

Rearing and instrumental insemination of *Apis florea* queens

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Introduction

Honey bee queens mate naturally high in the open air. During the mating flight, the drone's semen is injected into the queen's lateral oviducts in *Apis mellifera* (Ruttner 1953-1954, Woyke 1955) as well as in *A. cerana* (Woyke 1975). Queens of both species return from the mating flight with the mating sign. The mating sign consists of mucous and the chitinous plates of the drone's endophallus in *A. mellifera* (Ruttner 1953/1954, Woyke 1958), and of mucous only in *A. cerana* (Ruttner, Woyke and Koeniger 1973, Woyke 1975). After the queen returns to the colony, the spermatozoa are transferred from the oviducts into the spermatheca. Less than 10% of spermatozoa enter the spermatheca (Woyke 1960, 1962). The rest pass out of the reproductive organ (Trjasko 1951). It takes 10-20 hours to clear the oviducts (Woyke 1960). A similar process occurs after instrumental insemination. Queens of one species may be inseminated with semen of another species. The spermatozoa enter the spermatheca (Woyke 1973). The number of entering spermatozoa is characteristic for the species of the drone and not for the queen (Woyke 1973). However, eggs fecundated by spermatozoa of another species do not develop (Ruttner and Maul 1983).

According to Koeniger *et al.* (1989) the mating process of *A. florea* differs from that described above. During the mating flight, the spermatozoa are transferred directly from the drone's endophallus into the spermatheca.

During instrumental insemination of queen bees, the semen is also injected into lateral oviducts. Therefore investigations were undertaken to see whether it is possible to instrumentally inseminate an *A. florea* queen by injecting semen into lateral oviducts of the queen, as happens in other species, and to determine if the spermatozoa from the oviducts enter the spermatheca.

Table 1. Size of spermatheca and number of spermatozoa in it in naturally mated *Apis florea* queens of unknown age

Queen No.	Spermatheca		Spermatozoa	
	diam. mm	vol. mm ³	No. mil.	concentr. mil/mm ³
1	0.907	0.391	0.382	0.977
2	0.821	0.289	1.156	4.000
3	0.835	0.305	1.222	4.007
4	0.864	0.338	0.570	1.686
5	0.821	0.290	0.554	1.910
6	0.821	0.290	1.538	5.303
average	0.845	0.317	0.904	2.981

Materials and Methods

The investigations were conducted in Poona, India in 1974. Rearing of *Apis florea* queens was induced by removing the queen from the comb. Disturbances of colonies, *i.e.*, depriving them of queens or cutting part of the comb resulted in several abscondings. Next a queenless and combless colony was combined with another queenless colony which occupied a comb with brood of varying age. No fighting between workers was observed. Workers of the queenless colony constructed emergency queen cells.

The whole colony, together with the queen cells, was transported to Poland. Several days later, all queen cells but one were put into nursery cages in an incubator where the queens emerged. *A. florea* workers were added to take care of the virgins. The queens were instrumentally inseminated at the age of 5 days. Because *A. florea* drones were not available in Poland, the queens were inseminated with semen of *A. mellifera* drones. Two days later all queens but one were killed, and the number of sperm in the spermatheca was counted, using routine methods (Woyke 1975). The one queen taken from the colony was returned to it, after being instrumentally inseminated.

To get some idea about the number of spermatozoa in the spermatheca, six naturally mated *A. florea* queens of unknown age were killed and the spermatozoa

Table 2. Size of spermatheca and number of spermatozoa in four species of honey bees

Species	Spermatheca		Spermatozoa		Author
	diam.mm	vol.mm ³	No. mil.	conc.mil/mm ³	
<i>A. mellifera</i>	1.14	0.78	4.73	6.12	Woyke 1966
<i>A. cerana</i>	0.98	0.49	1.35	2.79	Woyke 1975
<i>A. florea</i>	0.78	0.25	0.90	3.66	Koeniger <i>et al.</i> 1989
<i>A. florea</i>	0.81	0.27	1.09	4.40	Lekprayoon <i>et al.</i> 89
<i>A. florea</i>	0.85	0.32	0.90	2.98	Woyke 1974/92
<i>A. dorsata</i> (2)*	1.10	0.70	3.67	5.24	Koeniger <i>et al.</i> 1990
<i>A. dorsata</i> (1)	1.15	0.80	2.60	3.25	Woyke 1974/92

*(2 or 1) indicates number of investigated queens

in their spermathecae were counted. Spermatozoa in the spermatheca of one *A. dorsata* queen were also counted.

Results

Table 1 shows that the diameter of the spermathecae of six *A. florea* queens ranged from 0.821 to 0.907 mm, and the average was 0.845 mm. The number of spermatozoa in the spermathecae of six naturally mated queens of unknown age ranged from 0.382 to 1.538 million, and the average was 0.904 million.

Table 2 shows that *A. florea* has the smallest spermatheca containing the lowest number of spermatozoa. However, the concentration of sperm in the spermatheca is relatively high and it is within the range characteristic for *A. cerana* and *A. dorsata*. Mating behavior of *A. cerana* is different from that suggested for *A. florea*.

Workers of the queenless *A. florea* colony constructed 21 emergency queen cells. Contrary to descriptions given by Sakagami and Yoshikawa (1973), all emergency queen cells were constructed on the lower edge of the comb. The queen cells were sealed four days after the old queen was removed.

The queens were instrumentally inseminated at the age of 5 days. After the abdomen was opened, two distinct lobes were visible at the ventral side of the vaginal orifice. Because *A. florea* drones were not obtainable, semen was collected from *A. mellifera* drones. It was easy to inseminate these queens. No difficulties occurred. The presence of spermatozoa in the spermatheca was checked after the

queens were killed two days later. The results show, that the sperm entered the spermatheca of all queens. The efficiency of the number of spermatozoa entering the spermatheca cannot be discussed because the semen originated from another species of honey bee. One instrumentally inseminated queen was not killed and it was returned to the colony. It survived until the colony perished.

These results show that the sperm enter the spermatheca in ways other than directly from the endophallus of drones as was suggested by Koeniger *et al.* (1989). The semen can be also injected into lateral oviducts, where the spermatozoa enter the spermatheca.

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Abstract

During natural as well as instrumental insemination of queen bees, semen is injected into lateral oviducts. Koeniger *et al.* (1989) suggested that direct sperm transfer from the drone's endophallus into the spermatheca occurs in *A. florea*. Investigations were undertaken to see whether *A. florea* queens can be inseminated by injecting the semen into lateral oviducts. *A. florea* virgins were reared by making the colony queenless. Workers constructed 21 emergency queen cells. The colony together with the queen cells was transported to Poland. Virgins five days old were inseminated with semen of *A. mellifera* drones. It was easy to inseminate *A. florea* queens. Results obtained showed that spermatozoa from semen injected into lateral oviducts enter the spermatheca of *A. florea* queens.

Key Words

Apis florea, queen rearing, instrumental insemination, semen migration, spermatheca, queenless colonies, emergency queen cells

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Asian Apiculture

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Cheshire, Connecticut 06410 USA

1993