

## Exocrine glands and the attractiveness of the ergatoid queen in the ponerine ant *Megaponera foetens*

B. Hölldobler, Ch. Peeters\* and M. Obermayer

*Theodor-Boveri-Institut, Lehrstuhl Verhaltensphysiologie und Soziobiologie der Universität, Am Hubland, 97074 Würzburg, Germany*

**Key words:** Caste, discrimination, queen pheromone, epidermis, setae.

### Summary

Queens in *Megaponera foetens* are permanently wingless. In a colony from Kenya we observed that the ergatoid queen was often surrounded by a large number of workers, all conspicuously facing her. To investigate the nature of this attraction, we divided the colony in half. When the queen was transferred from one group to the other, a large court of workers formed immediately. When marked workers were transferred from the queenright group to the other, they attracted much attention and were licked repeatedly. These data suggest that the queen produces a chemical signal which attracts workers, and this signal is transferred onto the workers. In a histological investigation of queen and workers, we found many intersegmental glands in both castes. However, only the queen had a thick glandular epithelium lining her entire body, and this may be the source of her signal. The numerous erect setae covering the queen (but not the workers) may help to dispense the secretions. Our results confirm that ergatoid queens in the Ponerinae are morphologically specialized, despite their external similarity with workers.

### Introduction

Ant queens are generally attractive to their workers, and this attraction is typically released by one or more exocrine secretions (Passera 1984, Fletcher and Ross 1985, Hölldobler and Bartz 1985). However, the occurrence of a retinue of workers around the queen has only been described in a few species, e.g. in the New World army ants (Rettenmeyer et al. 1978) or in the weaver ants (*Oecophylla*) (Hölldobler and Wilson 1983). Very little is known about queen attractiveness in phylogenetically less-advanced ants. In the context of a comparative study of reproductive division of labor in ponerine ants we have investigated several species having ergatoid queens (sensu Peeters 1991). Here we report our findings on queen attractiveness in *Megaponera foetens*.

---

\* Present address: CNRS URA 667, Laboratoire d'Ethologie, Université Paris Nord, F-93430 Villetaneuse, France

The African Matabele ant is the only species of its genus. It is a specialized predator which conducts highly organized raids on termite nests (Lévieux 1966, Fletcher 1973, Longhurst et al. 1978, 1979), and also readily emigrates to new nesting sites (Arnold 1915, Longhurst and Howse 1979 a). Its queen is permanently wingless, and consequently exhibits a simplified thorax which resembles that of major workers. Relatively little is known about the behavioral interactions between workers and ergatoid queen of *M. foetens*, although division of labor among the polymorphic worker caste is well documented (Villet 1990).

## Material and methods

A colony of *Megaponera foetens* was collected during May 1991 in the Shimba Hills reserve (Kenya; Kwale district, south of Mombasa). One queen, at least 950 workers, 35 males, 472 cocoons, many larvae and eggs, were found above ground while emigrating to a new nest site. The queen was quickly recognized because she was the only large individual that was carried. In the laboratory in Würzburg the colony was housed in a plastic nest box (30 × 22 cm) with some soil. A distinct nest was not built, and the ants remained in a bivouac-style congregation. Since we had no termites available for food we supplied pieces of mealworms. Workers and larvae fed on this, although the colony declined over the next six weeks.

For histological investigations, head, alitrunk with petiole, and gaster were fixed in alcoholic Bouin and stored in 75% ethanol. These parts were then embedded in Spurr's low viscosity medium (Spurr 1969), and serially sectioned at 1–2 µm or 4–6 µm using a Reichert-Jung microtome model 2050. Sections were attached to egg albumine-coated slides and stained on a hotplate either with Mallory's (1% methylene blue/1% azur II in 1% sodium borate), or with toluidine blue/basic fuchsin (Burns and Bretschneider 1981); the latter staining technique requires the removal of the plastic.

Our behavioral observations were supplemented by video recordings which made it possible to reanalyze particular interactions in greater detail. Lastly, a sample of workers were dissected to determine ovarian activity.

