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EVIDENCE AND ACTION OF CANNIBALISM SUBSTANCE IN *APIS CERANA INDICA* *

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Summary

Of 26 colonies investigated, 11 were headed by *Apis cerana indica* queens sibling-mated to several drones. The queens produced diploid drone larvae in about 25% of worker cells. Altogether, 9734 eggs were individually recorded, and the survival rate of brood was monitored on successive days. All the results showed that diploid drone larvae of *Apis cerana* are not eaten by nurse bees during the first day of larval life as in *Apis mellifera*. In unfavourable environmental conditions some were eaten in place of worker larvae. Many diploid drone larvae were eaten in their second day of life, but some were reared even until the fourth day, and in favourable conditions some probably reach the adult stage. This suggests that *A. cerana* diploid drone larvae secrete much less cannibalism substance at the beginning of larval life, but later produce it for a much longer period of life, than do *A. mellifera* diploid drones.

This is another specific difference between the two species of *Apis*, indicating an earlier evolutionary stage in *A. cerana*.

Introduction

Woyke (1979) showed that sibling-mated *Apis cerana* queens produced diploid drone larvae from fertilized eggs homozygous at sex locus *X*. These larvae did not disappear from the worker cells during their first day of life as did those of *Apis mellifera*. The present paper reports our investigations into the fate of diploid drone larvae in *A. cerana*.

In *A. mellifera*, diploid drone larvae are usually eaten by the workers within 6 h of hatching (Woyke, 1962, 1963*b*). This is caused by a lipid 'cannibalism substance' produced by the diploid drone larvae (Woyke, 1967) and thought by Dietz (1975) to be a type of pentane. It is secreted mainly during the first day of larval life, and in 1969 Woyke showed that larvae which had been separated from workers for 1-2 days could be returned to the colony, which then reared them normally. Some normal worker larvae are also eaten by the workers throughout the unsealed brood stage (Woyke, 1977).

Diploid drone larvae are produced by sibling-mated queens; however, as an *A. cerana* queen cannot be inseminated sufficiently by only one drone, semen must be collected from several drones before injection into the oviducts. A queen inseminated by several drones $X_1X_2 . X_i (i = 3, 4)$ produces X_1X_i and X_2X_i virgins and X_1 and X_2 drones. When a virgin is inseminated by many brothers ($X_1X_i . X_1, X_1, X_2, X_2$) she produces about 75% heterozygous eggs (X_1X_2, X_iX_1, X_iX_2) developing into female larvae, and about 25% homozygous eggs (X_1X_1) developing into diploid drone larvae.

If the fate of *A. cerana* diploid drone larvae is similar to that of *A. mellifera*, then the survival rate of brood produced in worker cells by *A. cerana* queens mated to several brothers should be 75%. The purpose of this investigation was to find out whether and when *A. cerana* diploid drone larvae are eaten by the workers.

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Materials and Methods

Investigations commenced in Poland using six colonies headed by sibling-mated *A. cerana* queens, of the hill variety. The bees originated in Peshawar, Pakistan, and were brought to Poland from the Beekeeping Institute in Oberursel, German Federal Republic. All other investigations, using the plains variety of *A. cerana*, were conducted at the Central Bee Research Institute, Pune, India, from March to September 1974.

Of the 26 colonies studied, 22 were *A. cerana* and 4 *A. mellifera*; half of them (11 and 2 respectively) were headed by sibling-mated queens. A total of 9734 eggs were individually recorded and the survival of brood was checked several times. Of these eggs 2144 were laid by free-mated *A. cerana* queens and 6686 by the sibling-mated *A. cerana* queens.

