

## Research article

# Sperm transfer in the ant *Carebara vidua* F. Smith (Hymenoptera: Formicidae)

H. G. Robertson\*

Zoology Department, University of the Witwatersrand, P.O. Wits 2050, Johannesburg, South Africa

*Key words:* *Carebara vidua*, sperm transfer, copulation, ovariole number.

## Summary

Sperm transfer in the myrmicine ant *Carebara vidua* was studied by making histological sections of newly mated queens. Queens are multiply inseminated, and longitudinal sections through the sperm-filled vagina show that the bundle of sperm from each male is enclosed in a layer of accessory gland secretion terminating in a plug of secretion in the female's vaginal orifice. This spermatophore breaks down soon after mating. The huge quantity of sperm packed into the spermatheca (ca. 16 mm<sup>3</sup>) suggests that the queen lays a large number of eggs in her lifetime, confirmed by the average of 1672 ovarioles in the two ovaries.

## Introduction

The process of sperm transfer in ants has received little attention in the literature. Studies have been limited to examining copulatory behaviour and positions in copulation (Hölldobler and Bartz, 1985; Robertson and Villet, 1989) and to studies of the morphology of male and female reproductive systems (Hermann and Blum, 1965; Hung and Vinson, 1975; Wheeler and Krutzsch, 1992; Ball and Vinson, 1984). Even within the Hymenoptera as a whole studies of sperm transfer seem to have been limited to the honey bee *Apis mellifera* (Koeniger, 1984, 1986; Koeniger et al., 1979) and an ichneumonid (Madel et al., 1990). In this study, sperm transfer in *Carebara vidua* was analyzed mainly by making histological sections of newly mated queens.

Mating behaviour in *Carebara vidua* has previously been described by Lepage and Darlington (1984) and Robertson and Villet (1989). The species is predominantly subterranean in habit and established nests have thousands of minute workers that prey on termites. After reasonably heavy rains, the workers construct emergence holes for the reproductives and these emerge in large numbers, sur-

---

\* Present address: Life Sciences Division, South African Museum, P.O. Box 61, Cape Town 8000, South Africa

rounded and covered by the tiny workers. After a short flight the queen settles on the branch of a tree and releases a pheromone to attract males. Males fly upwind to the female and having located her, mate for about four minutes (Robertson and Villet, 1989). Lepage and Darlington (1984) observed multiple mating with each female and Robertson and Villet (1989) reported up to four mating plugs in queens that had just mated. After the queen has finished mating she flies to the ground, breaks off her wings, and finds a site to excavate her nest.

Multiple mating in species with a female calling syndrome is unusual (Hölldobler and Bartz, 1985), but it is characteristic of species with populous colonies possibly evolving to avoid sperm depletion (Cole, 1983). *C. vidua* is a monogynous species (Lepage and Darlington, 1984) and the production of large numbers of tiny workers by the queen means that she needs to have a large quantity of sperm and many ovarioles to maintain a high egg-laying rate. The number of sperm in the spermathecae of queens was not determined in this study but I did count the number of ovarioles and used it in the formula of Tschinkel (1987a) to estimate the number of sperm.

