Journal of Apicultural Research 11(3) : 141–146 (1972)

REPRODUCTION IN *APIS CERANA*
1. MATING BEHAVIOUR

F. Ruttner, J. Woyke and N. Koeniger

Institut für Bienenkunde an der Universität Frankfurt am Main, 637 Oberursel/Ts, Germany (B.R.D.)

Manuscript received for publication 20th March 1972

Summary

Comparative studies were made in Germany with strains of *Apis cerana* from different parts of the Indian subcontinent and from China. Flight activity of drones and queens of *Apis cerana indica* was observed in an isolated place; 8 queens mated, 3 of them on two flights. Successful mating flights were longer than those of *A. mellifera* (average 30·8 minutes).

Introduction

During the past thirty years a number of facts have been established about the biology of reproduction in *Apis mellifera*. This new knowledge (multiple mating, mating flight distances, sex alleles) is extremely relevant to breeding methods. Very little is known in this respect about *Apis cerana*, and no direct analogy is permissible from the one species to the other, as they differ in a number of morphological and behavioural characteristics (Butler, 1962; Simpson, 1960; Bährmann, 1961).

One great difference is the number of spermatozoa produced by a drone. Ruttner and Maul (1969) found 1·5 million spermatozoa per drone for *Apis cerana*, only about 15% of the number for *A. mellifera*. On the other hand, although *A. cerana* colonies are in general smaller than those of *A. mellifera*, a *cerana* queen lays up to 1000 eggs per day. At the Oberursel Institute we kept a *cerana* colony for nearly 5 years among *mellifera* colonies; it was headed by one and the same queen (from Peking), and she laid well all this time. The system for storing spermatozoa in the queen is thus more or less as efficient in *cerana* as in *mellifera*, in spite of the smaller number of spermatozoa in *cerana* drones. This could be explained in one of two ways:

1. *cerana* queens receive fewer spermatozoa during mating, but the process of transfer to the spermatheca is less wasteful than in *mellifera* (where only about 10% reach the spermatheca), giving a final result which is about the same;
2. *cerana* queens mate with many more drones than *mellifera* queens do.

To resolve this question, and others, a study was made on the anatomy of the genitalia, on mating behaviour, and on the transfer of spermatozoa, in *Apis cerana*.

Apis cerana in its natural habitat
(North-West Frontier Province, West Pakistan)

Apis cerana is widespread throughout the continent of Asia. The material used in the 1971 experiments (*A. c. indica*) originated in the North-West Frontier Province of West Pakistan. Originally beekeeping was practised there only in the mountainous and hilly areas. The bees were (and are) kept in logs and earthern pitchers, which swarms occupy without human intervention. Except for cutting out some of the honeycombs at the end of the season, no other manipulation is carried out by the beekeeper. Colonies of *A. cerana* also live wild in the forests, preferring rotten trees or crevices in the rocks as nesting sites.

These regions are at an altitude of 2000–4000 metres, and the climate is temperate-mountainous, with 4 months of snow. Some colonies found during an excursion in the Swat area, in April 1971, were just at the beginning of the seasonal development.

In the plains around Peshawar the climate is subtropical, with mango, citrus, sugar cane, etc. *A. florea* and *A. dorsata* are common and, according to what we were told, there was originally no beekeeping there. So the natural distribution seems to be well separated, with *A. cerana* in the mountains and the two other species in the plains.

Modern beekeeping started some twenty years ago in the Peshawar district, at the initiation by the Agricultural Research Institute at Tarnab. At present there are around 200 colonies of *A. cerana* in Langstroth hives, for which modern equipment is used. Dr. M. Rashid Khan and his colleagues have developed a system of migratory beekeeping: from October to June the bees are kept in the plains of Peshawar, and for the hot part of the year they are taken to the mountains (Swat valley). In this way one of the most troublesome predators of *A. cerana* which is very common in the plains, the oriental hornet (*Vespa orientalis*), is avoided.

Under these conditions two swarming periods occur, the first at the end of February and into March in the plains and the second during August and September in the mountains. A colony produces up to 10 swarms during one swarming period, the average number being about 6. Swarming is the only procedure utilized by beekeepers for increasing the number of colonies. Usually a swarm will settle in a tree near the hive; it is captured by putting a skep on top of the cluster; the bees run up into it immediately, and are subsequently thrown into a hive containing one frame of honey and others with foundation only. The size of a *cerana* colony before swarming is around 4–6 Langstroth brood combs. The swarms are usually smaller than those of *A. mellifera* and occupy only 1 Langstroth comb.

Queen rearing, using the Doolittle method, was practised during April 1971 and was quite successful. The ripe queen cells were given to queenless colonies. Nearly all the colonies left the hives 3–9 days after the young queens emerged, in a swarming process. Drone flights were observed from 10 to 14 hr. No mating flights of queens were seen, but the swarms left the hives during the period of the day when mating occurred (April 1971).

Queen rearing

The first colonies of *A. cerana* to be introduced to the Institute at Oberursel were *A. c. cerana* obtained from Peking in 1966. Since 1970, *A. c. indica* colonies from Afghanistan, India and Pakistan have been used. Attempts were made from the beginning to obtain young mated queens, and from our experience the following conclusions can be drawn.

