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DO HONEYBEES EAT DIPLOID DRONE LARVAE BECAUSE THEY ARE IN WORKER CELLS?*

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SUMMARY

A total of 457 diploid larvae from eggs laid in worker cells by inbred queens were hatched in an incubator. They were then transferred into drone cells and put into queenless colonies, with a control group of 75 haploid drone larvae. Half the diploid larvae were eaten during the first few hours after transference, leaving 48% surviving until the next day, compared with 92% haploids. The bees sealed 65% of the cells containing haploids and 19% with diploids, of which 2·8% were drones. After emergence one drone was found to be diploid.

Since diploid drone larvae are eaten whether they are in drone or worker cells, the controlling factor is not that they are in the 'wrong' type of cell for their sex.

INTRODUCTION

Diploid drone larvae from inbred crosses of *Apis mellifera* L., which develop from eggs laid in worker cells (Woyke, 1963*a*, 1965*a*), are viable (1963*c*, 1965*b*), but are eaten by the worker bees shortly after hatching (1963*b*).

Mackensen (1951), in a test for biparental males, also tried to rear these drones in drone cells. But because the queens were forced to lay eggs directly, the proportions of fertilized and unfertilized eggs they laid were not known; thus the phenomenon of eating the diploid drone larvae (which at this time were not known to hatch) could not be investigated, nor was this Mackensen's intention.

In an attempt to rear and maintain diploid drones in a colony, and to investigate the possibility that they are eaten because they are reared in worker (instead of drone) cells, the following study was undertaken in 1962 and 1964.

MATERIAL AND METHODS

Five queens were used, three of which produced low-survival brood. Experiments were made with 532 larvae; 457 originated from inbred queens, and were transferred from worker to drone cells for further rearing in 6 different colonies.

After individual sibling mating, and testing for two-allele fraternity (Woyke, 1963*a*), the queens producing low-survival brood were chosen. One was homo-

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zygous for cordovan (*cd*) and one for chartreuse (*ch*) genes; both were inseminated with a wild brother (+). Because the two mutant genes *cd, ch* are recessive to wild, haploid offspring could be easily distinguished from diploid. The third queen was heterozygous, so haploid and diploid progeny of the same sex could not be distinguished. One of the homozygous queens (581) laid occasional unfertilized eggs in worker cells as well as fertilized ones. A control group included two wild queens producing normal brood.

Worker comb containing eggs from the inbred queens was put in a special box with water (or wrapped in a damp towel) and placed in an incubator. It was checked every 3 hours, and all hatched larvae not yet lying on bee milk were transferred on bee milk in drone cells from which haploid drone larvae of similar age had been removed. The larvae were transferred directly from one comb to the other, except in series 8 and 8*a* where the larvae were first grafted on royal jelly in queen cells and left for 11 hours in a dessicator in an incubator.

In the first series of experiments the control group consisted of the other 50% of diploid larvae i.e. the females. In the next series a group of haploid drone larvae was included. It proved very difficult to get diploid and haploid larvae from the same inbred queen, hatching at the same time. So in later experiments controls were haploid drone larvae originating from a wild queen, and in the final series (11*a*) larvae which were not transferred but had hatched in the drone comb in a colony also headed by a wild queen.

In order to locate individual larvae after transference, a piece of cardboard with a rectangular hole was used, and the row and cell numbers were noted. The pin holes in the frame showed exactly where the cardboard had been placed.

So that the transferred larvae should not be the only item foreign to the rearing colony, they were transferred to a drone comb taken from a different colony. The only exceptions were series 1 and 2 (own comb) and 10, 11*a* (comb in the rearing colony 2, 3 days earlier). These drone combs were deliberately taken

TABLE 1. Percentage survival of young low-survival larvae at different times after transference to drone cells in a colony

Series No.	*No. larvae transferred*	*3–4 hr.*	*6 hr.*	*9 hr.*	*Next day*
1.1	14	57	—	57	50
1.2	22	—	59	—	55
3.1	24	79	—	—	50
3.2	12	83	—	—	58
3·3	11	—	55	—	46
5.1	18	56	—	—	39
6.1	22	—	59	—	5
6.2	29	—	—	41	46
6.3	14	64	—	—	43
6.4	23	—	—	52	52
Total after:					
3 hr.	82	68	—	—	48
6 hr.	55	—	58	—	33
9 hr.	66	—	—	49	47
1 day	189	—	—	—	43

from colonies producing haploid drones phenotypically different from those being transferred.

