity for an average of 23.5 ± 9.2 weeks in the laboratory with a minimum of six weeks (for the only queenless colony obtained in July 2009) and a maximum of 34 weeks for a colony obtained in October 2008 (Fig. 3). On average, colonies with queens survived for 27.0 ± 6.0 weeks ($\chi^2 = 14.4$, $P < 0.0002$, 95 % CI, 19.6–34.4) before they declined to a few individuals. High ant mortality (6 to 48 %) was observed in the first week of captivity (usually between the first to the third day) in the laboratory (Fig. 3). These high mortalities could be attributed to factors such as stress or injuries sustained during excavations, transportation to rearing facility at ICIPE from Mpala (a journey of 250 km), as well as to problems in adapting to conditions in the rearing facility. Soon after this acclimation period, the colonies became stable, with an average weekly mortality of 4.0 ± 3.6 % (24 ± 22.5 ants per colony).

DISCUSSION

As described in previous studies (Longhurst 1977; Villet 1990), *P. analis* is difficult to maintain in the laboratory. However, our set-up significantly improved the time colonies could be kept alive

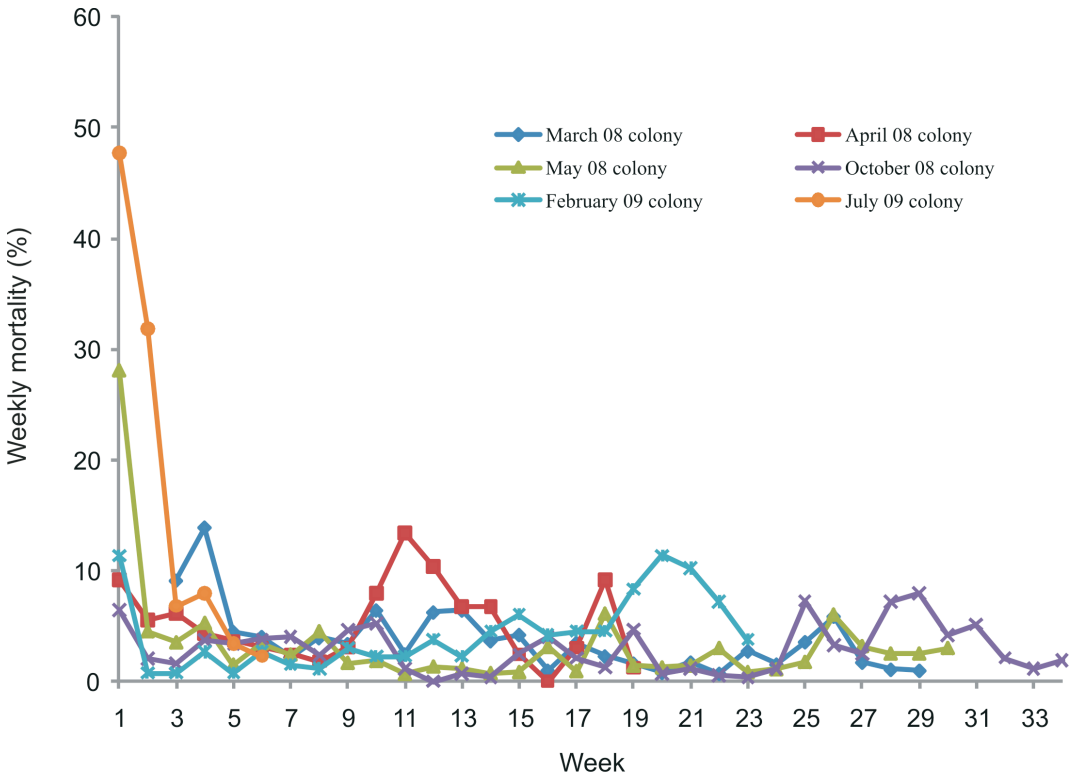

Fig. 3. Mortality data per week per colony for the six colonies of *Pachycondyla analis* maintained in the laboratory. The colony excavated in July 2009 was queenless, hence the sharp decline and high weekly mortality rates.

in the laboratory, from six weeks as reported in Hölldobler *et al.* (1994), up to about 34 weeks. Such a significant increase would allow ample time (4.5 times longer) for studying *P. analis* in the laboratory.

Maintaining ant colonies under similar conditions of temperature and relative humidity as obtained in the field is desirable and may be one of the factors leading to the success recorded in this study. Another important factor that contributed to the successful maintenance of the ants in the laboratory was keeping them on their normal diet of termites as opposed to feeding them insects such as mealworms. Dejean *et al.* (1999) showed that *P. analis* is strictly termitophagous and only ate cockroaches that lived commensally within its nest as alternative prey.