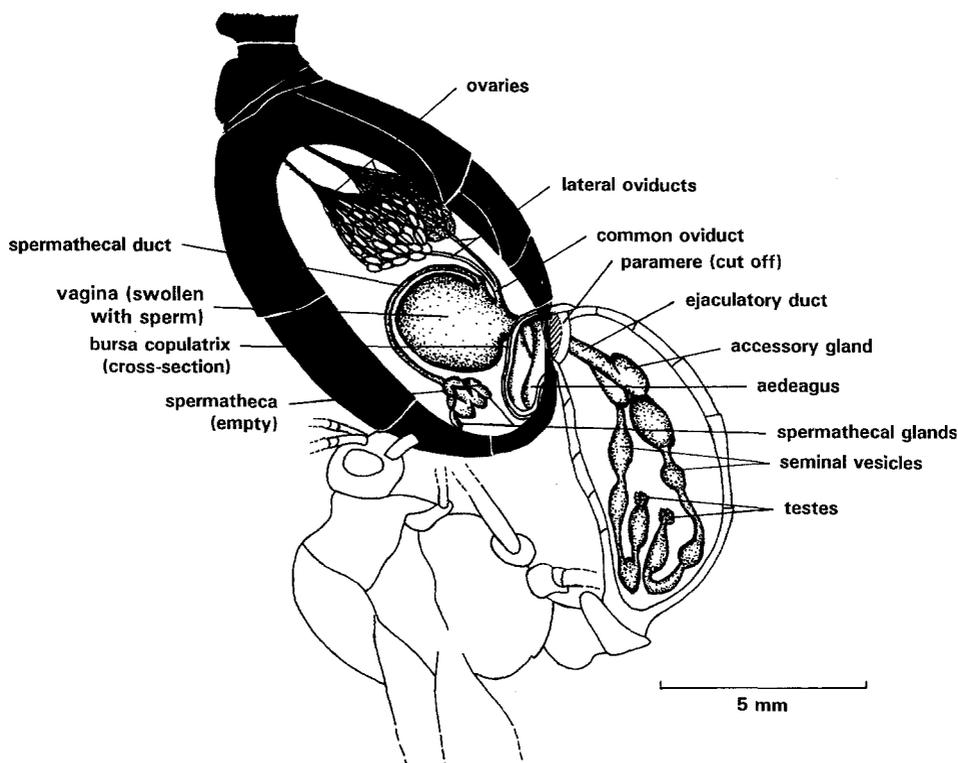
### Materials and methods

Specimens used in this study were collected in Mkuze Game Reserve (27°36'S 32°13'E) from 29 September – 10 October 1987 and on the 15 October 1988. On the first occasion, virgin queens and males were placed in a container with sticks to perch on. One pair which started mating was killed instantly in copula by placing it in liquid nitrogen. On the second occasion, queens were collected just after they had finished mating and broken off their wings. Within three hours the reproductive systems of three specimens were dissected out and fixed in formalin. Other queens, and males as well, were kept live and taken back to the University of the Witwatersrand where they were dissected.

The three reproductive systems of freshly mated queens were embedded in wax, sectioned longitudinally and stained with Mallory's one step stain (Humason, 1972). Virgin males, two days after their emergence, were dissected and their reproductive systems fixed, embedded, sectioned and stained in the same way as those of the queens. The copulatory pair that had been killed in liquid nitrogen was embedded in "historesin" glycol methacrylate rather than wax to ease sectioning through their tough integuments. Two ovaries were embedded in wax, sectioned longitudinally and stained with Mallory's. These sections were used to determine where the ovary needed to be cross-sectioned in order to count the ovarioles. A further 10 ovaries of which seven were used, were embedded for cross-sectioning. The number of ovarioles in these cross-sections was counted under a stereo microscope with an ocular graticule.

### Results

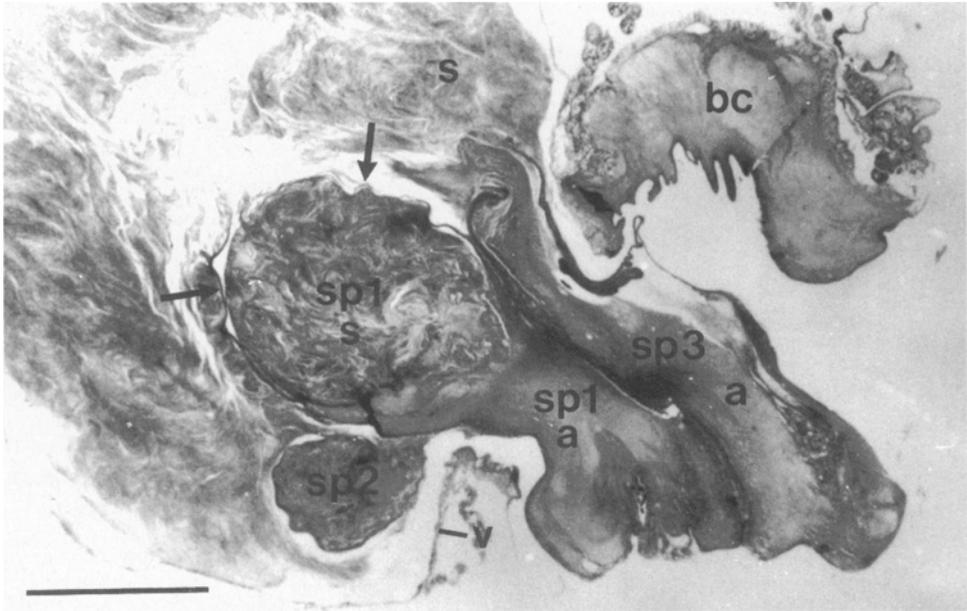
Examination of the pair frozen in copula revealed that the male inserts his aedeagus into the bursa copulatrix of the female (Fig. 1) with the digitus and cuspis fitting



