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REARING DIPLOID DRONES ON ROYAL JELLY OR BEE MILK*

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Summary

Low-survival larvae produced by two inbred queens were used to investigate whether a diet of royal jelly protected diploid drone larvae from being eaten by the worker bees. The larvae were hatched in an incubator, and one group was fed on bee milk and the other on royal jelly for 1, 2 or 3 days before being transferred to drone cells in a colony.

Out of 689 larvae transferred 48·2% survived after one day in the colony, and 31·1% were finally sealed. Significantly more diploid drones in the groups left in an incubator for 2 days were sealed than in the groups kept there for 1 or 3 days, but there was no significant difference between the percentages of diploid drones reared and sealed in the groups fed on royal jelly and on bee milk. Royal jelly does not, therefore, confer any additional protection on the diploid drone larvae, and bee milk can be used equally well.

Introduction

The author has shown in previous papers that diploid drone larvae can arise in the honeybee (1963*a*), but that the larvae are eaten by the workers within six hours of hatching from the eggs (1963*b*). The larvae are viable (1965*a*) and can be reared outside the colony (1963*c*), although this method is very inefficient. In order to rear these drones efficiently to the imago, factors controlling their destruction in a colony were studied; the author showed that the workers eat diploid drone larvae in either worker (1963*b*) or drone cells (1965*b*), but that they eat fewer larvae when these are placed on royal jelly within queen cells (1965*c*) or when the larvae are initially reared for two days on royal jelly in an incubator and are then transferred to drone cells in a colony (1969). The questions therefore arise whether the royal jelly diet protects the drone larvae from being eaten, and if they *must* be fed on royal jelly before being transferred to drone cells in the colony or whether they can be fed on bee milk.

Materials and Methods

Diploid drone larvae were obtained from two sibling-mated queens producing low-survival brood, which consists of 50% females and 50% diploid drones (the latter normally being eaten as newly hatched larvae by the workers).

The low-survival larvae were hatched in an incubator and then divided into two groups in each experiment: (1) those fed on royal jelly (R), and (2) those fed on bee milk (B). The royal jelly was taken from queen cells with a jelly spoon, and bee milk was collected from worker cells with a micropipette; larvae of the same age as those to be fed were present in both types of cells from which food was taken.

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In the first four experiments all the larvae were reared in the incubator for 3 days and then transferred to drone cells in the colony. In later experiments the larvae were again reared in the incubator, but they were transferred (on the same day), when 1, 2, or 3 days old, to drone cells in a queenless colony. Each cell with a transferred larva was marked, and the survival of these larvae was checked (1) the next day, (2) after 2 days and (3) after the cells were sealed, when the sex could be easily determined from the convex capping on drone larvae and the flat capping on worker larvae.

The "t" test was applied to results from the two groups (R and B) of larvae transferred at the age of 3 days, and an analysis of variance was applied to all the data of Experiments 4–8, in which larvae were kept in an incubator for 1–3 days. In one case when no data were available (Experiment 5, larvae 3 days old) an estimate of the missing value was calculated by the equation $X = \frac{rB + tT - G}{(r-1)(t-1)}$ where r and t were, respectively, the numbers of replications and treatments, B and T were, respectively, the numbers of the observations made in the replication and treatment containing the missing unit, and G was the total number of all observations. Least significant differences (0·05) were calculated only where the F value for the treatment was significant. In the tables, lsd (0·05) are given only for columns containing all data. Lsd for columns with missing data were calculated (they were a little higher), but were used for interpretation only. Table 1 shows the numbers of larvae transferred to the drone cells in the colony, and these totals formed the basis for the percentages given in all other tables.

TABLE 1. Number of low-survival larvae fed with bee milk (B) or royal jelly (R) in an incubator for 1, 2 or 3 days, and then transferred to drone cells in a colony.

Exp. no.	*No. of days in incubator*						*Total no. larvae*
	1		2		3		
	B	R	B	R	B	R	
1	—	—	—	—	10	10	20
2	—	—	—	—	21	11	32
3	—	—	—	—	16	9	25
4	38	44	29	19	26	41	197
5	21	28	28	25	—	—	102
6	27	14	15	6	14	6	82
7	27	23	21	20	17	16	124
8	22	21	17	16	15	16	107
Total	135	130	110	86	119	109	689

Results

Acceptance of larvae after one day

Out of 689 low-survival larvae transferred to the colony, only 332 larvae (48·2%) were accepted by the worker bees, and the rest were eaten during the first day.

