

NATURAL AND INSTRUMENTAL INSEMINATION OF *APIS CERANA INDICA* IN INDIA*

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Summary

The investigation was conducted on the India plains variety of *A. c. indica*. The queens flew between 13.30 h and 15.30 h, and mating occurred between 14.00 h and 15.00 h. The mean duration of a successful mating flight was 27 minutes, and a queen mated with an average of 10 drones on one flight. The mating sign is formed of a twisted and compressed thread of solidified mucus, surrounded by sticky orange membranes. After one mating flight a well mated queen had 1.5–3.0 mm³ (average 1.9 mm³) semen in the oviducts, and a young laying queen had a mean 1.3 million spermatozoa in the spermatheca.

Queens instrumentally inseminated twice with a total of 6 mm³ semen had on average 1.4 million spermatozoa in the spermatheca. Queens inseminated twice with a total of 4–6 mm³ laid only fertilized eggs for one year; observation of such queens for a longer period is desirable.

Introduction

To increase the productivity of *Apis cerana indica*, controlled mating must be assured. But a successful method of instrumental insemination of this species can be worked out only when the details of its reproduction, and especially of its mating behaviour, are better known. The reproduction biology of *A. mellifera* has been studied by many authors, whereas such studies on *A. cerana* have only just started.

Sharma (1960) and Adlakha (1971) carried out investigations in India on *A. c. indica*, and Ruttner, Woyke and Koeniger (1972, 1973) and Woyke (1973) worked on this species imported into the German Federal Republic, where the queens mated in rather unusual conditions. *A. cerana* queens start to fly when younger than *A. mellifera* queens (Sharma: 3.6 days; Adlakha: 2.9 days). Adlakha reported matings on one flight only, whereas Sharma, and Ruttner, Woyke and Koeniger (1972) reported matings on two flights. The amount of semen in the oviducts was measured by Ruttner, Woyke and Koeniger (1973) in Germany, in one queen returning from a mating flight, and they counted the number of spermatozoa in the spermatheca of two queens mated naturally in Germany and of one naturally mated in Pakistan.

The first successful instrumental insemination of *A. c. indica* queens were made by Woyke (1973) in Germany, and the egg-laying activity of these queens was investigated in Poland. But it was not possible to compare the results of instrumental insemination with the results of natural matings.

Materials and Methods

This investigation was carried out at the Central Bee Research Institute in Poona, India, and observations of mating flights of queens were made at the Apicultural

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Institute's hill station at Mahableshwar, 120 km south of Poona (altitude 1370 m).

Observations on the common yellow plains variety of *A. c. indica* were made in April and May 1974, after the main swarming season (in March) was finished. The author was informed that there were about 6000 colonies in the neighbourhood, which should have provided sufficient drones for natural mating.

Queen cells were introduced into small colonies in mating hives, containing 4 combs (17×23 cm) of brood and honey. The entrance of each mating hive was closed with queen excluder fastened to special entrance bars, making a kind of small veranda at the front of the hive. Hive entrances were watched by several observers from 12.00 to 16.00 h. When the queen appeared on the inner side of the excluder, it was lifted up, and the queen flew out; the entrance was then shut again. The times of departure and return were recorded. Altogether 15 flights by 13 queens were observed. All queens which returned from a flight were examined, and those with a mating sign were killed and dissected in saline solution. Three measurements, perpendicular to one another, were made under a binocular microscope of each oviduct filled with semen; from these results, the volume of semen in both oviducts was calculated. After the mating signs and oviducts of 9 queens had been examined, the queen excluders were removed from the entrances of the other mating hives and the queens were allowed to fly freely. Queens which started to lay eggs were killed, to allow examination of the spermatheca and the number of spermatozoa in it. In addition, 4 queens mated in the apiary in the swarming season were examined. The spermatozoa from the spermatheca of *A. cerana* queens were found to disperse badly in tap water, and the best results were obtained with a solution containing 1% NaCl and 2% sugar. The spermatheca was transferred to a drop of this solution, and after removal of the tracheal cover the diameter was measured. Next the spermatheca was crushed, the spermatozoa mixed with the solution, and more solution up to 1 cm³ added. After mixing, 3–4 cm³ of tap water were added, mixed thoroughly again, and then the spermatozoa were counted in 10 squares of a Fuchs-Rosenthal counting chamber, i.e. in 2 mm³ liquid. The total number of spermatozoa in the spermatheca was then calculated.