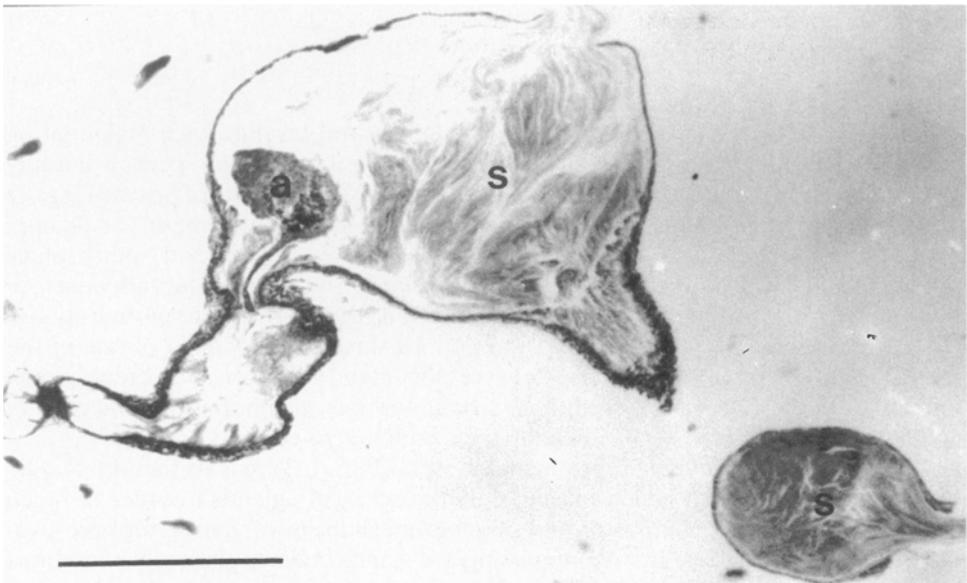
**Figure 1.** Diagrammatic cross-section through mating pair of *Carebara vidua*

round the ventral edge of the fourth (terminal) gastral tergite. Each male mating with the female leaves a mating plug in her vaginal orifice and the sperm is initially accumulated in the vagina which becomes a large swollen ball of sperm (Fig. 1) measuring, in mid-longitudinal section,  $4.41 \times 3.74$  mm (Mean diameter = 4.08 mm;  $n = 1$ ). The longitudinal sections through the swollen vagina and sperm plugs reveal that the accessory gland secretions forming the sperm plug also enclose the bundle of sperm which the male has injected, thus forming a spermatophore (Fig. 2). However, it was apparent from sections through the vagina of one of the females, that this enclosing envelope of accessory gland secretion soon breaks down and mixes in with the sperm so that no spermatophore is apparent. The accessory gland secretions stained blue indicating their high mucus content.

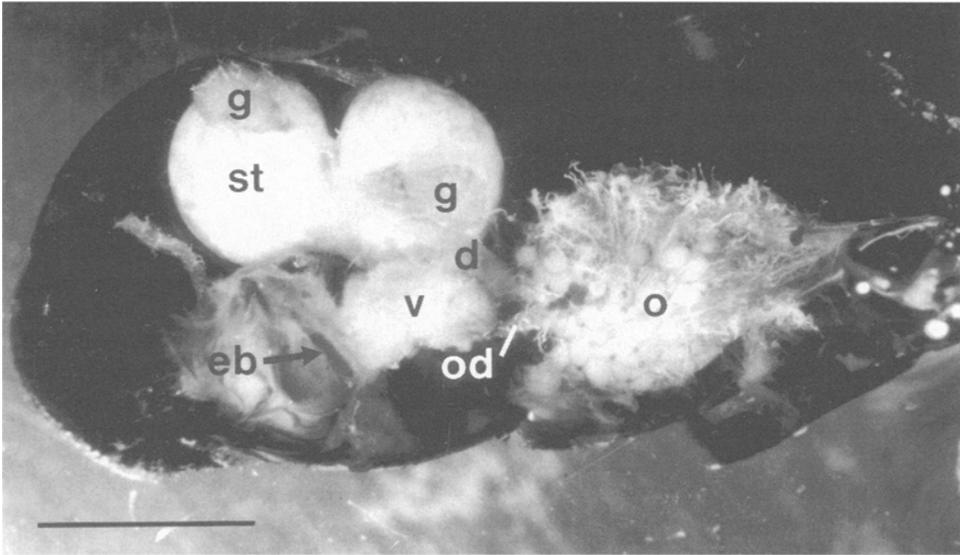
The structure of the male reproductive system (Figs. 1, 3) reveals that the bundle of sperm in the seminal vesicles has to pass the accessory glands in order to reach the ejaculatory duct. Therefore, one possible mechanism of spermatophore production is that the sperm bundle in passing the glands collects glandular secretions on its perimeter which remain when the sperm enters the vagina of the female. After the ejaculation of the sperm the male would then expel secretions to form the mating plug. A much more likely scenario, however, is that once the male's genitalia have