## Results

### *Queen behavior and interaction with workers*

Although the queen of *M. foetens* is wingless and therefore lacks the enlarged thorax of a typical winged queen, she nevertheless can easily be recognized by her large gaster. Usually it was held slightly up in the air, and often waved faintly from side to side while the queen was standing still in the nest box. The queen was frequently surrounded by a court of workers which was variable in size. Especially when the nest was disturbed, more workers surrounded the queen and sometimes they occurred in a multilayered retinue, standing very close to her with their heads facing her, but with only



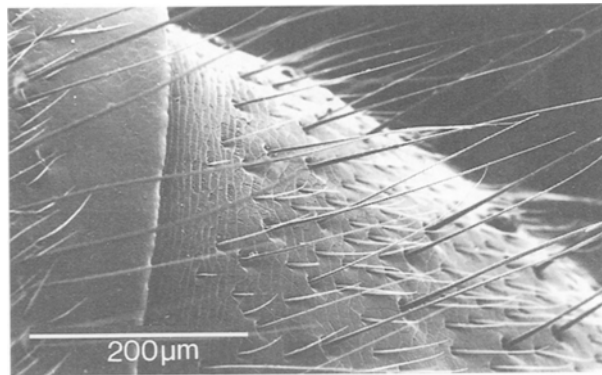
**Fig. 1.** The queen of *M. foetens*, with her gaster held up slightly, is surrounded by a retinue of workers, all with their heads directed towards the queen. Note the dense cover of erect setae over the entire body of the queen

occasional physical contact. Many of these were majors, but minors were also present among them. The workers either stood with their antennae folded back or they antennated slowly over the body of the queen (Fig. 1). Workers further back in the retinue also faced the queen. We did not determine the turn-over rate of workers in the retinue, but from focussing on individual workers we documented that the duration of a worker's presence in the retinue varies greatly, from a few seconds to more than 20 minutes. We noticed only occasional grooming of the queen, and no trophallaxis.

Both when the queen was stationary and while walking, some major workers physically interacted with her, antennating rapidly while simultaneously jerking forward. The queen usually did not respond to this, but on two occasions this interaction led to carrying behavior. A major grabbed the queen's mandibles and pulled her away, then the queen assumed a pupal posture and was carried. These relatively frequent attempts to initiate queen transport may have been a consequence of the laboratory conditions, because this behavior was especially obvious when we illuminated the nest box.

When laying eggs, the queen continued to hold her gaster slightly upwards. The egg was discharged within less than a minute and it was immediately taken by a minor worker and placed onto the egg pile. The queen often stayed near or even on the egg pile.

In order to further investigate the attractiveness of the queen we conducted the following experiments. We divided the colony into two parts. Group A contained 568 workers with the queen, group B had 378 workers without queen. After 10 days the queen was transferred to group B. She instantly attracted a large retinue of workers



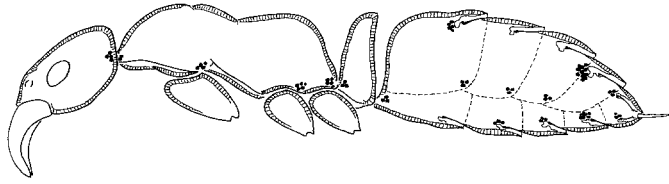
**Fig. 2.** SEM-photograph of part of the gaster in a *M. foetens* queen, showing the long setae which cover the whole body

(2–4 times the size of a normal retinue). All workers in the retinue stretched out their antennae toward the queen, and those immediately next to her seemed to sweep with their antennae closely over the queen's body, although we could not clearly determine whether they touched the body. We speculate that the workers' antennae get into brief contact with the tips of the erect hairs which cover the entire body of the queen (see Fig. 1 and 2). After about 60 minutes this marked response of the workers slowly declined and reverted to normal.

We repeated this procedure twice, reintroducing the queen after 2 days to group A, and after 4 days again to group B. In each case we made similar observations. Whenever the queen was absent for a few days, she elicited a remarkable response on her return to the queenless worker group.

In the following experimental series we exchanged workers which were marked with group-specific color dots (Testor's PLA paints). While the queen was in group B, we divided group A to create a second queenless group C. After 8 days we simultaneously introduced into the queenless group A one worker from C and one worker from the queenright group B. The behavior towards the worker from C appeared indifferent, whereas the worker from B elicited a strong attraction with the resident ants, and was repeatedly licked and carried. We repeated this procedure a total of 15 times, each time using a different pair of test ants and introducing them into either group C or A. The results were always the same. We quantified this effect by counting the workers surrounding the introduced ants during 1-minute intervals (in the first 10 minutes). The mean number of individuals being attracted to the workers from the queenright group was  $2.52 \pm 1.45$  ( $x + \text{SD}$ ) and to the control workers  $0.42 \pm 0.31$  ( $p \ll 0.01$  paired non-parametric rank test). The unusual attractiveness of ants taken from the queenright group lasted almost 3 hours in some cases.

