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REARING DIPLOID DRONE LARVAE IN QUEEN CELLS IN A COLONY*

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SUMMARY

Investigations were made on 586 larvae of high and low survival rates; 363 of the latter were hatched in an incubator and transferred on royal jelly in queen cells in several rearing colonies. Of these, 55% reached the age of 5 days, giving 55% females and 45% diploid males. The efficiency of rearing young larvae was raised after it was found that the workers would rear several female larvae in one queen cell for several days; this was also possible with diploid drone larvae. Although no adult diploid drones were obtained, for the first time many diploid drone larvae were reared *in colonies*, at least to the time of sealing.

The fact that the larvae are on royal jelly in queen cells prevents their being eaten, and this suggests that some special substance governs the eating phenomenon. The results obtained make it possible to develop a method of rearing imago diploid drones in the colony.

INTRODUCTION

Previous investigations by the author have shown that in colonies of the honeybee (*Apis mellifera*) diploid drone larvae (1963*a*, 1965*a*) in either worker (1963 *b*) or drone cells (1965*c*) are eaten by the workers within a few hours of hatching. Diploid drones can be reared in an incubator to the imago (1963*c*), but only with great difficulty; similar difficulties were found with rearing haploid drones in an incubator (1965*b*).

An attempt has therefore been made to rear diploid drones in queen cells in the colony.

MATERIALS AND METHODS

Of the 586 larvae used, 223 were offspring of naturally mated queens and 363 were from 7 queens whose brood in worker cells included 50% diploid drone larvae that were eaten by the worker bees. Nine different rearing colonies were used.

The inbred queens were produced, and the survival rate of their brood tested, by methods already described (1963*a*).

First a test was made of the degree of rearing success obtainable by transferring normal females and males to queen cells. Eight normal larvae of each sex, about 1 day old, were transferred to queens cells from which the larva had been removed (double grafting). One larva was put into each queen cell. The number of survivals was noted each day until sealing.

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Then 100 larvae 1 day old originating from a partially drone-laying queen were used. The sex of survivals in queen cells was determined at 4 – 5 days.

In the next experiment 265 larvae of low-survival rate were hatched in an incubator and similarly transferred to queen cells from which the queen larvae 1 – 2 days old had been removed. The queen cells were then returned to the rearing colony, whose combs contained either larvae of all ages, or only sealed brood. In one series (7) the low-survival larvae were transferred to emergency queen cells built on drone comb. In the preliminary tests (series 1 – 6) the number of survivals was checked every few days. In the second year's tests (series 7 – 14) it was checked every day, and the sex of larvae was determined when they were 4 – 5 days old. All the queen cells were caged shortly before the adults emerged.

Attempts were then made to rear more than one larvae per queen cell, in the colony. At first 107 female larvae from two naturally mated queens were transferred, 4 or 7 per queen cell, to two queenless colonies, by double grafting. The numbers of live larvae that survived were recorded daily.

The next investigation was conducted in a similar way. But here 98 larvae originating from three queens producing 50% diploid drone brood were hatched in an incubator, and three or four larvae were grafted into each queen cell and given to two queenless colonies. The sex of the larvae that survived was determined four days after grafting. The sealed queen cells were caged and treated as given above. Three queen cells in which the diploid males were capped were dissolved in warm xylene, to discover whether there was a cocoon.

RESULTS

Rearing normal females and males in queen cells

Of the 8 female and 8 male larvae grafted, only 3 of each were lost before sealing; 5 females and 5 males were sealed. Unfortunately the number and sex of the imagines that emerged were not noted.

When 100 larvae from a partially drone-laying queen were used, determination of their sex showed that many drone larvae had been accepted. But the results were similar to those obtained by Vuillaume (1957) and Naulleau (1962): the number of drone larvae in queen cells decreased 4 days after grafting, and n adult drones emerged from the capped cells. Nevertheless the workers did accept and rear male larvae in queen cells, in the presence of female larvae in other queen cells.

Rearing larvae of low-survival rate in the colony

Results of rearing brood of low survival rate in queen cells are given in Table 1. In series 1 – 3, 45% of the grafted larvae survived after 4 days. In series 4 – 6 this low percentage of survival was reached one day after grafting, and only 21% of grafted larvae survived after 5 days. All the bees emerging from the cells in series 1 – 6 were queens. The low percentage of survivals after 1, 4 and 5 days, and the sex of the imagines, suggested that the diploid drone larvae were being eaten shortly after hatching, as they are in worker and drone cells. But the percentage of imagines that emerged was generally lower than the percentage

surviving to 4 – 5 days, so although the sex of these was not determined, some of them may have been diploid drones.