**Figure 2.** Longitudinal section through the vagina of a newly mated *C. vidua* queen showing a complete section through a spermatophore and partial sections through the plugs of two others. Abbreviations: a: male accessory gland secretion forming plug and perimeter (marked by arrows) of spermatophore; bc: bursa copulatrix; s: sperm; sp1–sp3: spermatophores 1–3 (only sperm-filled portion of spermatophore 2 is visible while for spermatophore 3, the sperm-filled portion has burst and only the plug is clearly visible); and v: wall of vagina. Scale line = 1 mm



**Figure 3.** Longitudinal section through part of a convoluted seminal vesicle of a *C. vidua* male, showing sperm (s) and accessory gland secretion (a). Scale line = 1 mm

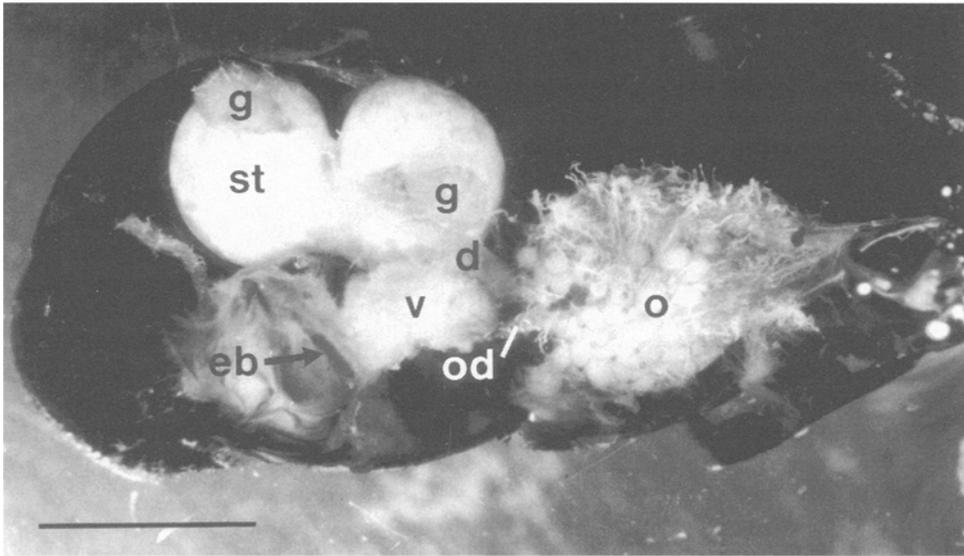


**Figure 4.** Dissection of the reproductive system of a newly mated *C. vidua* queen (lateral view) after the sperm has been shunted from the vagina to the spermatheca. Abbreviations: eb: entrance to bursa copulatrix; d: spermathecal duct; g: spermathecal gland; o: left ovary (right ovary removed); od: oviduct; st: sperm-filled spermatheca; v: vagina. Scale line = 3 mm

been inserted into the bursa copulatrix and the orifice of the ejaculatory duct is opposite the female's vaginal orifice, the male puts a globule of accessory gland secretion into the female's orifice and then ejaculates the bundle of sperm and sends it through the secretion so that the secretion clings to the outside of the sperm bundle. At the end of the process, some more accessory gland secretion is expelled which places the final seal on the spermatophore. Sections through the spermatophores show that whisks of sperm sometimes extend through the centre of the mating plug and there is clear evidence of a final quantity of secretion being placed terminally on the plug presumably to seal it properly (Fig. 2).

After completion of mating, the sperm is shunted from the vagina into the spermatheca presumably through the action of haemolymph pressure. The spermatheca becomes distended with sperm forming a large bilobed structure while the vagina, now empty, becomes a small shrunken body (Fig. 4) The spermathecal duct is broad, measuring 0.23 mm in diameter ( $n = 1$ ).