This experimental set-up clearly demonstrated an improvement in the time (up to approximately 8½ months) that this ant could be kept and maintained in the laboratory. This was achieved by providing similar conditions as prevailed in the field, including feeding the ants on their usual diet of termites. This is a very important step towards solving one of the puzzles in the study of this important ant species in several attempts to maintain them in the laboratory. The results also showed the importance of maintaining insects on their normal diets where possible, against substituting with alternatives which could lead to shorter lifespan and possibly cause behavioural changes.

ACKNOWLEDGEMENTS

We acknowledge K.L. Crous and three anonymous reviewers for their comments on an early version of the manuscript, the Mpala Research Centre, Nanyuki, Kenya, for permission to work on their premises, M. Stüben of the University of Würzburg, Germany for confirming the identity of the ants, and P. Malusi of the Animal Rearing and Quarantine Unit at ICIPE for his help with ant rearing. Funding was provided by the Dutch SII project 2004/09 Activity No. 10799 to ICIPE, the University of Pretoria and a DAAD fellowship to A.A.Yusuf.

REFERENCES

ABBASI, B., AHMED, K., KHALIQUE, F., AYUB, N., LIU, H., KAZMI, S. & AFTAB, M. 2007. Rearing the cotton bollworm, *Helicoverpa armigera*, on a tapioca-based artificial diet. *Journal of Insect Science* **7**: 35.

ANDERSON, T.E. & LEPPLA, N.C. 1992. *Advances In insect Rearing for Research and Pest Management*. Westview Press, Oxford, and IBH Publishing Co., India.

BAYLISS, J. & FIELDING, A. 2002. Termitophagous foraging by *Pachycondyla analis* (Formicidae, Ponerinae) in a Tanzanian coastal dry forest. *Sociobiology* **39**: 103–121.

CREWE, R.M., PEETERS, C.P. & VILLET, M. 1984. Frequency distribution of worker sizes in *Megaponera foetens* (Fabricius). *South African Journal of Zoology* **19**(3): 247–248.

DEJEAN, A., SCHATZ, B., ORIVEL, J., BEUGNON, G., LANCHAUD, J.P. & CORBARA, B. 1999. Feeding preferences in African ponerine ants: a cafeteria experiment (Hymenoptera: Formicidae). *Sociobiology* **34**: 555–568.

HÖLLDOBLER, B., PEETERS, C.H. & OBERMAYER, M. 1994. Exocrine glands and the attractiveness of the ergatoid queen in the ponerine ant *Megaponera foetens*. *Insectes Sociaux* **41**: 63–72.

LEPAGE, M. 1981. Étude de la predation de *Megaponera foetens* (F.) sur les populations recoltantes de Macrotermitinae dans un ecosystème semi-aride (Kajiado-Kenya). *Insectes Sociaux* **28**: 247–262.

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

LIVINGSTON, D. 1857. *Missionary Travels and Researches in South Africa*. John Murray, London.

LONGHURST, C. 1977. Behavioural chemical and ecological interactions between West African ants and termites. Ph.D. thesis, Southampton University, Southampton, U.K.

LONGHURST, C., JOHNSON, R.A. & WOOD, T.G. 1978. Predation by *Megaponera foetens* (Fabr.) (Hymenoptera: Formicidae) on termites in the Nigerian Guinea Savannah. *Oceologia* (Berl) **32**: 101–107.

LONGHURST, C. & HOWSE, P.E. 1979. Foraging, recruitment and emigration in *Megaponera foetens* (Fab.), (Hymenoptera: Formicidae) from the Nigerian Guinea Savannah. *Insectes Sociaux* **26**: 204–215.

VILLET, M.H. 1990. Division of labour in the Matabele ant *Megaponera foetens* (Fabr.) (Hymenoptera Formicidae). *Ethology, Ecology and Evolution* **2**: 397–417.

WHEELER, W.M. 1936. Ecological relations of ponerine and other ants to termites. *Proceeding of the American Academy of Arts and Science* **71**: 159–243.

YUSUF, A.A. 2010. Termite raiding by the ponerine ant *Pachycondyla analis* (Hymenoptera; Formicidae): behavioural and chemical ecology. Ph.D. thesis, University of Pretoria.

YUSUF, A.A., PIRK, C.W.W., CREWE, R.M., GORDON, I., NJAGI, P.G.N. & TORTO, B. 2010. Nestmate recognition and the role of cuticular hydrocarbons in the African termite raiding ant *Pachycondyla analis*. *Journal of Chemical Ecology* **36**: 441–448.

Accepted 13 September 2012