Altogether 15 queens were instrumentally inseminated (twice), with a total of 4.0–6.7 mm³ semen collected from 20–36 drones. After insemination, the queens were returned to their colonies. Queen excluders were kept on hive entrances all the time. Some queens were killed for counting the spermatozoa in the spermatheca and the remainder were left for production of brood.

Results

General observations

The plains variety of *A. c. indica* of central India has much smaller colonies than the hill variety of northern India and Pakistan, which is kept in Langstroth hives. The plains variety is kept in hives containing 10 smaller combs (17×23 cm), and during the honey flow one or two shallow boxes of comb are added.

The foragers were active mainly in the morning, until about 10.00 h. By mid-day or afternoon only single workers left the hive, or else none flew. *A. florea* and especially *A. dorsata* workers were, however, seen foraging on the flowers throughout the day.

During the period of day when mating flights were made, about 10–20 workers came out of the hive, on to the flight board and around the entrance, before the queen appeared in the entrance. In contrast to *A. mellifera*, few workers flew around the

hive, but sometimes so-called "play flights" occurred about this time. No workers were seen on the flight board of hives from which the queen did not fly.

Queen flights

Queens departed on flights between 13.30 h and 15.30 h (Table 1A), and the greatest number of flights occurred between 14.15 h and 14.30 h. Mating took place on flights starting between 14.00 h and 15.00 h, but not on the earliest and latest flights. The non-mating flights could be separated into the very short ones lasting 2–3 minutes, and those of a similar duration to mating flights (Table 1B). The mean duration of a successful mating flight was 27 minutes.

TABLE 1. Time and duration of 15 flights by 13 queens of *Apis cerana indica* in Mahableshwar.

A. Time of day at which flights started

| <i>15 min. commencing</i> | <i>No. flights without mating</i> | <i>No. flights with mating</i> |
|---------------------------|-----------------------------------|--------------------------------|
| 13.30 | 1 | — |
| 13.45 | — | — |
| 14.00 | 1 | 2 |
| 14.15 | 2 | 3 |
| 14.30 | — | 2 |
| 14.45 | 1 | 2 |
| 15.00 | — | — |
| 15.15 | 1 | — |
| Total | 6 | 9 |

B. Duration of flights

| <i>Duration (min)</i> | <i>No. flights without mating</i> | <i>No. flights with mating</i> |
|-----------------------|-----------------------------------|--------------------------------|
| 0–4 | 2 | — |
| 5–19 | — | — |
| 20–24 | 3 | 4 |
| 25–29 | 1 | 1 |
| 30–34 | — | 2 |
| 35–39 | — | 2 |
| Total | 6 | 9 |

The mating sign

The mating sign was easily visible, protruding from the queen's sting chamber. It consisted of a plug of mucus, normally with a white thread of mucus on its exterior

