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SEX DETERMINATION IN *APIS CERANA INDICA*

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Summary

Each of 11 *Apis cerana indica* queens was mated instrumentally to 5-29 of her brothers. Any queen mated to more than 15 brothers laid only fertilized eggs in worker cells. Unlike *A. mellifera*, almost all *A. cerana* larvae produced by sibling-mated queens survived their first day of larval life. The youngest larvae were collected from worker cells for microscopical investigation. Examinations of slides prepared from 572 larvae (originating from all the sibling-mated queens) showed that 27·4% were drones, most of which must have developed from fertilized eggs. This percentage is close to the expected 25%, and it is concluded that the sex determination mechanism in *A. cerana* is similar to that in *A. mellifera*, where there is one sex locus *X* with several alleles. Heterozygosity results in females, homozygosity in diploid males, and hemizygosity in haploid males. The fate of diploid drone larvae in the colony is different in *A. cerana* and *A. mellifera*.

Introduction

In *Apis mellifera* zygotic diploid drones develop from eggs homozygous at the sex locus *X* (Woyke, 1963*a*), but the larvae are eaten by worker bees (Woyke, 1963*b*) within 6 h of hatching (Woyke, 1962).

The first step towards investigating the mechanism of sex determination in *Apis cerana* was the development of a method for individual controlled mating in this species by instrumental insemination (Woyke, 1973*a*, 1973*b*, 1975). The drones produce only about 0·2 mm^3 of semen, one-sixth of the volume produced by *A. mellifera* drones. An *A. cerana* queen inseminated with semen from a single drone would have only traces of sperm in her spermatheca. Even queens inseminated with semen from 5 drones (1·0 mm^3) produced some drones in worker cells among worker brood. Thus sibling mating of a queen with one brother could not be conducted with *A. cerana* as it can with *A. mellifera*.

If the sex determination mechanism is the same in *A. cerana* as in *A. mellifera*, then an $ab \times c$ queen would produce *ac* and *bc* virgin queens and *a* and *b* drones. Half the virgin queens inseminated by a brother ($ac \times a$) would produce 50% fertilized homozygous eggs (*aa*) and 50% heterozygous eggs (*ac*). The remaining queens ($ac \times b$) would produce only heterozygous eggs. If virgin queens are inseminated by several brothers ($ac \times a, a, b, b$), then most of them would produce 25% homozygous eggs (*aa*) resulting in diploid drones, and 75% heterozygous eggs (*ab*, *ac* and *bc*) resulting in females. The percentage of homozygous eggs, producing diploid drones, may vary theoretically from 0 to 50% depending upon the proportion of drones carrying an *a* or *b* allele, but the average should be 25%.

Materials and Methods

This investigation commenced in 1971 at the Beekeeping Institute in Oberursel, German Federal Republic, using the hill variety of *Apis cerana indica* from Peshawar, Pakistan. Six colonies headed by sibling-mated *A. cerana* queens were then brought to Poland where

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the first part of our work was concluded. Most of the investigation, however, was conducted during 1974 at the Central Bee Research Institute in Mahableshwar and Pune, India, using the plains variety of *A. c. indica.* Slides of fixed larvae were prepared and examined in Poland, a total of 572 larvae originating from 11 sibling-mated queens being sexed microscopically.

The queens were reared by grafting larvae into artificial queen cups in queenless *A. cerana* colonies. The queens emerged in nursery cages, and were clipped and marked before being introduced into nucleus colonies. *A. cerana* workers are much less willing than *A. mellifera* workers to accept a new queen, and several queens were lost. Only after the queens were introduced in the presence of alcohol were satisfactory results obtained. The queens were prevented from leaving the hive by a queen excluder placed over the entrance. These were specially designed for *A. cerana,* with slots 4 mm for bees from Pakistan and 3·6 mm for bees from India. The drones emerged in their colonies, and were prevented from leaving by the same queen excluders.

All queens were mated instrumentally to their brothers. In Oberursel (1971) the queens were inseminated once or twice, with altogether only 1·0 mm^3 to 4·2 mm^3 of semen collected from 5-21 drones. In India all queens were inseminated twice, using a total volume ranging from 3·3 mm^3 to 5·7 mm^3, collected from 17-29 drones. After the queens started to lay eggs, the survival rate of larvae 1 day old was measured. A strip of plastic was attached to the comb containing eggs, and every cell row was numbered and marked on the strip (Fig. 1). Each cell containing an egg was individually recorded, using a binocular microscope. The comb was returned to the colony, and each cell was checked daily for presence or absence of an egg or a larva. Comb with brood was always covered with a moist towel when outside the colony.