Queens of the hill variety were inseminated once or twice, with a total volume of 1·0 to 4·2 mm^3 of semen, collected from 5-21 drones. Queens of the plains variety were inseminated twice with a total volume of 3·3 to 5·7 mm^3 of semen collected from 17-29 drones. The queens were marked, and part of one wing was clipped off. Queen excluders were attached to the entrances of all hives for the duration of the experiment. Queens mated naturally or artificially with unrelated drones were used for comparison. After egg laying commenced the survival rate of the brood was checked using the following method. A plastic strip 1 cm wide was attached to one side of a comb containing eggs of unknown age (Woyke, 1976). Each cell row was given a number, recorded on the strip, and the presence or absence of eggs or larvae in successive cells along each row was noted before the marked comb was returned to the hive. The comb was removed from the hive daily, or at 3-4 day intervals, to record remaining eggs and larvae. The eggs were distributed among cells with scattered larvae and sealed pupae.

Since the age of the eggs was unknown, individual checks had to be made for 8 days until the last larva was sealed. A more practical method, used with *A. mellifera*, of introducing an empty comb in which the queen could lay her eggs (of known age) caused much disturbance to the colony and loss of time while waiting for the eggs to be laid. Queens of *A. cerana* were more likely to lay eggs between brood than in empty combs, and the nurse bees reared the larvae more successfully in combs with pollen and honey than in empty combs. In the early experiments queens were separated for the first 3-4 days from the experimental combs by a vertical queen excluder or by a large queen-excluder cap pushed into an adjacent comb, which was removed later. In the final experiments queens were never separated from the experimental combs.

Initially each brood comb investigated was in a separate colony, but later comparisons were made by using two brood combs simultaneously in the same colony: one comb with eggs deposited by an open-mated queen, and the other with eggs from a sibling-mated queen. This almost eliminated errors in recording eggs and larvae. If on rare occasions an egg disappeared between two daily checks and was replaced by a new one, then an egg would be present in a cell for 4 or more days. If a larva disappeared, then an egg would be found at the next check. For comparison, two repetitions were made in Poland using two *A. mellifera* queens each sibling-mated to one brother.

Data on the results of instrumental insemination of *A. cerana* queens and the production of diploid drones are taken from an earlier paper (Woyke, 1979).

Results

Instrumental insemination and production of diploid drones by sibling mated queens

Table 1 shows that *A. cerana* queens of the hill variety, inseminated with less than 3 mm^3 of semen, produced some haploid drones among sealed worker brood at the end of the

TABLE 1. Characteristics of sibling-mated *Apis cerana* queens and brood.

Queen no. A	*Total vol. semen injected* (mm^3) B	*% Haploid drones among sealed worker brood* C	*Microscopical investigation % drone larvae*	
			Total D	*Probably diploids* D-C
	A. cerana hill variety			
1	1·0	high	14·3	?
2	1·0	0·8	23·1	22·3
9	3·5	0·0	23·5	23·5
17	3·0	22·0	46·7	24·7
19	3·0	9·1	19·1	10·0
43	4·2	0·0	16·7	16·7
	Mean		23·9	19·4
	A. cerana plains variety			
1/195	3·3	0·0	38·1	38·1
1/260	4·5	0·0	36·9	36·9
2/260	5·7	0·0	24·0	24·0
2/341	4·1	0·0	33·3	33·3
3/341	4·8	0·0	28·4	28·4
	Mean		32·1	32·1
	Mean for both varieties			25·8

season. This means that they were inseminated insufficiently, and laid some unfertilized eggs in worker cells.

A. cerana queens of the plains variety, inseminated with larger volumes, laid only fertilized eggs in worker cells. Microscopical investigation of the youngest larvae collected from worker cells of both varieties showed that 14·3-46·7% were drones in the hill variety, and an average of 32·1% in the plains variety. Since some queens of the hill variety produced haploid drones in worker cells, only 10-24·7% of the drone larvae could be assumed to be diploid. However, all drones found among larvae produced by the plains variety must be considered diploid. Thus all the 11 sibling-mated queens produced an overall mean of 74·2% workers and some haploid drones in worker cells and 25·8% diploid drones. The fate of these diploid drones was investigated.

Survival rate of brood in different colonies headed by sibling - and open - mated queens

The queens were separated by a queen excluder from the experimental comb. To prevent excessive disturbance, the first check for surviving larvae was made 4 days after the eggs of unknown age were recorded, and when the larvae should be 2-4 days old. Table 2*a* shows that 36-97% surviving larvae were found, but never the expected 75%.