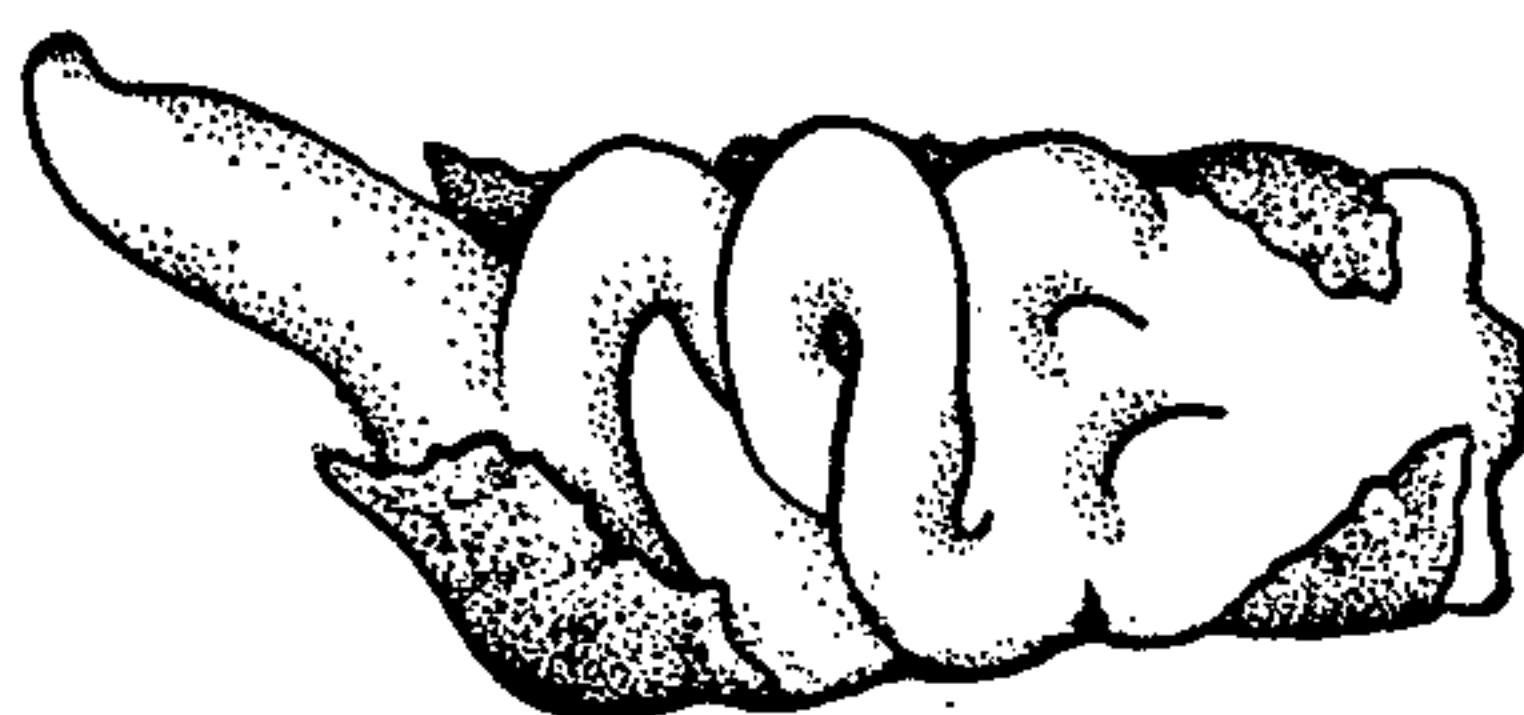


FIG. 1. Mating sign of *Apis cerana indica*, ventral view.

end. After the sign was removed from the sting chamber it could be seen to consist of a twisted and compressed thread of solidified mucus (Fig. 1); it was 1 mm wide, 0.6 mm thick and 2.0–2.5 mm long. The dorsal part was destroyed by the movements of the sting. Orange sticky membranes were attached laterally to the mating sign, partly overlapping the ventral and dorsal sides. There was no evidence that these membranes might have originated from several drones.

Volume of semen in the oviducts of newly mated queens

Queens returning from mating flights with a mating sign had 0–5.39 mm³ semen in their oviducts (Table 2). Each drone produced about 0.20 mm³ semen (see later), and the data indicate that the queens mated on average with about 10 drones during one flight. The two extreme values in Table 2 (0 and 5.39 mm³ semen) seem to be rather exceptional. Although at the time when the high value was recorded (10th April) there were far more drones in the colonies than in May, it still appears unduly large, but unfortunately re-examination was impossible. It also looks anomalous that one queen returning with a mating sign had no semen in the oviducts; some semen was found in the vagina and bursa copulatrix anterior to the mating sign; the queen had, therefore, mated with at least one drone. These two extreme values did not greatly affect the overall mean.