We also introduced workers from a queenless group into the queenright group. The introduced individuals were initially treated as hostile by the resident ants; they were attacked with mandible strikes and they responded by fleeing or by assuming a pupal position. In the latter situation they were then often picked up by the attacking



**Fig. 3.** Schematic drawing of a queen of *M. foetens*, showing the location of the numerous intersegmental glands (black dots) and the glandular epidermis lining the entire body (hatched areas)

ant and carried to the refuse heap where they were released. This occurred in 8 out of 15 tests. The agonistic behavior toward the introduced ant eventually declined, and the “newcomer” was finally accepted into the colony. This “initiation” period varied greatly from a few minutes to more than 1 hour.

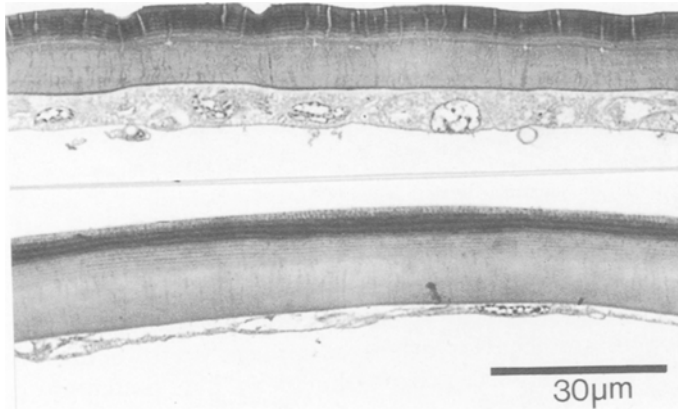
These observations strongly suggest that the queen produces chemical signals which make her attractive to the workers and which are transferred onto the workers. Workers which lack this queen signal are discriminated against by their nestmates. We next investigated the possible anatomical source of these queen pheromones.

#### *The possible source of queen pheromones*

Superficially the appearance of an ergatoid queen of *M. foetens* resembles that of a major worker, except for the grossly swollen gaster. However, closer inspection reveals that the queen differs from the workers in that all surfaces of her head, body and appendages are clothed with numerous erect hairs of uniform length (Arnold 1915, B. Bolton pers. comm.). In comparison, workers have only a few such hairs.

Histological investigations revealed many intersegmental exocrine glands in workers and the queen (schematically illustrated in Fig. 3). The most common intersegmental gland in ponerine ants is the dorsal pygidial gland. It is located between the VIth and VIIth abdominal tergites and consists of two lateral clusters of secretory cells. This gland has been discovered in all ponerine species investigated by Hölldobler and Engel (1978) and by Jessen et al. (1979), and it has also been detected in *M. foetens* workers (Villet et al. 1984; Fanfani and Valcurone 1986). It is also present in the queen of *Megaponera*. In addition, the queen and the workers possess a spiracular plate gland (postpygidial gland) between the VIIth and VIIIth abdominal segments, and another tergal gland between the IIIrd and IVth abdominal tergites.

Fanfani and Valcurone (1986) noted intersegmental sternal glands in workers between the IVth and Vth, Vth and VIth, and the VIth and VIIth abdominal sternites and two pleural glands in the IVth and Vth abdominal segments. We can confirm these findings but found that the most posterior sternal gland (VI/VIIth segment) is considerably larger than the other sternal and pleural glands. Further we report that identical glands are present in the queen. In addition we found in both the queen and workers intersegmental glands between VII and VIII abdominal sternal segments, between petiole and alitrunk, between coxae and thoracic sternites, and between head