More frequent checks were then made. Two days after egg recording, few eggs were still unhatched. Table 2*b* shows that 76-94% of survivals were found at that time. This high variation occurred even in brood originating from the same queen (2), and it could be caused in particular tests by different ages of larvae present in the cells 2 days after an egg count. Nevertheless, in most cases more than 75% of the brood survived. An average of only 44·3% of the larvae survived the 4 days after egg recording, and only 34·0% were finally sealed. Thus more larvae than expected survived for 2 days, but fewer than expected survived to the sealing stage.

TABLE 2. Survival rate of brood produced in Poland by sibling-mated *Apis cerana* queens from Pakistan.

Queen no.	Egg count		Brood surviving (%)					
	Date	No. eggs	Days after egg count					
			1	2	3	4	5	sealed
(*a*) Larvae checked after 4 days								
1	28·6	119				97		
2	12·8	400				51		
9	2·8	575				82		
19	9·8	226				36		
Total/Average		1320				66·5		
(*b*) Brood checked after 2 days								
2	13·9	350		94	71	43		30
2	16·9	146		85	69	48		42
2	28·9	178		76	54	41		28
9	12·9	130		94	69	45		36
Total/Average		804		87·3	65·8	44·3		34·0

The brood was then checked daily, in order to collect more detailed data, and the numbers of larvae of the same age were recorded. Table 3*a* shows that 91-98% of Pakistani *A. cerana* larvae survived their first day of life in the colonies in Poland. On the second day 82-83% of larvae were still alive in colony 9. Since that queen produced 23·5% diploid drone larvae and 76·5% females (Table 1), at least 5·8-6·5% of diploid drone larvae were present among those still surviving 2 days after hatching. However, Table 3 shows that on average only 52·3% of larvae were finally sealed. This low percentage was thought to be due to the lateness of the season.

Further investigations in India (Table 3*b*) gave results very similar to those obtained in Poland. More than 75% of expected survivals were found 3 days after hatching in three colonies (1/260, 2/341, 3/341), and 4 days after hatching in two colonies (2/341, 3/341). Thus at least a few per cent of the diploid drone larvae survived the first 4 days.

In two colonies the percentage of sealed brood was far below the expected 75%; 54% in 1/195 and 0% in 2/260. As no disease was detected, further experiments were conducted to investigate this low result. Comparison of survival rates of brood from three open-mated queens (Table 3*c*) showed that even more of their brood was cannibalized than of that from sibling-mated queens. Thus is was doubtful at first whether the diploid drone larvae of *A. cerana* secreted any cannibalism substance, and whether any differences existed between the survival rates of larvae originating from sibling- and open-mated queens.

To establish whether separating the queen from the brood nest influenced the care of larvae, the queens were left unrestricted. Table 3*d* shows that 94-98% of larvae then survived their first day, a higher percentage than for those of restricted queens. However, even more larvae disappeared on the following days than in the previous experiment (Table 3*b*).

Colonies headed by sibling-mated queens were not so populous as some of those with open-mated queens. However, when the survival rate of brood from sibling-mated queens was investigated in normal-size colonies (Table 3*e*), even fewer larvae survived, and none beyond the 3rd day of life. Several factors could be responsible for this: lack of pollen flow, introduction of a foreign comb, frequent disturbance of the colony, e.g. for daily counts of brood.

TABLE 3. Daily survival rate of brood produced by sibling- and open-mated *Apis cerana* queens.