In all the next series (7 – 14) the number of survivals was noted each day; the greatest losses occurred the first day after grafting.

Two pairs of parallel series (9 – 10 and 11 – 12), all run at the same time, differed only in the presence (9, 11) or absence (10, 12) of unsealed brood in the rearing colonies. They showed that the survival rate in queen cells was higher when the colonies had only sealed brood.

The most important result, however, is that altogether more than 50% of the larvae survived beyond the fifth day of rearing; in two series even 82% and

TABLE 1. Results of rearing brood of low survival rate (hatched in an incubator) in queen cells in a colony. *F* = female; *M* = male

Series No.	*No. larvae grafted*	*Percentage survival on successive days*							*Imagines emerged*	
		1	2	3	4	5				
						Total	*F*	*M*	*%*	*No. Sex*
1	11				36		—	—	9	1 F
2	15				40		—	—	33	5 F
3	14				57		—	—	36	5 F
Total 1-3	40				45					
4	25	72				40	—	—	32	8 F
5	25	36				16	—	—	16	4 F
6	25	32				8	—	—	4	1 F
Total 4-6	75	47				21				
7	11	45	45	45	45	45	45	0	27	3 F
8	20	100	100	95	95	90	35	55	35	7 F
9	17	53	47	47	47	47	—	—	no drones	
10	17	71	71	65	65	54	—	—	no drones	
11	23	30	26	26	26	26	17	9	17	4 F
12	22	45	45	41	41	41	18	23	18	3 F
13	17	82	82	82	82	82	59	23	59	10 F
14	23	78	65	61	61	57	30	27	9	2 F
Total 7-14	150	63	60	57	57	55	31	25		

90% did so. In all but one of the series (7) in which the sex was determined, males as well as females were found after 5 days of rearing. (In series 7, where emergency queen cells built on drone comb were used, no males were found in them.) In all, 36 females (55%) and 29 males (45%) were found among the 65 larvae that were sexed. Thus, out of the 116 larvae originally grafted in these series, 31% known to be female and 25% known to be male reached the age of 5 days. (The sum of these percentages differs from the 55% of survivals, because of the two series in which the sex was not determined.)

The high percentage of males found, differing little from that of females, shows that at least a large part of these males must have been diploid.

Most queen larvae were sealed 5 days after grafting. Some of the drone larvae

disappeared within the first few days; some fell down and were seen to be eaten by the workers. Some others slid down the side of the queen cell; the bees lengthened some of these cells and sealed them; in others the larvae were observed partly eaten. But many of the drone larvae were sealed normally, 6-7 days after grafting. These capped cells containing (diploid) drone larvae did not differ from those containing queen larvae on the first day, but on the second day they were surrounded with burr comb in some colonies, whereas cells containing queen cells were not. Queen cells containing diploid drones were therefore easily distinguishable from those containing queens.

Before the queens started to emerge, the queen cells containing drones were opened by the bees from the distal end. In one colony all queen cells were caged 7 days after grafting, but no adult drones emerged. To discourage the workers from destroying queen cells containing drones, all sealed cells containing queens were removed from the colony in series 14; only unsealed queen cells with drones and two unsealed with queens, were left. The former were not opened until 10 days after grafting. By this time an overlooked emergency queen had emerged, and the bees destroyed all the queen cells—the two containing queen larvae being opened from the side and those containing drones from the distal end. When three of the latter were dissolved in xylene, it was found that no cocoons had been spun in them. It seemed that whereas the bees removed the tip of the capped queen cells in the usual way, cells without a cocoon were left open.

TABLE 2. Rearing several female larvae per queen cell in a colony

No. days after grafting	*No. larvae per queen cell*							*Total numbers*		
	7	6	4	3	2	1	0	*No. queen cells with larvae*	*Larvae*	
	No. queen cells containing this number of larvae								*No.*	*%*
					Experiment I					
0			11	—	—	—	—	11	44	100
1			5	3	3	—	—	11	35	80
2			5	2	3	1	—	11	33	75
3			1	3	5	1	1	10	24	55
4			—	1	5	2	3	8	15	34
5			—	—	1	3	7	4	5	11
					Experiment II					
0	1	—	14	—	—	—	—	15	63	100
1	1	—	8	4	1	1	—	15	54	86
2	—	1	3	6	4	—	1	14	44	70
3	—	—	3	6	4	1	1	14	39	62
4	—	—	—	—	2	7	6	9	11	17
5	—	—	—	—	—	6	9	6	6	10

The results do not entirely exclude the possibility of rearing diploid drones in queen cells, but they do show that it is not an efficient method for producing adult diploid drones. Nevertheless these drones can be reared longer in queen cells than in worker or drone cells.