In *Apis mellifera* a considerable quantity of the sperm injected by the males is discarded and only about 10% reaches the spermatheca (Woyke, 1979; Ruttner, 1985). Is there excess sperm in *C. vidua*? The average diameter of sperm-filled vagina of one of the females in longitudinal section was 4.08 mm and as it forms virtually a sphere, one can estimate from this that the sperm in the vagina occupied a volume of about 35 mm<sup>3</sup>. The volume of the sperm filled spermatheca is more difficult to estimate because it is bilobed but in another female examined, the average diameter of a lobe was 2.48 mm and assuming the volume approximates two spheres each of this diameter, the volume of sperm in the spermatheca would



**Figure 5.** Longitudinal section through the ovary of a newly mated *C. vidua* queen showing central calyx with ovarioles radiating from it. Scale line = 1 mm

be about  $16 \text{ mm}^3$ . From such limited data one can only draw tentative conclusions but it appears that the queen of *C. vidua* receives more sperm than is necessary to fill the spermatheca.

Longitudinal sections through an ovary reveal that it is made up of a sphere-like calyx measuring 0.68 mm in diameter with ovarioles radiating from it (Fig. 5). The number of ovaries was counted in cross-sections made just above the calyx so as not to miss any. At this level the ovarioles arising from the base of the calyx are tiny thin tubes but are still sufficiently visible to be counted. There was a mean of 836 ovarioles per ovary (range = 744–898; sd = 56; n = 7) so that queens had a mean of 1672 ovarioles in their two ovaries.

## Discussion

The spermatophore in *C. vidua* is of a temporary nature but it possibly plays a role in containing the sperm so that it does not leak out of the vagina, a very like event considering how swollen this structure can get (Fig. 1) and the copulatory activities of subsequent males mating with the female. Woyciechowski et al. (1994) present evidence that the mating sign in *Apis mellifera* prevents sperm leakage. This mating sign is not a spermatophore but is a plug in the vaginal orifice of the queen, made up of accessory gland secretions and the expelled chitin plates of the drone's endophallus (Koeniger, 1986). Sperm transfer in *A. mellifera* is

entirely different to that in *C. vidua* in that it is performed in flight and lasts only about 1.5 seconds (Koeniger et al., 1979) as opposed to four minutes in *C. vidua* (Robertson and Villet, 1989).

In the protracted time that it takes a *C. vidua* male to mate with a queen, I propose that it injects accessory gland secretion first and the sperm second and that the spermatophore is formed by the viscous mucus-containing secretion clinging to the outside of the sperm bundle as it is injected.

The only record I can find of the formation of spermatophores in the Hymenoptera is that of Madel et al. (1990) who found that a well-defined stalked spermatophore is formed in the accessory glands of the male genital system in the ichneumonid wasp *Diadegma semiclausum* Hellen. The spermatophore is placed into the female's vagina with the stalk coming into contact with the entrance of the spermathecal duct. The spermatophore is therefore entirely different to that in *C. vidua* being more permanent, functional, and being formed in the male, not in the female.

*C. vidua* queens are clearly egg-laying machines par excellence and the 1672 ovarioles is a record for the subfamily Myrmicinae, with *Atta texana* coming a poor second with about 300 ovarioles (estimated from graph in Tschinkel, 1987a). However, it pales into insignificance relative to the 15 000 ovarioles evidently recorded for *Dorylus* sp. (Hölldobler and Wilson, 1990).

Unfortunately, because of insufficient specimens and the lack of opportunity to observe another nuptial flight of *C. vidua*, I was not able to count the number of sperm in the spermatheca using standard procedures (Tschinkel, 1987b). Instead, the number of sperm was estimated using the following formula supplied in Tschinkel (1987a):  $\log y = 3.12 + 1.59 \log x$  where  $y$  is the number of sperm in the spermatheca and  $x$  is the number of ovarioles in the ovaries. Based on the average of 1672 ovarioles in *C. vidua*, this formula predicts that a full spermatheca would contain about 176 million sperm. As the number of ovarioles is far beyond the values used in Tschinkel (1987a), this estimate should be regarded with circumspection. Taking these reservations into account, if the number of sperm needed to produce one worker is 3.5 as in *Solenopsis invicta* (Tschinkel, 1987a), then a single *C. vidua* queen in her lifetime could produce a maximum of about 50 million workers. Lepage and Darlington (1984) counted 124 000 workers in the largest of three colonies they collected but they did not find the queen, clearly showing that this is a substantial underestimate of the total colony size. To anyone who has witnessed the emergence of hundreds of the huge queens and males from a nest hole, surrounded by thousands of the tiny workers, it is obvious that the colonies of *C. vidua* are extremely large and the queens need to have a very efficient egg laying apparatus and extraordinary reserves of sperm as shown in this study.