Queen no.	Egg count: Date	Egg count: No. eggs	Larvae surviving (%): Days after hatching 1	2	3	4	5	sealed
(*a*) Sibling-mated queens from Pakistan separated in Poland for 3-4 days from combs investigated, by queen excluder								
2	1·10	107	94	68	39	22		20
9	12·9	222	98	83	71	62		34
9	29·9	198	95	82	79	75		75
17	1·10	65	92	92	88	85		83
19	14·10	66	91	77	61	42		20
43	20·9	302	98	88	85	84		82
Total/Mean		960	94·7	81·7	70·5	61·7		52·3
(*b*) Sibling-mated queens in India separated for 3-4 days from combs investigated, by queen excluder								
1/195	18·5	91	92	67	59	57	54	54
1/260	18·5	60	92	90	78	75	75	75
2/260	21·5	115	92	58	19	0	0	0
2/341	22·5	72	86	81	79	76	74	71
3/341	18·5	150	95	86	82	79	69	68
Total/Mean		488	91·4	76·4	63·4	57·4	54·4	54·2
(*c*) Open-mated queens separated for 3-4 days from combs investigated by queen excluder								
25/75	22·5	105	88	70	67	67	65	64
4/75	23·5	243	98	96	89	72	34	26
85	23·5	111	98	91	87	70	59	52
Total/Mean		459	94·7	85·7	81·0	69·7	52·7	47·3
(*d*) Sibling-mated queens free in hive								
3/341	24·5	226	98	89	81	60	31	29
1/260	24·5	296	98	80	71	67	61	59
1/195	24·5	265	94	70	64	57	50	49
Total/Mean		787	96·7	79·7	72·0	61·3	47·3	45·7
(*e*) Brood of sibling-mated queens transferred to normal wild colonies (1/195 in 4/75; 2/260 in 89)								
1/195	27·5	193	92	42	5	0	0	0
2/260	27·5	94	81	53	26	0	0	0
Total/Mean		287	86·5	47·5	15·5	0	0	0

Survival rate of brood from sibling- and open-mated queens, when compared in the same colony

Since wide variations were found in the results obtained from different colonies, the survival rate of brood from sibling- and open-mated queens was compared within the same colony. To reduce the strange smell of the comb removed from another colony, both experimental combs with eggs were left in the test colony for one day to familiarize the bees with it. During that time the queen was restricted by a queen excluder, but the workers had free access to the combs.

The next day the remaining unhatched eggs were recorded. To avoid disturbance, the number of surviving larvae was checked three days later, when the queen was also released. The larvae were now 2-3 days old. A second check was made when the brood was sealed. This investigation was conducted at Pune in the apiary near Mulla river, where more pollen was available than by the Institute.

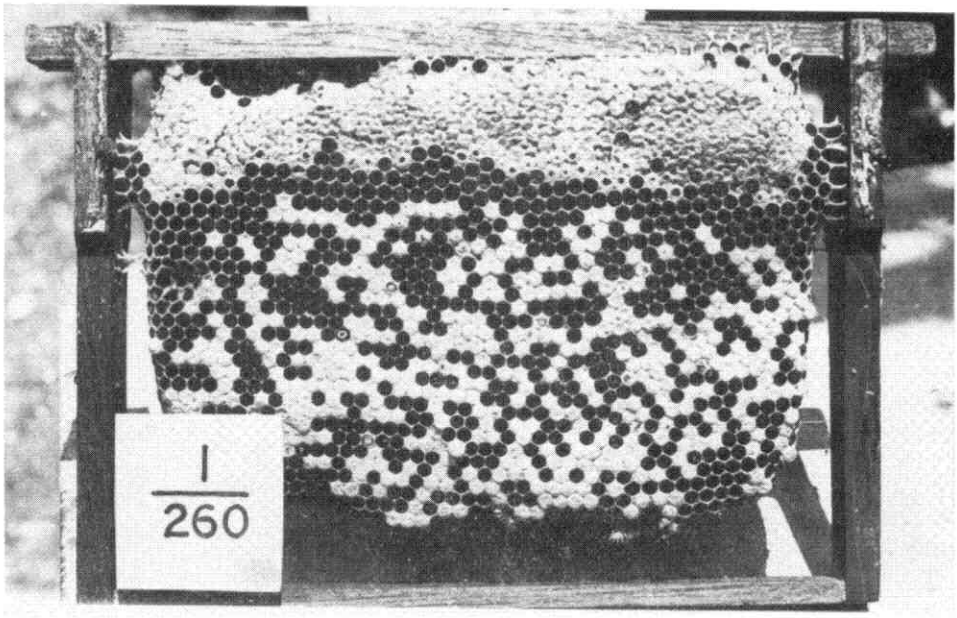


FIG. 1. Scattered brood of a sibling-mated *Apis cerana* queen.