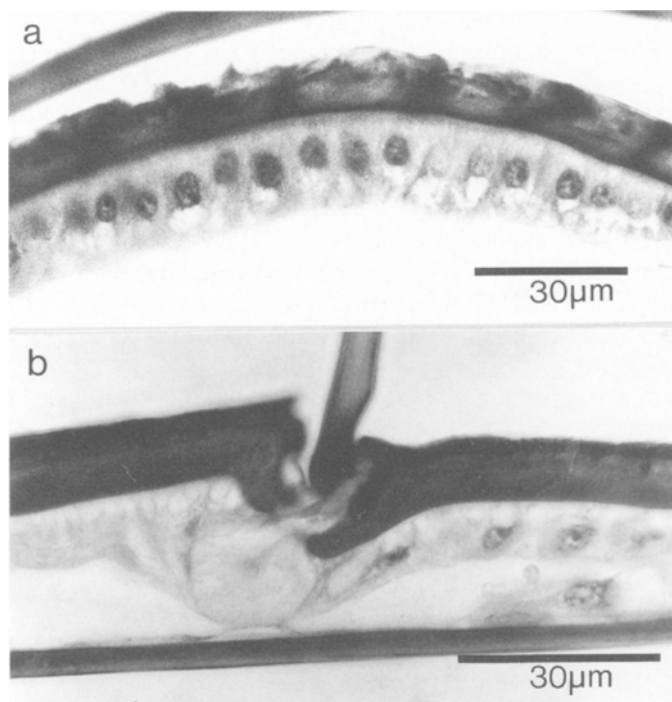
**Fig. 4.** Cross-sections (1  $\mu\text{m}$  thick) of the cuticle and epidermis from the gaster of both a queen (above) and a worker (below) of *M. foetens*. Note the glandular epithelium in the queen, with numerous ducts penetrating the cuticle. In contrast, the epidermis in the worker is much thinner, and ducts are not seen

and thorax. No structural differences in these intersegmental glands could be detected between workers and queen.

Striking differences between queen and worker castes exist, however, in the cuticle and epidermal epithelium. In the workers the epidermis is a thin layer of collapsed cells, whereas in the queen it is a well developed thick glandular epithelium with large nuclei and many vacuoles (Fig. 4, 5). The cuticle of the queen is penetrated by dense networks of dermal gland ducts (Fig. 4). The large erect setae are innervated. At their base are large cells with vacuoles which might be tormogen and trichogen cells (Fig. 5b). Immediately around the pits and the base of the setae, many dermal gland ducts open, suggesting that the long hairs might also serve as dispensers of the secretions. The queen's epidermal lining is particularly well developed underneath those cuticular areas which are exposed to the outside. For example, in each gaster segment, only those external parts that are not covered by the sclerites of the preceding segment have the enlarged glandular epithelium (Fig. 3).

#### *Ovarian activity of workers*

The occurrence of oogenesis by workers of *M. foetens* has been reported previously (Crewe et al. 1984). In this study we dissected 32 workers (mostly large) from a queenright group, and found immature yolky oocytes in one third of these. We also dissected 38 workers from a group which had been isolated from the queen for three weeks. A greater proportion (50%) of these workers exhibited oogenesis, and the developing oocytes found in each ant were larger and more numerous (4 ants had one mature oocyte). Similarly, Villet and Duncan (1992) found that over 50% of large orphaned workers had developing oocytes, and that eggs were laid by some of these.



**Fig. 5.** (a) Section (4  $\mu\text{m}$  thick) through a coxa of a *M. foetens* queen, showing the columnar glandular epithelium. Note the large nuclei and vacuoles. (b) Section (4  $\mu\text{m}$  thick) through the cuticle and erect seta in the gaster of a *M. foetens* queen. Note the thick glandular epidermis and the large cells at the base of the seta

## Discussion

The ergatoid queen of the ponerine ant *Megaponera foetens* is highly attractive to her workers. She is often surrounded by a large court of workers, and behavioral experiments suggested that her attractiveness is based on chemical signals. A histological investigation was aimed at finding possible sources for the queen pheromones. *Megaponera* workers as well as the queen are endowed with numerous intersegmental glands, but we did not find important differences between the castes. Some of these intersegmental glands may serve for social communication, as demonstrated with the pygidial gland, which secretes recruitment pheromones in *Megaponera* workers (Hölldobler et al. in prep.). Most of them, however, might be lubrication glands as suggested by Jessen and Maschwitz (1983) for *Pachycondyla tridentata*, a ponerine also richly endowed with intersegmental glands. A lubrication function does not exclude other roles, and it is conceivable that some of the glands evolved a secondary activity in defense or communication. This has to be explored in the future.

A striking difference between workers and the ergatoid queen exists, however, in the surface pilosity and in the glandular epidermal lining. The numerous erect hairs

which cover the entire surface of the queen's body probably function as mechanoreceptors, but they also appear to be associated with the glandular epithelium and may help to disperse the secretions. We suggest that this epidermal glandular epithelium is the source of the queen signal. The mechanisms of queen substance dispersal have been studied in honeybees (Seeley 1979); airborne dispersal is relatively unimportant compared to direct queen-worker contacts or worker transport. This needs to be investigated in *M. foetens*, keeping in mind that licking among nestmates is relatively rare and trophallaxis does not occur. It is not known whether this queen signal is also involved in the inhibition of ovarian activity in the workers.