TABLE 2. Characteristics of natural mating of *Apis cerana indica* queens in Mahabaleshwar.

| Date | Duration of mating flights (min) | Vol. semen in oviducts (mm ³) | Estimated no. drones mated |
|------|----------------------------------|---|----------------------------|
| 10.4 | 38 | 5.39 | 27 |
| 30.4 | 20 | 2.51 | 13 |
| 1.5 | 30 | 2.73 | 14 |
| 4.5 | 23 | 1.86 | 10 |
| 4.5 | 21 | 1.40 | 7 |
| 5.5 | 20 | 0.98 | 5 |
| 5.5 | 36 | 0.50 | 3 |
| 6.5 | 26 | 0.00 | 1 |
| 7.5 | 33 | 2.08 | 11 |
| Mean | 27 | 1.94 | 10 |

The data presented in Table 2 suggest that well inseminated *A. c. indica* queens normally had 1.8–2.8 mm³ semen, and that they mated with 9–14 drones. Since the queens were killed after return from the first successful mating flight, it could not be established how many of them would have mated on a second flight.

No correlation was found between the duration of the mating flight and the volume of semen in the oviducts after that flight.

Number of spermatozoa in the spermatheca of naturally mated queens

Table 3 shows that queens mated about 10th May from the mating apiary had 0.655–1.995 million spermatozoa in the spermatheca. The number of drones was then much lower than in the swarming season in March, and 4 additional queens mated from the main apiary in March were, therefore, examined for comparison: the numbers of spermatozoa in the spermatheca of these queens were within the range

TABLE 3. Numbers of spermatozoa in the spermatheca of naturally and instrumentally inseminated *Apis cerana indica* queens.

| <i>Diameter of spermatheca (mm)</i> | <i>Vol. of spermatheca (mm³)</i> | <i>No. spermatozoa (millions)</i> | <i>Concentration (millions/mm³)</i> |
|--|---|-----------------------------------|--|
| <i>Queens mated from mating apiary in May</i> | | | |
| 0.965 | 0.471 | 0.655 | 1.391 |
| 0.979 | 0.492 | 0.800 | 1.626 |
| 1.008 | 0.537 | 0.965 | 1.797 |
| 0.950 | 0.449 | 1.143 | 2.546 |
| 0.950 | 0.449 | 1.228 | 2.735 |
| 0.979 | 0.492 | 1.340 | 2.724 |
| 0.930 | 0.421 | 1.548 | 3.677 |
| 0.936 | 0.430 | 1.571 | 3.653 |
| 0.930 | 0.421 | 1.995 | 4.739 |
| Mean 0.959 | 0.462 | 1.249 | 2.765 |
| <i>Queens mated from main apiary in March</i> | | | |
| 0.994 | 0.515 | 1.169 | 2.270 |
| 1.037 | 0.584 | 1.470 | 2.517 |
| 0.950 | 0.449 | 1.543 | 3.437 |
| 1.008 | 0.537 | 1.638 | 3.050 |
| Mean 0.997 | 0.521 | 1.455 | 2.818 |
| <i>Queens inseminated instrumentally twice with 3 mm³ semen</i> | | | |
| 0.965 | 0.471 | 1.180 | 2.505 |
| 0.950 | 0.449 | 1.440 | 3.201 |
| 0.930 | 0.421 | 1.704 | 4.048 |
| Mean 0.948 | 0.447 | 1.441 | 3.251 |

estimated for the May queens. The average number of spermatozoa in March queens was a little higher than that in May queens, but the spermatheca of the latter was larger than that of the May queens, and Woyke (1966, 1971) found that the number of spermatozoa in the spermatheca of naturally mated queens depended very much upon the size of spermatheca. The concentration of spermatozoa in the spermatheca (Table 3) shows less difference than their number, which is therefore thought to be related here also to the size of the spermatheca, and developmental conditions being better during the swarming season than later. The mean number of spermatozoa in the spermatheca in all 13 naturally mated queens was 1.313 million, and Table 3 shows that the spermatheca of well inseminated queens contained about 1.4 million.