TABLE 4. Survival rate of brood from open-mated (O) and sibling-mated (S) *Apis cerana* queens investigated in the same colony. Queen released 3 days after egg count.

Queen no.	*Colony no.*	*Egg count* *Date*	*No. eggs*	*Larvae surviving after 3 days (2-3 days old)* %	O%-S%	S/O%	*Brood sealed* %	O%-S%	S/O%
O 63	63	5·6	127	98	17	83	85	17	80
S 1/195	63	5·6	99	81			68		
O 77	77	5·6	136	95	23	76	62	13	79
S 1/260	77	5·6	167	72			49		
O 1	1	5·6	94	93	21	77	77	23	70
S 2/341	1	5·6	57	72			54		
O 106	106	5·6	100	92	6	93	8	−3	138
S 3/341	106	5·6	176	86			11		
O 77	13	8·6	184	96	25	74	0	0	—
S 2/260	13	8·6	255	71			0		
O 16	96	31·8	216	99	26	74	95	32	66
S 1/260	96	31·8	116	73			63		
Total/Average O			857	95·5	19·7	79·5	54·5	13·7	72·2
S			870	75·8			40·8		

Table 4 shows that the survival rate of brood 3 days after the egg count was lower in larvae from sibling-mated queens than in those from open-mated queens. However, larvae originating from open-mated queens disappeared also. The difference between average percentages of larvae surviving from both groups of queens was only 19·7%, not 25% as expected. This means that nurse bees did not eat the 25% of diploid drone larvae in addition to the percentage of worker larvae eaten for other reasons, but ate a few per cent of diploid drone larvae in place of some of the worker larvae. The proportion between the number of surviving larvae originating from sibling-mated queens and the number of those surviving from open-mated queens was almost 75% in four tests, and higher in one other. Probably some diploid drone larvae survived for 3 days or more after the egg count.

Table 4 shows that many larvae originating from both groups of queens were eaten throughout the unsealed stage. The difference between sealed survivals was mostly lower than 25%. However, the survival rate of brood from sibling-mated queens in relation to that of sealed brood from open-mated queens was close to 75% in four tests, as was the average. This suggests that the nurse bees had eaten the larvae originating from sibling-mated queens for two reasons. (*a*) Due to environmental conditions the total percentage of worker and diploid drone larvae consumed was similar to that of worker larvae originating from open-mated queens. (*b*) Additionally they ate some diploid drone larvae among those that remained. However, the proportion of diploid drone larvae available for eating was no longer 25% of those hatched, but at its maximum 25% of those surviving.

Therefore the difference between survival rates of larvae originating from the two types of queens was usually lower than 25%, but the ratio between the two survival rates was about 75%. The difference of 32% sealed survivals found in the last test (Table 4, O-S) may be explained by production of an even higher percentage of diploid drones (36·9%) by the sibling-mated queen 1/260 (Table 1).

When the homozygous larvae disappear

In order to discover the exact age at which the diploid drone larvae were eaten, a daily comparison of the survival rates for both types of larvae was necessary. To increase the population of the test colonies, several brood combs with emerging workers had been added a few days earlier. Table 5 shows that the larvae in colonies 2/17 and 96 were eaten so rapidly that there were no survivors after the 3rd or 4th day of life. Larvae in colony 2/17 from an open-mated queen were eaten much more quickly than those from the sibling-mated queen. It was not possible in such unfavourable conditions to discover which part of the brood was eaten due to homozygosis at the sex locus. Both repetitions showed that in unfavourable conditions, when the workers eat many normal larvae, they do not eat additional homozygous larvae, but homozygous instead of normal. This accounts for the fact that the difference is much lower than was expected, or even zero (Fig. 2*a*).

The low rate of brood rearing could be caused by the diminishing pollen flow. However, the main reason was the effect of the added combs with emerging workers, and was contrary to what was expected. The queens deposited eggs in all empty cells from which adults had emerged. Consequently an increased number of larvae hatched, which the nurse bees were not able to feed.