The combs with transferred larvae were put into a queenless colony. To determine survival rates, larvae were counted several times the next day, and at the time of sealing; the capping (flat or convex) then identified the sex.

Before emergence, the cells were screened so that the adults could be caught and investigated.

RESULTS AND DISCUSSION

The age at which young diploid larvae in drone cells are eaten

Table 1 shows that about 60 – 80% of brood survived 3 – 4 hours after transference. By 9 hours less than 60% survived, but relatively few further larvae had been eaten by the next day, when 43% still survived, or 48% if series 6.1 is omitted. Thus the ages at which the larvae are eaten in drone cells and in worker cells (Woyke, 1962) are very similar.

The 24-hour survival rate and its variation

Results for both years (Table 2) show that about 50% of transferred diploid

TABLE 2. Results of transferring low-survival and haploid larvae (hatched in an incubator) into drone cells

Series No.	*Rearing colony*	*Queen No.*	*Genotype of parents*	*Cell in which larvae hatched**	*No. larvae grafted*	*% survived next day*	*% capped*	*% drones*	*No. drones reared*	*Phenotype of drones reared*
				1962						
1	77	581	*cd*/*cd* × +	W	72	63	35	1	1	*cd*
2	77	581	*cd*/*cd* × +	W	22	64	45	5	1	*cd*
3	80	581	*cd*/*cd* × +	W	60	47	23	5	3	all *cd*
4	80	581	*cd*/*cd* × +	W	20	45	35	0	0	—
5	80	581	*cd*/*cd* × +	W	38	42	21	11	4	all *cd*
6	85	581	*cd*/*cd* × +	W	88	35	0	—	—	—
	Total 1962			W	300	48	21	3	9	
				1964						
7	6	753	*ch*/*ch* × +	W	34	53	0	—	—	—
7*a*	6	753	*ch*/*ch* × +	D	27	100	50	50	11	all *ch*
8	71	753	*ch*/*ch* × +	W	50	58	6	4	2	*bk* & +
8*a*	71	75	+/+ × +	D	17	65	41	41	7	all +
9	91	854	*cd*/+ × *cd*	W	26	15	0	—	—	—
10	91	854	*cd*/+ × *cd*	W	22	59	36	0	0	—
11	91	854	*cd*/+ × *cd*	W	25	44	44	8	2	both +
11*a*	91	87	+/+ × +	D	31	100	100	100	31	all +
	Total 1964			W	157	47	14	2·5	4	
				D	75	92	65	65	49	
	Total 1962 + 1964			W	457	48	19	2·8	13	

cd = cordovan; *ch* = chartreuse; + = wild (black)

* W denotes that the larvae came from worker cells, D from drone cells

larvae survived until the next day. Although in 1962 all larvae originated from the same queen, some variation in survival is apparent. This may be due to difference in rearing colonies, but the highest survival rate was in series 1 and 2, representing larvae transferred to a drone comb originating from the rearing colony itself. The same phenomenon occurred in 1964 in series 9 – 11. Series 9 was transferred to a foreign comb which resulted in very low survival rate; series 10 and 11 were transferred to the same comb after it had already been 2 and 3 days in the rearing colony.

These results indicate that the worker bees eat the larvae in foreign comb for some reason other than low-survival rate (smell, different region of the brood area, etc.); better results, with higher survival rate, can be obtained with combs that have already been in the rearing colony for a few days.