(*a*) Artificial queen rearing from grafted larvae gives the same results as with *A. mellifera*. The diameter of the wax cup must not be greater than 8 mm. A medium-size queenless colony can rear 12–20 young queens.

(*b*) It is not possible to rear *cerana* queens in *mellifera* colonies. On one occasion, using the method of double grafting (first graft *mellifera* larva, second graft *cerana*), 8 cells out of 30 were fed throughout the larval stage and the cells capped. But the larvae did not undergo metamorphosis, and they were dead 10 days after grafting.

(*c*) It is impossible to make mating nuclei by artificial swarms, because the bees abscond very soon after they are allowed free flight. The only practicable method is to make nuclei with combs containing brood. Even then, in the first few days some of the small colonies are likely to abscond or unite with each other.
(*d*) If there were any drones of *A. mellifera* in the area, the virgin *cerana* queens did not lay at all, or they became drone layers. This was true even if the nearest *A. mellifera* colonies with drones were no nearer than 3 km. At the time there were many *cerana* drones in full-strength *cerana* colonies where the *cerana* queens were located. Several queens started to produce worker brood, but they became drone layers within 3 weeks. This phenomenon can be explained by the facts that the same sexual attractant is effective for both species (Butler, Calam & Callow, 1967; Ruttner & Kaissling 1968; Shearer et al., 1970), and that *mellifera* drones are attracted by *cerana* queens nearly as much as by *mellifera* queens. It seems probable that the attracted *mellifera* drones interfere in the mating process of *cerana* queens and prevent insemination with *cerana* sperm. Successful natural matings were obtained when at last we were able to use a sufficiently isolated mating place.
(*e*) Instrumental insemination is feasible with *A. cerana*, but it is time-consuming because of the small quantity and high viscosity of the semen produced.

Observations on mating flights

The mating place used was 1000 m above sea level in the Graswang valley of the Bavarian Alps, about 15 km south of Oberammergau. This mountainous region has only very sparse human habitation. The nearest apiary (with 6 colonies) was 10 km to the north. In the other direction the distances were 11, 12 and 15 km, across high mountain ranges.

The location is used as a mating station by the Bayerische Landesanstalt für Bienenzucht in Erlangen, and had been tested for isolation with the cordovan mutation (Böttcher, 1969): 24 out of 26 queens were purely mated, the other 2 giving mixed progeny. Assuming that each queen mated with 10 drones, then only 4 of the 260 drones involved came from outside, i.e. less than 2%. According to our experience, this mating station is one of the most isolated from drones on the European continent.

Queens of *A. cerana* were reared in the usual way in a queenless colony, larvae being obtained from a colony brought from the region of Peshawar in India. Twenty nuclei were established at Oberursel with frames of brood, and a ripe queen cell was added to each on 1st June. (It was very difficult to get the nuclei established, on account of absconding, drifting and robbing.)

The nuclei were taken from Oberursel to the Graswang Valley (500 km) on 9th June. An abundance of *cerana* drones were introduced to the site in 4 queenright colonies (2 queens mated and 2 virgin). The nuclei and colonies were placed in pairs, several metres apart, in a sunny place between young trees.

Queen flights were observed by the method of Alber, Jordan, Ruttner and Ruttner (1955): an entrance "channel" was fitted to each hive, with a glass cover on top and a queen excluder at the front. Bees coming and going could be observed and, in particular, a queen trying to leave the hive would be seen in the channel, and could be released by removing the queen excluder.

General observations

A. cerana workers found much more difficulty in getting used to the new entrance than *A. mellifera* bees. Orientation to the hive from close by seems to be governed to a great extent by odour, and secondarily by recognition of a dark "hole". To begin with, the bees insisted on trying to enter the hive at the front (where the channel was fixed), or under the roof, or from underneath the box. Four queens which joined the workers were lost on account of this behaviour.

A second difficulty was that the bees absconded with the queen on her mating flight. When the queen started to fly, the whole colony left the nucleus, settling on a tree, or finally uniting with other colonies. Once 3 nuclei united to form a single swarm. In all, 8 colonies swarmed (1 twice). Several others tried to do so, but were prevented. Four queens were lost through swarming, and a fifth flew away after returning from a mating flight.

In evaluating this behaviour, it must be taken into account that weather conditions were very unfavourable during our experiment. During the whole experimental period (10th to 29th June) there were only 5 days favourable for mating flights (14th, 20th, 22nd, 23rd, 25th); on these days the maximum temperature reached 20°C or more, and the sun shone for at least a short time. On the other days it was rainy and cold, with maximum temperatures below 12°—on some days not more than 8–9°. But when the clouds broke and the sun came out, the temperature rose quickly, and the bees immediately became very active; as soon as the queen flew off the workers left the hive with her. If there had been a consistent series of favourable days, the behaviour of the bees would perhaps have been different, and the results of the mating period more regular. Certainly in the region where the *A. cerana* bees were indigenous such contrasts of temperature would never occur in the mating season. Similar difficulties had not arisen in earlier years with the Peking strain of *A. cerana cerana*. It is true that mating flights then took place in warmer weather, but genetic differences are probably also of importance.