Instrumental insemination

At first, some difficulties were encountered in collecting semen from the drones of *A. cerana indica*. When the thorax was squeezed, as is the practice with *A. mellifera* drones, the endophallus was not everted at all. So the next method adopted was to excite the drones greatly by light and by flight (while held by the legs) and then to press the thorax twice—once lightly, and then strongly enough to kill the drone. The endophallus was everted completely, but ruptured at the tip, with a consequent mixing of haemolymph and semen. Such semen could not be used for insemination, and the method was satisfactory only for weak drones, which had been separated for a

long time from the worker bees. The collection of a sufficient amount of semen from a greater number of drones seemed hopeless at this time. But at last a satisfactory method was worked out, involving gradually increasing pressure on the thorax until the drone died. Nevertheless, the amount of semen produced by each drone is very small, it was very laborious work to collect a sufficient amount for instrumental insemination.

Detailed records of 26 batches of semen collected for instrumental insemination are available. Each batch consisted of 1.8–3.6 mm³ semen, and the total amount was 74.3 mm³ semen from 358 drones, an average of 0.208 mm³ semen from one drone. However, the measured amount of semen also contained the fluid from the endophallus of 1–3 additional drones in each batch; this fluid was added to dilute the dense semen. Subtracting this amount, one can state that one drone produced about 0.20 mm³ of semen. In collecting the semen 908 drones were killed, 2.5 times the number that yielded semen: 12–13 drones must be killed to obtain 1 mm³ semen yielded by about 5 of the drones.

Of 15 queens inseminated instrumentally: 4 were not accepted by the colony or died for another reason; 2 flew out during inspection of the colony and did not return; 3 were killed for investigation; 6 were left to produce brood. The 3 queens killed for counting the number of spermatozoa in the spermatheca had been inseminated twice with 3 mm³ semen. The total amount of semen needed for 1 queen (6 mm³) was collected from 30–40 drones. Table 3 shows that the queens had 1.2–1.7 million spermatozoa in the spermatheca. The first queen was inseminated with the semen collected from the smallest number of drones, and the last one with semen from the greatest number of drones; the concentration of spermatozoa in the semen injected into the oviducts of different queens was probably not the same.

The average number of spermatozoa in spermatheca of instrumentally inseminated queens was almost the same as that found in queens mated naturally from the main apiary, and a little higher than in queens mated from the mating apiary. But the queens inseminated instrumentally had small spermathecae, the concentration of spermatozoa in them was higher than in both groups of naturally mated queens.

The 6 queens left for the production of brood were inseminated twice between 5th–9th April with a total of 4–6 mm³ semen, collected from 20–30 drones. All laid only fertilized eggs till the end of September (when the author left India); afterwards some colonies swarmed. The remaining queens were still laying only fertilized eggs in the following year.

Discussion

The queens made mating flights between 13.30 h and 15.30 h in Mahableshwar, a time similar to that found by Sharma (1960) and by Ruttner, Woyke, and Koeniger (1972). The mean duration of successful mating flights (27 minutes) was similar to that of 22.3 minutes found by Sharma (1960) and 30.8 minutes by Ruttner, Woyke and Koeniger (1972), but was longer than the 19 minutes of Adlakha (1971).