Our data confirm that ergatoid queens possess all the specialized attributes of the reproductive caste, despite their external resemblance to workers (Peeters 1991). What is the adaptive significance of the marked queen attractiveness in *Megaponera*? The existence of worker retinues has not been observed in other ponerine ants which we have investigated over the years. *Megaponera* is a distinctive species not only because the queen is ergatoid, but also because the colonies are relatively large for ponerine species (see review in Peeters 1993). Over 40 colonies have been collected in various parts of Africa, and average numbers of workers have ranged from 500 (Ivory Coast) to 1475 (Kenya) (Lévieux 1967, Lepage 1981, Longhurst and Howse 1979 b, Peeters 1991). All colonies studied have been monogynous (above references and Villet 1990). Thus the attractiveness of *Megaponera* queens must be interpreted in the context of large colony sizes and monogyny. *Megaponera* colonies readily emigrate, and it may be hypothesized that in ants with a nomadic lifestyle, the queens require especially powerful attractants to signal their presence to their workers during emigrations.

Extreme queen attractiveness is well known in certain phylogenetically advanced ant species that form large monogynous colonies, such as the New World army ants (*Ecitonini*) (Rettenmeyer et al. 1978), the weaver ants (*Oecophylla*) (Hölldobler and Wilson 1983), and leaf cutter ants *Atta* (Hölldobler and Wilson unpublished results). In *Eciton*, when the queen was experimentally removed during emigrations, her absence was communicated within hours among at best 100 000 workers (probably the entire colony) (Rettenmeyer and Watkins 1978). Army ant queens have been shown to possess large intersegmental exocrine glands and epithelial glands in their gasters (Hagan 1954, Whelden 1963, Franks and Hölldobler 1987). In species of the genus *Leptanilla*, another legionary ant, Hölldobler et al. (1989) found the ergatoid queen to be endowed with a series of large, paired intersegmental tergal and sternal glands which are absent in the workers.

The specialized morphology of ergatoid queens needs to be documented in more species of ants. This may help to understand the adaptive significance of evolving queens which are permanently wingless.

## Acknowledgements

We thank Helga Heilmann for the photographic dark room work, Joseph Mugambi for field assistance, and Dr. Richard Bagine (National Museums of Kenya) for organizing fieldwork in Shimba Hills. This work was supported by the Deutsche Forschungsgemeinschaft (Leibniz Prize to B. H.).