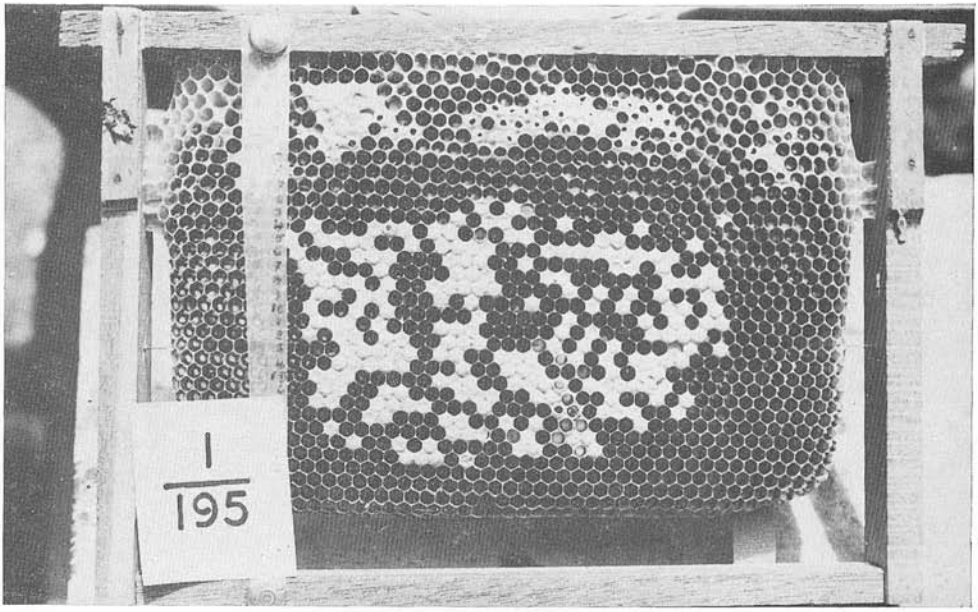

FIG. 1. Scattered brood produced by a sibling-mated *A. cerana* queen. There is no sealed drone brood among the worker brood.

Two procedures were adopted for determining the sex of larvae. First, a comb containing eggs 3 days old was wrapped in a moist towel, and placed in an incubator, so that workers could not eat the newly hatched larvae. The comb was checked every 3 h, and all newly hatched larvae were collected, and to facilitate sex determination, larvae were grown for 1 or 2 days on royal jelly in the incubator.

In India, the youngest larvae, hatched while still in the colony, were collected directly from the worker cells and grown for 1 or 2 days in the incubator. All the larvae were fixed in Gilson's fluid before being embedded in paraffin wax for sectioning. They were then stained with haematoxylin and eosin, and mounted in Canada balsam under a cover slip. The larvae were sexed by the characters described by Woyke (1963*a*) for *A. mellifera* brood.

Results

Brood produced by instrumentally sibling-mated queens

Some of the brood cells in worker cells produced by queens inseminated in Oberursel were sealed with a domed capping, indicating that they contained drone pupae. The percentage of drone brood increased towards the end of the season. Of 6 queens only 2 did not produce drone brood in worker cells at the end of the season. Table 1 shows that only queens inseminated with more than 3 mm^3 of semen (collected from more than 15 drones) produced exclusively worker brood in worker cells. The other queens were probably inseminated insufficiently, and thus laid some unfertilized eggs in worker cells, giving rise to haploid drones. The percentage of drones was lower in early and mid season than at the end.

All queens in India were inseminated with more than 3 mm^3 of semen, and they produced worker brood in worker cells until the end of the season. This was true also for queen 1/195, inseminated with the lowest volume of semen (3·3 mm^3); see Table 1.

The sealed brood of sibling-mated *A. cerana* queens (Fig. 1) resembled that of sibling-mated *A. mellifera* queens in that it was scattered. The survival rate of larvae which emerged in their colonies was surprising: Table 1 shows that 90·9%-99·1% survived their first day of life. A lower survival rate (86·1%) was found in only one colony (2/341). Of a total of 1369 individual larvae, 95·1% survived their first day of life in the colony. The first conclusion was that the sex determination mechanism must be different in *A. cerana* from that in *A. mellifera*. Since *A. mellifera* homozygous diploid drone larvae are eaten within 6 h of hatching, an average 75% survival rate for larvae 1 day old was expected for *A. cerana*.

Sex of larvae produced by sibling-mated queens

After it was found that relatively few larvae disappeared from cells retained in the colony, larvae were no longer hatched in an incubator, but collected directly from the colonies (Table 1).

Examination of sections of prepared larvae showed them to be of two types. The first type had smaller rudiments of gonads, and the two mesodermal cords extending from these gonads ended on the ventral side of segment 10; three pairs of imaginal discs were found on the ventral side of abdominal segments 10, 11 and 12. Larvae of the second type had larger rudiments of gonads, the mesodermal cords extending from the gonads ended on the ventral side of segment 12, and there was only one imaginal disc on the ventral side of segment 12. A very distinct invagination ran from this disc towards the endings of the mesodermal cords. These characteristics are the same as those described for *A. mellifera* larvae (Woyke, 1963*a*). Consequently larvae of the first type were females and those of the second type were males.