Table 5 shows that colony 85, supplied with extra pollen combs, reared more larvae. No significant difference was found between the survival rates for the first day of larval life of brood from open- and sibling-mated queens. More larvae were eaten from sibling- than from open-mated queens on the 2nd and 3rd day of larval life. Since the differences

TABLE 5. Survival rate of brood of open-mated (O) and sibling-mated (S) *Apis cerana* queens. Poor pollen flow. Queens free, daily checks.

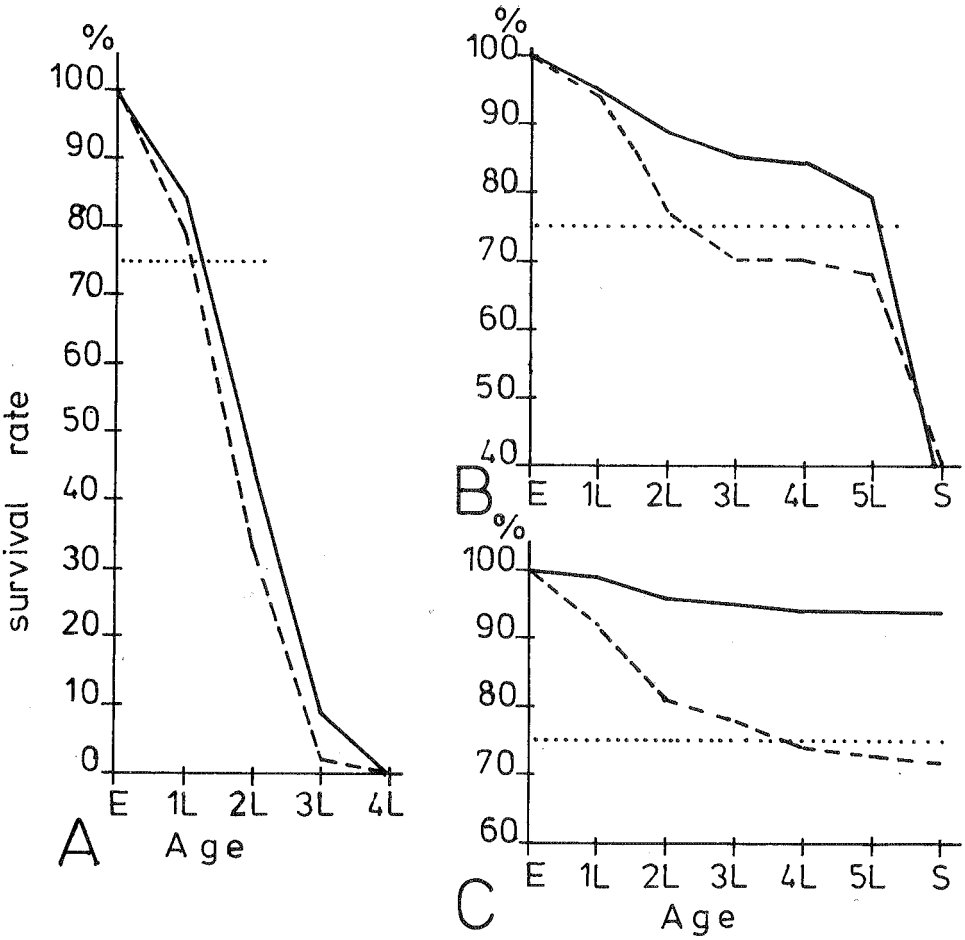
Queen no.	*Colony no.*	*Date*	*No. eggs*	*Brood surviving (%) Days after hatching* 1	2	3	4	5	*sealed*
Supplied with combs of brood									
O 1/ 17	2/17	10·6	132	85	54	17	—	—	—
S 2/341	2/17	10·6	142	87	54	25	2	—	—
O 85	96	10·6	111	84	44	9	—	—	—
S 1/260	96	10·6	104	79	33	2	—	—	—
S 3/341	3/341	11·6	136	96	69	47	2	—	—
Supplied with pollen combs									
O 1	85	10·6	141	95	89	85	84	79	38
S 2/260	85	10·6	188	94	77	70	70	68	40
Difference O-S				1	12	15	14	11	−2
S/O%				99	87	82	83	86	105
S 1/260	1/260	11·6	179	96	83	73	71	55	41

between survivals from both groups of queens were lower than 25%, and one was more than 75% of the other, it must be concluded that diploid drone larvae were eaten in place of worker larvae.