## Acknowledgements

I am most grateful to Professor Robin Crewe for supporting this study, Colleen Walther for her expertise and time in producing the histological preparations, Professor Shirley Hanrahan for supplying laboratory facilities and for her advice about histological preparations, Michelle van der Merwe for printing the photographs, Michael Allsopp for information on honeybees, and the Natal Parks Board for giving me permission to work in Mkuze Game Reserve.

## References

- Ball, D. E. and S. B. Vinson, 1984. Anatomy and histology of the male reproductive system of the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). *Int. J. Insect Morphol. & Embryol.* 13: 283–294.
- Cole, B. J., 1983. Multiple mating and the evolution of social behavior in the Hymenoptera. *Behav. Ecol. Sociobiol.* 12: 191–201.
- Hermann Jr., H. R. and M. S. Blum, 1965. Morphology and histology of the reproductive system of the imported fire ant queen, *Solenopsis saevissima richteri*. *Ann. ent. Soc. Am.* 58: 81–89.
- Hölldobler, B. and S. Bartz, 1985. Sociobiology of reproduction in ants. In: *Experimental Behavioral Ecology* (B. Hölldobler and M. Lindauer, Eds.), G. Fischer Verlag, Stuttgart. pp. 237–257.
- Hölldobler, B. and E. O. Wilson, 1990. *The Ants*. Belknap Press of Harvard University Press, Cambridge.
- Humason, G., 1972. *Animal tissue techniques*. 3rd Edition. W. H. Freeman & Co., San Francisco, 641 pp.
- Hung, A. C. F. and S. B. Vinson, 1975. Notes on the male reproductive system in ants (Hymenoptera: Formicidae). *J. N. Y. ent. Soc.* 83: 192–197.
- Koeniger, G., 1984. Funktionsmorphologische Befunde bei der Kopulation der Honigbiene (*Apis mellifera* L.). *Apidologie* 15: 189–204.
- Koeniger, G., 1986. Mating sign and multiple mating in the honeybee. *Bee World* 67: 141–150.
- Koeniger, G., N. Koeniger and M. Fabritius, 1979. Some detailed observations of mating in the honeybee. *Bee World* 60: 53–57.
- Lepage, M. G. and J. P. E. C. Darlington, 1984. Observations on the ant *Carebara vidua* F. Smith preying on termites in Kenya. *J. Nat. Hist.* 18: 293–302.
- Madel, G., D. Muhlen and M. Happe, 1990. *Diadegma semiclausum* (Hym., Ich.) Begattung, Spermatophorentransfer und Nachkommenschaft. *Z. angew. Zool.* 77: 347–355.
- Robertson, H. G. and M. Villet, 1989. Mating behaviour in three species of myrmicine ants (Hymenoptera: Formicidae). *J. Nat. Hist.* 23: 767–773.
- Ruttner, F., 1985. Reproductive behavior in honeybees. In: *Experimental Behavioral Ecology* (B. Hölldobler and M. Lindauer, Eds.), G. Fischer Verlag, Stuttgart. pp. 225–236.
- Tschinkel, W. R., 1987a. Relationship between ovariole number and spermathecal sperm count in ant queens: a new allometry. *Ann. ent. Soc. Am.* 80: 208–211.
- Tschinkel, W. R., 1987b. Fire ant queen longevity and age: estimation by sperm depletion. *Ann. ent. Soc. Am.* 80: 263–266.
- Wheeler, D. E. and P. H. Krutzsch, 1992. Internal reproductive system in adult males of the genus *Camponotus* (Hymenoptera: Formicidae: Formicinae). *J. Morph.* 211: 307–317.
- Woyciechowski, M., L. Kabat and E. Król, 1994. The function of the mating sign in honey bees, *Apis mellifera* L.: new evidence. *Anim. Behav.* 47: 733–735.
- Woyke, J., 1979. Effect of the access of worker honeybees to the queen on the results of instrumental insemination. *J. apic. Res.* 19: 136–143.

Received 24 January 1995;

revised 8 May 1995;

accepted 5 June 1995.