TABLE 1. Larvae produced in worker cells by *Apis cerana indica* queens mated instrumentally to their brothers.

Queen			*Brood in colony*		*Microscopical investigation*			
No.	*No. inseminations*	*Total vol. semen injected (mm^3)*	*% drones in sealed worker brood at end of season*	*% surviving after 1 day*	*No. larvae sexed*	*% females*	*% males*	*Where hatched*
				Bees from Pakistan				
1	1	1·0	high	98·3	7	85·7	14·3	incubator
2	1	1·0	0·8	93·5	26	76·9	23·1	colony
9	2	3·5	0	99·1	68	76·5	23·5	incubator
17	2	3·0	22·0	92·3	15	53·3	46·7	colony
19	2	3·0	9·1	90·9	89	80·9	19·1	incubator
43	2	4·2	0	97·7	78	83·3	16·7	incubator
			Total	96·7	283	78·8	21·2	
				Bees in India				
1/195	2	3·3	0	92·3	97	61·9	38·1	colony
1/260	2	4·5	0	91·7	65	63·1	36·9	colony
2/260	2	5·7	0	92·2	25	76·0	24·0	colony
2/341	2	4·1	0	86·1	21	66·7	33·3	colony
3/341	2	4·8	0	95·3	81	71·6	28·4	colony
			Total	92·2	289	66·4	33·6	
			Overall	95·1	572	72·6	27·4	

Table 1 shows that 14·3%-46·7% of larvae collected from worker cells of the Pakistan bees were drones. The highest percentage of drone larvae was found among brood originating from queen 17, which produced 22·0% of haploid drones in worker cells at the end of the season. Some drone brood was also found in worker cells earlier; some haploid drones must therefore have been present among the drone larvae found on the slides. Nevertheless in all cases the percentage of drone larvae on the slides was much higher than that of sealed drone brood in worker cells.

Of the slides prepared from 283 larvae originating from six Pakistan queens, 21·2% were drones. Since the queens laid mostly fertilized eggs in worker cells, and the percentage of drones found on slides was much higher than that of the sealed haploids found in worker cells, it must be concluded that most of the drone larvae collected from those cells originated from fertilized eggs. The real percentage of diploid drone larvae produced by the queens was not much lower than the 21·2% of drones found on the slides, since the proportion of haploid drone brood to worker brood was highest in colonies 1 and 17 from which fewest larvae (7 and 15) were collected for investigation.

Table 1 shows that the percentages of drone larvae found on prepared slides of larvae collected in India (24·0-38·1%) were within the expected range.

The slides made from 289 larvae collected from worker cells in colonies headed by 5 sibling-mated Indian queens contained 33·6% of drone larvae. Since the queens laid fertilized eggs in worker cells, and no haploid drone brood was detected there, it must be concluded that all the drone larvae found originated from fertilized eggs. The real percentage of diploid drone larvae produced by some queens could be slightly lower. Certain prepared slides were discarded, when the larvae had been sectioned incorrectly, or had been damaged. It was impossible to distinguish the sex in these samples; however, the same proportion of males and females should theoretically have been discarded, leaving the sex ratio unaltered. Nevertheless, we found it much easier to identify the males in poor slides due to the large invagination on segment 12, and it is probable that more female larvae were excluded.

Table 1 shows that 24% of the 572 larvae collected from worker cells in colonies headed by all the 11 sibling-mated *A. cerana* queens were drones, and most of them must have developed from fertilized eggs.

Table 1 also shows that the percentage of males found on the slides was similar in larvae collected directly from the colonies and in those protected larvae that emerged in the incubator. Consequently the few larvae which were eaten during the first day must have included both male and female.

Conclusions

Eggs laid by sibling-mated *A. cerana* queens in some of the worker cells produced drone larvae. Since the queens lay fertilized eggs in these cells, all the drone larvae produced by well inseminated queens must have developed from fertilized eggs. The range of the percentage production of diploid drones (out of all offspring) by particular queens was within the range expected, and the overall mean of 27·4% was very similar to the expected means (25%). This indicates that sex determination in *A. cerana* must be similar to that in *A. mellifera*, i.e. sex is determined by one sex locus *X* with several alleles. Heterozygosity (*ab*) results in females, homozygosity (*aa*) in diploid drones, and hemizygosity (*a*) in haploid drones.

Diploid drone larvae of *A. cerana* are not eaten during the first day of larval life, and their fate is thus different from that of *A. mellifera* diploid drone larvae. It will be discussed in a forthcoming paper.

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