Later so many larvae of both types were eaten (just before sealing) that no difference was apparent after sealing (Fig. 2*b*). More than 75% of the larvae were still present on the second day of larval life in colony 1/260 headed by a sibling-mated queen. This means that some diploid drone larvae had survived although the pollen supply was not optimal. The monsoon arrived during the second half of June, and the pollen supply was so poor that the bees stopped rearing brood. Further work was commenced at the end of September when the monsoon finished. Table 6*a* shows that colony 1/260 headed by a sibling-mated queen was still small at the beginning of September, and the workers did not collect much pollen. Of larvae in the foreign combs, more from the open-mated queen were eaten than from the sibling-mated queen. As the conditions improved pollen became readily available, and the colonies were fed extra syrup. The largest colonies prepared to swarm, and the queens laid eggs into queen cups.

Table 6*b* shows that 96% of larvae from open-mated queens survived their first day of larval life under these conditions in colony 96, and as many as 94% were finally sealed. Larvae from the sibling-mated queen survived the first day of life in the same percentage as those from the open-mated queen. The difference in survival between the two groups appeared on the second day of larval life and increased until the fifth.

To provide optimal conditions for the final test, a colony headed by sibling-mated queen No. 3/341 was specially prepared. Access to the brood nest was restricted with queen excluder, so that the free queen could not lay many eggs and the nurse bees did not have many larvae to feed. A foreign comb with eggs from an open-mated queen was added to the nest with the free queen. Table 6*c* shows that 99% of larvae from the open-mated queen survived the first day of life and 94% were sealed. However, 81% of larvae from the sibling-mated queen survived the second day. The differences between survival rates of larvae from both groups of queens increased gradually until the time of sealing (Fig. 2*c*). Table 6 shows that the sums of percentages of sealed worker larvae from sibling-mated queens and of diploid drone larvae detected on prepared slides (67 + 37, 63+ 38, and 72 + 28) were very close to 100%. This indicates that, here, practically only diploid drone larvae were eaten. If so, some diploid drone larvae must have been present in the comb cells until the 4th or even the 5th day of life.



2. Survival rate of brood of wild (solid line) and sibling-mated (broken line) *A. cerana* queens in different pollen conditions:
A—poor (Table 5, colony 96, 10 June)
B—average (Table 5, colony 85, 10 June)
C—good (Table 6, colony 3/341, 7 September).
Ordinate: survival rate of brood
Abscissa: age of brood in days: E—eggs, L—larvae, S—sealed brood.
Dotted line: expected 75% survival rate.

Rearing of diploid drones to the adult stage in *Apis cerana* colonies

During the very heavy pollen flow, a few cells with sealed drone brood appeared among scattered worker brood in colonies headed by sibling-mated queens. The cells were sealed with domed cappings, and drone pupae (determined by the eyes) were found after opening the cells; adult drones emerged from other domed cells. The author did not have much experience with instrumentally inseminated *Apis cerana* queens, and the production of diploid drone larvae in this species. He supposed that the reserve of spermatozoa in the spermatheca of such queens was exhausted. After the pollen flow decreased, no more drone brood was found among worker brood, despite efforts to repeat this phenomenon. Other queens inseminated by the same method, and then killed and examined (Woyke,

TABLE 6. Survival rate of brood from open-mated (O) and sibling-mated (S) *Apis cerana* queens. Good pollen flow. Queens free.