No clear evidence was found that the mating sign of *A. c. indica* consisted of parts of several drones, as in one queen mated in Germany (Ruttner, Woyke & Koeniger, 1973). Since the semen is pushed out of the endophallus by mucus, and no chitinous plates are present in the bulb of this species, it would be expected that each drone which mates with the queen would leave a mucus mating sign, and it is difficult to imagine how the next drone could remove the mating sign before copulating with the queen on the same flight. An explanation might be that the mucus of *A. c. indica* drones solidifies much more quickly in the air than that of *A. mellifera* drones.

The one queen investigated in Germany (Ruttner, Woyke & Koeniger, 1973) after a mating flight had as much semen in her oviducts (2.79 mm^3) as a well mated Mahableswar queen. But all *A. c. indica* queens returning from mating flights had much less semen in the oviducts than returning *A. mellifera* queens (11.6 mm^3 , Woyke, 1960). Nevertheless, *A. c. indica* queens mated with an average of 10 drones in one flight, whereas *A. mellifera* queens mate on average with 8 drones (Woyke, 1960).

Queens mated in India had 0.655–1.995 million spermatozoa in the spermatheca, more than in the two queens mated in Germany (0.270, 0.610 millions), but fewer than the 2.665 million found in one queen mated in Pakistan (Ruttner, Woyke & Koeniger, 1972). The average number of spermatozoa found in the spermatheca of naturally mated *A. c. indica* queens (1.313 million) was one quarter that found in *A. mellifera* queens (5.340 million) (Woyke, 1960).

Queens inseminated instrumentally in India with 6 mm^3 semen had more spermatozoa in the spermatheca (1.441 million) than queens inseminated by Woyke (1973) with 4 mm^3 in Germany (1.195 million). The spermatheca of both groups of instrumentally inseminated queens contained numbers of spermatozoa within the range for naturally mated queens (1.313 million). But instrumentally inseminated *A. c. indica* queens had many fewer spermatozoa than *A. mellifera* queens inseminated with the same total amount of semen (4.120 – 4.324 million, Woyke 1960).

A. c. indica queens inseminated with either 4 mm^3 semen (Woyke, 1973), or with 4 – 6 mm^3 as described here, produced exclusively worker brood during the first year. Examination of brood from instrumentally inseminated *A. c. indica* queens laying for a longer period of time would be very desirable.

Conclusions

The mating biology of *A. c. indica* queens differs in some respects from that of *A. mellifera*. It is shown that the smaller, plains variety of *A. c. indica* can be instrumentally inseminated as satisfactorily as the hill variety. But the procedure with *A. c. indica* is much more time-consuming than with *A. mellifera*.

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References

- ADLAKHA, R. L. (1971) Preliminary studies on the mating behaviour of *Apis mellifera* and *Apis indica* queens and their age of starting oviposition. *XXIII Int. Beekeep. Congr.* : 420–425
- RUTTNER, F., WOYKE, J. & KOENIGER, N. (1972) Reproduction in *Apis cerana*. 1. Mating behaviour. *J. apic. Res.* 11(3) : 141–146
- (1973) Reproduction in *Apis cerana*. 2. Reproductive organs and natural insemination. *J. apic. Res.* 12(1) : 21–34
- SHARMA, P. L. (1960) Observation on the swarming and mating habits of the Indian honeybee. *Bee World* 41(5) : 121–125
- WOYKE, J. (1960) Naturalne i sztuczne unasienianie matek pszczelich [Natural and artificial insemination of queen honey bee]. *Pszczel. Zesz. Nauk.* 4(3/4) : 183–275
- (1966) Wovon hängt die Zahl der Spermien in der Samenblase der auf natürlichen Wege begatteten Königinnen ab? *Z. Bienenforsch.* 8(7) : 236–247
- (1971) Correlations between the age at which honeybee brood was grafted, characteristics of the resultant queens, and results of insemination. *J. apic. Res.* 10(1) : 45–55
- (1973) Instrumental insemination of *Apis cerana indica* queens. *J. apic. Res.* 12(3) : 151–158