## References

- Arnold, G., 1915. A monograph of the Formicidae of South Africa. *Annals of the South African Museum* 14:1–766.
- Burns, M.S. and A. Bretschneider, 1981. *Thin is in*. American Society of Clinical Pathologists, Educational Products Division, Chicago.
- Crewe, R. M., C. Peeters and M. Villet, 1984. Frequency distribution of worker sizes in *Megaponera foetens* (Fabricius). *S. Afr. J. Zool.* 19:247–248.
- Fanfani, A. and M. D. Valcurone, 1986. Glandole delle ponerine e ricerche sulle glandole del gastro di *Megaponera foetens* (Fab.) (Hymenoptera: Formicidae). *Accademia Nazionale dei Lincei* 260:115–132.
- Fletcher, D. J. C., 1973. “Army ant” behaviour in the Ponerinae: a reassessment. Proc. 7th Congr. IUSSI (London, 1973), 116–121.
- Fletcher, D. J. C. and K. Ross, 1985. Regulation of reproduction in eusocial Hymenoptera. *Ann. Rev. Entomol.* 30:319–343.
- Franks, N. R. and B. Hölldobler, 1987. Sexual competition during colony reproduction in army ants. *Biol. J. Linnean Soc.* 30:229–243.
- Hagan, H. R., 1954. The reproductive system of the army-ant queen, *Eciton* (*Eciton*), part 3: The oocyte cycle. *Am. Mus. Novit.* 1665, 20 pp.
- Hölldobler, B. and H. Engel, 1978. Tergal and sternal glands in ants. *Psyche* 85:285–330.
- Hölldobler, B. and E. O. Wilson, 1983. Queen control in colonies of weaver ants (Hymenoptera: Formicidae). *Ann. Ent. Soc. Am.* 76:235–238.
- Hölldobler, B. and S. Bartz, 1985. Sociobiology of reproduction in ants. In: *Experimental behavioral ecology and sociobiology* (B. Hölldobler and M. Lindauer, eds.). Sinauer Assoc., Sunderland, Mass., pp. 237–257.
- Hölldobler, B., J. Palmer, K. Masuko and W. L. Brown, 1989. New exocrine glands in the legionary ants of the genus *Leptanilla* (Hymenoptera, Formicidae, Leptanillae). *Zoomorphol.* 108:255–261.
- Jessen, K. and U. Maschwitz, 1983. Abdominaldrüsen bei *Pachycondyla tridentata* (Smith) (Formicidae, Ponerinae). *Ins. Soc.* 30:123–133.
- Jessen, K., U. Maschwitz and M. Hahn, 1979. Neue Abdominaldrüsen bei Ameisen. I. Ponerini (Formicidae: Ponerinae). *Zoomorphologie* 94:46–66.
- Lepage, M. G., 1981. Etude de la prédation de *Megaponera foetens* (F.) sur les populations récoltantes de Macrotermitinae dans un écosystème semi-aride (Kajiado - Kenya). *Ins. Soc.* 28:247–262.
- Lévieux, J., 1966. Note préliminaire sur les colonnes de chasse de *Megaponera foetens* F. (Hymenoptera: Formicidae). *Ins. Soc.* 13:117–126.
- Lévieux, J., 1967. Recherches écologiques dans la savane de Lamto (Côte d’Ivoire): données préliminaires sur le peuplement en fourmis terrioles. *La Terre et la Vie* 3:278–296.
- Longhurst, C., R. A. Johnson and T. G. Wood, 1978. Predation by *Megaponera foetens* (Fab.) (Hymenoptera: Formicidae) on termites in the Nigerian Southern Guinea Savanna. *Oecologia* 32:101–107.
- Longhurst, C. and P. E. Howse, 1979 a. Foraging, recruitment and emigration in *Megaponera foetens* (Fab.) (Hymenoptera: Formicidae) from the Nigerian Guinea Savanna. *Ins. Soc.* 26:204–215.
- Longhurst, C. and P. E. Howse, 1979 b. Some aspects of the biology of the males of *Megaponera foetens* (Fab.) (Hymenoptera: Formicidae). *Ins. Soc.* 26:85–91.
- Longhurst, C., R. Baker and P. E. Howse, 1979. Termite predation by *Megaponera foetens* (Fab.) (Hymenoptera: Formicidae). Coordination of raids by glandular secretions. *J. Chem. Ecol.* 5: 703–719.
- Passera, L., 1984. *L’organisation sociale des fourmis*. Privat, Toulouse. 360 pp.
- Peeters, C., 1991. Ergatoid queens and intercastes in ants: two distinct adult forms which look morphologically intermediate between workers and winged queens. *Ins. Soc.* 38:1–15.
- Peeters, C., 1993. Monogyny and polygyny in ponerine ants with or without queens In: *Queen number and sociality in insects* (L. Keller, ed.) Oxford University Press.
- Rettenmeyer, C. W., H. Topoff and J. Mirenda, 1978. Queen retinues of army ants. *Ann. Ent. Soc. Am.* 71:519–528.

- Rettenmeyer, C. W. and J. F. Watkins, 1978. Polygyny and monogyny in army ants (Hymenoptera: Formicidae). *J. Kans. Entomol. Soc.* 51:581–591.
- Seeley, T. D., 1979. Queen substance dispersal by messenger workers in honeybee colonies. *Behav. Ecol. Sociobiol.* 5:391–415.
- Spurr, A. R., 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* 26:31–43.
- Villet, M., 1990. Division of labour in the Matabele ant *Megaponera foetens* (Fabr.) (Hymenoptera Formicidae). *Ethol. Ecol. Evol.* 2:397–417.
- Villet, M., C. P. Peeters and R. M. Crewe, 1984. The occurrence of a pygidial gland in four genera of ponerine ants (Hymenoptera: Formicidae). *J. Georgia Ent. Soc.* 19:413–416.
- Villet, M. and F. D. Duncan, 1992. Reproductive abilities of orphaned workers of two ponerine ant species (Hymenoptera, Formicidae). *J. ent. Soc. sth. Afr.* 55:280–282.
- Whelden, R. M., 1963. Anatomy of adult queen and workers of army ants *Eciton burchelli* Westw. and *E. hamatum* Fabr. (Hymenoptera: Formicidae). *J. N. Y. Ent. Soc.* 71(1):14–30; (2):90–115; (3):158–178; (4):246–261.

Received 22 February 1993;  
revised 4 May 1993;  
accepted 12 May 1993.