Queen no.	*Colony no.*	*Date*	*No. eggs*	% *Larvae surviving Days after hatching*						% *Diploid drones on microscope slides*
				1	2	3	4	5	*sealed*	
(*a*) Small colony collecting little pollen										
O 13	1/260	2.9	135	87	74	67	64	56	55	
S 1/260	1/260	2.9	210	94	79	70	69	68	67	37
O-S difference				–7	–5	–3	–5	–12	–8	
(*b*) Colony preparing to swarm										
O 16	96	6.9	128	96	96	95	95	95	94	
S 1/195	96	6.9	108	96	71	68	66	63	63	38
O-S difference				0	25	27	29	32	31	
S/O%				100	74	72	69	66	67	
(*c*) Brood nest restricted with queen excluder										
O 4	3/341	7.9	181	99	96	95	94	94	94	
S 3/341	3/341	7.9	103	92	81	78	74	73	72	28
O-S difference				7	15	17	20	21	22	
S/O%				93	84	82	79	78	77	
(*d*) *Apis mellifera* in Poland										
O 369	79	2.7	238	97	96	95	95	93	93	
S 313	79	2.7	134	56	53	53	53	53	53	—
O-S difference				41	43	42	42	40	40	
S/O%				58	55	56	56	57	57	
O 371	99	2.7	323	99	99	99	99	99	99	
S 322	99	2.7	209	58	51	51	50	50	50	—
O-S difference				41	48	48	49	49	49	
S/O%				59	52	52	51	51	51	

1975), had even more spermatozoa in the spermatheca than naturally mated queens. Also, no more drone brood was found later among worker brood. This phenomenon could therefore not be caused by shortage of stored spermatozoa in the queen's spermatheca. It may be suggested that *Apis cerana* colonies probably rear diploid drones to the adult stage when good nectar and pollen flows are available.

Daily survival rate of *Apis mellifera* larvae

To compare the above results with the survival rate in *A. mellifera*, identical investigations were conducted at the same time with open-mated queens and queens instrumentally inseminated from one brother each. In the latter, a survival rate of 50% was expected. Table 6*d* shows a very sharp decrease in survival during the first day of larval life. Only a few more larvae survived than the expected 50%.

Larvae less than a few hours old, which are not normally eaten by the worker bees, could still be present among surviving larvae 1 day old. However, from the second day of larval life practically no change in survival occurred until the time of sealing. This confirmed the conclusion that *A. mellifera* diploid drone larvae are eaten by the nurse bees at a much younger age than are those of *A. cerana*.

Discussion and Conclusions

Inefficient brood rearing by *Apis cerana* nurse bees (Woyke, 1976) prevented us from detecting any decrease in the survival rate of larvae caused by homozygosis at the sex

locus. Comparison of survival rates of two groups of brood, originating from sibling-mated and open-mated queens, must be conducted in the same colony. In unfavourable environmental conditions the nurse bees ate some of the diploid drone larvae, but instead of eating worker larvae. Therefore the lower survival of brood homozygous at the sex locus was almost undetectable during the poor pollen flow (Fig. 2*a*); it could be detected on the third day of larval life, but not later, when a minor pollen flow occurred (Fig. 2*b*), and was detectable from the third day up to the time of sealing during a good pollen flow (Fig. 2c).

All the results showed that the diploid drone larvae of *Apis cerana* were not eaten by nurse bees during the first day of larval life, as in *A. mellifera.* Many are eaten in their second day of life, but some are reared until their fourth day and in favourable conditions, a few probably reach the adult stage. This suggests that *A. cerana* diploid drone larvae secrete little or no cannibalism substance during the first day of life, but afterwards secrete it for much longer period than do the diploid drone larvae of *A. mellifera.* This is another difference between the two species of *Apis*. One can imagine that, in an early evolutionary development stage, both diploid sexes were reared till the imago. After a mutation occurred which caused elimination of unproductive diploid drones, colonies with such a mutation survived in competition with others. Rearing of undesirable larvae by *A. cerana* for a longer period than by *A. mellifera* is a waste of energy, and it may indicate an earlier stage of evolutionary progress. Many *A. mellifera* subspecies living in hard environmental conditions conserve energy much more effectively by eating diploid drones at their earliest development stage. Perhaps this was one of the factors which allowed *A. mellifera* subspecies to spread over such a vast territory of the earth's land masses.

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