Of the haploid drone larvae in the control groups 100% survived in two series, one of which consisted of transferred larvae (7*a*) and the other not (11*a*). In one series (8*a*) only 65% survived, which was however more than in the other part of this series (8) consisting of diploid larvae.

A total of 219 out of 457 diploid larvae transferred to drone cells survived until the next day (48%). About twice as many larvae in the control groups of haploid drone larvae survived (92%). It is doubtful, however, whether the final series (11*a*) with untransferred larvae is comparable.

Larvae surviving until they were sealed

The method used did not allow determination of the sex of the surviving larvae until they were sealed; the shape of the capping then showed that 41 – 100% of the haploid larvae survived (Table 2), (larvae in series 11*a* not being transferred). On the average 65% of the haploid larvae were capped; this phenomenon of disappearance of some of the haploid larvae is already known.

Of the low-survival brood 0 – 45% was capped, an average of 19% of the larvae originating from worker cells being sealed in drone cells. Altogether the survival was much less than that of haploid brood. The very low survival rate in some series (0 – 6%) may be due to factors not investigated here. Most commonly 30 – 40% of these larvae were capped.

The most important question is *which* of the diploid larvae survived. Most of the cells containing larvae originating from worker cells were sealed flat, indicating that workers were inside, but 2·8% of the cells to which the low-survival larvae had been transferred (457 in all) were sealed with convex cappings, indicating the presence of drones.

Type of adult drone originating from eggs laid in worker cells and emerging from drone cells

All 9 drones emerging in 1962 were cordovan. Since the mother (581) was homozygous cordovan and mated to a wild drone, these 9 drones must have developed from unfertilized eggs. This queen also laid some unfertilized eggs in worker comb.

Four of the drones reared in 1964 are of special interest. Drone A, reared in

series 8, was brick; since the mother (753) was homozygous chartreuse mated to black, the drone could not have developed from either an unfertilized or a fertilized egg laid by this queen. It could have developed only from one of the larvae which should have been removed before the low-survival larvae were transferred; these larvae were brick-eyed. A mistake was made here, which thanks to the method used was brought to light.

Drone B was from the same queen and series. It was wild, and so could not have been the result of a mistake (drone A was brick). The mother was homozygous chartreuse mated to a wild drone, so drone B must have developed from a fertilized egg. This was confirmed from the structure of reproductive organs, which was different from that in haploids and identical with that in diploid drones reared by us later. Other genetic characters indicated also that the drone was really diploid and not a result of androgenesis.

Drones C and D, reared in series 11, were wild; since the mother (854) was heterozygous it was not possible to state genetically if they developed from fertilized or unfertilized eggs. But the records showed that two larvae were found in one cell the day after transferring, and that drone C emerged from this cell. Perhaps the haploid larva had not been properly removed before the diploid one was inserted, or perhaps only one larva was removed out of two haploids hatched in this cell; this can happen. The structure of reproductive organs of drones C and D showed them to be haploids. The drone D was probably a result of an incomplete insemination of the queen with the semen of one drone only.

Thus only one drone of those reared was diploid.

Why a single diploid drone survived in the colony

The question arises why a diploid drone developed in only one out of all the series, and why in series 8. There was in fact one distinct difference between this series and the others: the larvae were first transferred on royal jelly into queen cells and then left for 11 hours in an incubator. Thus when the larvae were transferred to the colony, the oldest were 14 hours old, and it may be that one of the oldest diploid drone larvae survived on royal jelly in the incubator long enough to pass the critical period of being eaten by the workers in the hive.

CONCLUSIONS

No diploid drone survived in any series in which the larvae were transferred from worker comb directly to drone comb immediately after hatching. Results are presented on: (1) the time young diploid larvae are eaten; (2) the 24-hour survival rate (50%, most being females); (3) the sex (female) of capped and emerged bees indicate that diploid drone larvae in drone cells are eaten by the bees, just as those in worker cells.

We can therefore conclude that diploid drone larvae are eaten by the bees irrespective of whether the larvae are in drone or worker cells: the 'correctness' of the cell does not affect the issue.

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