Rearing several larvae in one queen cell

The results above simplify the procedure of rearing the diploid drones during the first few days of their life. But this method was inadequate even for producing larvae, since in the colony only one was reared per cell, whereas in the incubator it became possible to rear 8 or more.

When only female larvae were grafted (4, or on one occasion 7, per queen cell) the majority of queen cells contained the full complement of larvae on the day after grafting; the number decreased slowly on the next day (Table 2). Only 2 or 3 larvae remained in most cells on the third day and the number decreased rapidly thereafter.

It was observed that 2 days after grafting the bees put royal jelly only on the walls of queen cells containing four regularly spaced larvae, leaving the centre of the cell unsupplied with fresh food. This did not happen when the larvae were irregularly placed, or when fewer than 4 larvae survived. During subsequent days, some of the larvae slipped down the wall of the cells, and only the larva located most centrally remained on the royal jelly at the base of the queen cell. Larvae

TABLE 3. Results of rearing larvae of low survival rate 3 and 4 per queen cell in a colony

Days after grafting	*No. larvae per queen cell*					*Total no. queen cells with larvae*	*No. larvae alive in cells*		*Total no. larvae alive*	
	4	3	2	1	0		*with 4 grafted*	*with 3 grafted*	*No.*	*%*
	No. queen cells containing this number of larvae									
					Experiment I					
0	7	7	—	—	—	14	28	21	49	100
1	3	10	1	—	—	14	24	20	44	90
2	2	10	2	—	—	14	23	19	42	86
3	1	7	4	2	—	14	17	18	35	71
4	—	4	7	2	1	13	12	16	28	57
					Experiment II					
0	7	7	—	—	—	14	28	21	49	100
1	4	6	4	—	—	14	24	18	42	86
2	1	5	8	—	—	14	18	17	35	71
3	—	4	8	2	—	14	15	15	30	61
4	—	1	8	5	—	14	13	11	24	49

which fell down were eaten by the workers. Unless larvae were replaced in their regular positions after each inspection, only one would usually remain in the cell at the time of sealing.

Of 107 grafted larvae, 83%, 72% and 59% survived on subsequent days, and the percentage then decreased rapidly. But the 63 larvae surviving three days, which were grafted in 26 queen cells, give an average of more than 2 per cell. So it was worth while to graft and rear several larvae per queen cell.

Rearing larvae of low survival rate, several per queen cell, in the colony

The previous section shows that, 3 days after grafting, more queen cells contained 3 larvae than contained 4. So in experiments with larvae of low survival rate, the efficiency of rearing 4 or 3 larvae per queen cell was also investigated.

Out of a total of 98 grafted larvae, 88%, 79%, 66% and 53% survived on subsequent days (Table 3). These percentages are similar to those for females with normal survival, or even higher. (The higher percentage may be partly due to grafting only 3 larvae per cell in half the cells.) Table 3 also shows that the day after grafting more larvae can be obtained from the same number of cells by grafting 4 per cell than by grafting 3. Two days after grafting only a few more larvae were obtained in these cells, but by 3 days it did not affect the survival whether 3 or 4 larvae had been grafted per cell. *Relatively* more larvae survived in queen cells with 3 grafted.

Of all 98 larvae, 65 and 52 reached the age of 3 and 4 days respectively; by 4 days the average was about 2 per cell (52 in 28 cells).

When the sex of these larvae was determined, 6 drones were found in the first experiment and 5 in the second, indicating much less than 50% survival. The queen larvae grow much more rapidly than drones at 2-3 days of age. So when drone and queen larvae are together in the same cell, the drone larvae are at a disadvantage. It can be assumed that more diploid drones survive for the first two days after grafting than later on.

After four days of rearing, by removing the females or transferring the males, each cell was left with only one drone larva in it. No adult diploid drones were reared in these queen cells.

CONCLUSIONS

By no means all diploid drone larvae grafted on royal jelly in queen cells are eaten by the workers in the colony, and they can be reared at least to the time of sealing. For the first few days, several larvae can be reared per queen cell by the workers in a colony.

The fact that the larvae are on royal jelly in queen cells prevents their being eaten. This suggests that some special substance governs the phenomenon of eating diploid drone larvae by the workers. These results provide a basis for developing a method for rearing diploid drones to the imago in the colony.

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