A method was quickly devised to prevent absconding. As soon as the queen was released for flight and the colony started to follow her, the entrance was closed. A few minutes later, the colony being stabilized again and the queen out of its immediate environment, the entrance could be reopened without danger. At least a partial success was achieved in this way.

Drone flights

Although our main attention was paid to the queens and their nucleus colonies, regular observations were also made on the four drone colonies.

Drone flights started rather early, usually between 11 and 12 hr; on sunny days a little flight activity by the drones might start as early as 10.30 hr. Maximum drone flight was observed between 12.00 and 14.00 hr. Then the frequency decreased, and at 15.00 hr flight activity had practically ceased. This was surprising, as on several days the maximum temperature was not reached until 15.00–16.00 hr. The flight rhythm observed in Graswang does not seem to be characteristic of the *A. cerana* species in general, as in earlier years we found *A. cerana cerana* drones (from Peking) flying even after 16 hr.

Temperature had much less influence on drone flight activity than in *A. mellifera*. At 12–15°C there was a continuous busy flight activity of drones, and even on rainy days with temperatures as low as 8° some colonies had a short period of drone flight (about 15 minutes). Drones left the hive in groups, made a circle about 2 m in diameter around the hive, and returned. The temperature threshold of the start of flight activity, strongly marked at about 16–18° in *A. mellifera*, does not exist in the same way in this strain of *A. cerana*.

Queen flights

During the experiments at Graswang we observed 35 flights of 13 queens. Of the 13, 2 absconded with a swarm, 1 on the first and 1 on the second flight; 2 others did not return from a flight (1 of them had left again after returning with a mating sign); 2 queens made several flights before the end of the experiment, but without mating, one further queen did not fly at all.

So only 7 queens remain for a complete flight analysis during their mating period. These queens made 1–7 flights, average number 3·3:

Number of flights	1	2	3	4	5	6	7
Number of queens	1	2	2	–	1	–	1.

The queens emerged on 3rd–4th June; they were 10–11 days old on the first day of flight and mating (14th June) and 21–22 days on the last (25th June).

No flight started before 13.00 hr. The 35 flights of 13 queens started during the following periods:

Time	13.00	13.30	14.00	14.30	15.00	15.30	16.00 hr
Number of flights	3	10	10	5	5	2	

Maximum flight activity was thus between 13.30 and 14.30 hr. Queen flight started and ended later than drone flight: almost no drones were seen leaving or entering the hive entrance after 15.00 hr.

A mating sign was observed on 5 queens once and on 3 queens twice. According to this evidence most matings took place on the first three flights:

Flight no.	1	2	3	4	5	6	7
Number of queens seen with a mating sign	3	3	3	1	–	–	1.

No successful mating flight started earlier than 13.26 hr:

Time flight started	13.00	13.30	14.00	14.30	15.00	15.30 hr
Number of queens mated	1	2	6	1	1	

The 12 flights after 14.30 hr thus resulted in only 2 matings, although the weather was mostly more favourable then. A likely reason is the lack of drones in flight at this time.

Flights from which the queen returned without a mating sign lasted from 4 to 60 minutes (average 13·7). If the single flight with the unusual length of 60 minutes is omitted, the average duration drops to 11·00 minutes, about the same as for *A. mellifera* (Alber et al., 10·0; Roberts, 10·4).

Flights from which the queen returned with a mating sign lasted from 17 to 46 minutes (average 30·8). This is much longer than the flight times recorded for *A. mellifera*. In the experiments on Vulcano (Alber et al., 1955) we found an average of 18·0 minutes; Roberts (1944) gave 15·8, Woyke (1960) 21. The high average for *A. cerana* is not brought about by a few exceptionally long flights, but by a large number of flights of this long average duration:

Flight duration (min)	17–19	20–29	30–39	40–46
Number of flights	1	2	7	1.

Temperature and flight activity of queens

No queen flights were observed at temperatures lower than 16°C. But such flights must have taken place at the beginning of the experiment, because on 12th June one queen was found under the tar-board of a hive roof, and another in a little cluster of bees outside the entrance, though the temperature on this day was not higher than 15° and on the day before did not exceed 12°. On 21st June, 3 queens made short flights lasting 4–10 minutes, at temperatures of 16–17°. All other flights observed were at temperatures above 17–18°.

As in *A. mellifera*, matings occurred generally only in a sunny spell when the temperature was above 20°. The exception was on 20th June, when two successful mating flights were observed while the temperature was 17·5–18·5°.

Multiple mating

With 3 queens (out of 8) we observed a mating sign on two different days. One was on the next day, and another on the next day but one. The third queen (KV 47) had one mating sign on 14th June and a second on 23rd June. However, the original colony of this queen was very weak, and she was transferred to a queenless colony on 23rd June. This long interval may therefore be due to unusual circumstances; nevertheless, it shows a very strong urge to make another flight. Immediately after the second mating flight this queen was dissected to determine the quantity of sperm.

[*Discussion, Acknowledgements and References will follow at the end of Part 2.*]