Table 2 indicates that there was a tendency for the larvae fed on bee milk in the incubator to have a rather better initial acceptance rate in the colony than the larvae

TABLE 2. Percentage acceptance of low-survival larvae one day after transference to the colony; see Table 1.

Exp. no.	*No. of days in incubator*					
	1		2		3	
	B	R	B	R	B	R
1	—	—	—	—	70·0	60·0
2	—	—	—	—	85·6	36·3
3	—	—	—	—	31·2	55·5
4	42·1	36·3	72·4	68·4	38·4	29·2
5	47·6	42·8	64·3	52·0	—(1)	—(2)
6	51·8	64·2	80·0	50·0	50·0	33·3
7	66·6	65·2	47·6	50·0	47·0	43·7
8	40·8	29·5	35·5	18·7	33·3	18·7
Mean (diet)						
Exp. 1–8					50·8	39·5
Exp. 4–8	49·8	47·6	60·0	47·8	42·2	31·2
			lsd (0·05) = 15·77			
Overall mean (diet)						
Exp. 4–8	B, 51·2		R, 43·0			
Mean (age)						
Exp. 4–8	48·7		53·9		36·7	
			lsd (0·05) = 12·16			

(1), (2) Missing data are, respectively, 42·7 and 31·7 (see text).

fed on royal jelly, but this difference was not statistically significant in any of the three age groups.

The effect of the length of time for which the larvae were reared in the incubator is also shown in Table 2 (Experiments 4–8). The 2-day group had the highest acceptance rate (53·9%), and this was significantly higher than that of the 3-day group, but not significantly higher than of the 1-day group. Larvae fed on bee milk for 2 days had a significantly greater acceptance rate than those fed on it for 3 days, and also a highly significantly greater acceptance rate than those fed on royal jelly for 3 days.

Thus low-survival larvae fed on bee milk in an incubator are as readily accepted in a colony as larvae fed on royal jelly.

Further rearing of larvae

Two days after transferring the larvae into the colony, 275 of them (39·9%) still survived instead of 48·2%, so 8·3% alive on the previous day had now been eaten. Comparing the percentages of larvae accepted initially (Table 2) with those of larvae reared further (Table 3), heavy losses occurred in Experiments 5 and 6, indicating that factors other than age and food were operative.

The means given in Table 3 show that a slightly higher percentage of larvae fed on bee milk than on royal jelly were reared for two days in the colony, but the difference was not statistically significant. In Experiments 4–8 there was no

TABLE 3. Percentage of low-survival larvae reared for two days after transference to the colony; see Table 1.

Exp. no.	*No. days in incubator*					
	1		2		3	
	B	R	B	R	B	R
1	—	—	—	—	70·0	50.0
2	—	—	—	—	85·6	36.3
3	—	—	—	—	31·2	55·5
4	42·1	36·3	72·4	68·4	34·6	29·2
5	23·8	17·8	57·1	48·0	—(1)	—(2)
6	7·3	7·1	13·3	33·3	7·1	0·0
7	66·6	65·2	47·6	50·0	47·0	43·7
8	40·8	28·5	35·5	18·7	26·6	18·7
Mean (diet) Exp. 4–8	36·1	31·0	45·2	43·7	28·8	22·9
Overall mean (diet) Exp. 4–8	B, 37·3		R, 32·2			
Mean (age) Exp. 4–8	33·5		44·4		25·9	
			lsd (0·05) = 9·71			

(1), (2) Missing data are, respectively, 29·8 and 25·4 (see text).

significant difference between the two diet groups, but significantly more of the larvae transferred at two days old were reared by the bees than those of the younger and older age groups.

Sealing of worker and diploid drone brood

Of 689 larvae transferred initially, 214 (31·1%) were sealed; there were 122 drones (57·0%), 85 workers (39·7%) and 7 unclassified (3·3%). After pupation the drones were dissected, and the size of the testes confirmed that all were diploids. The number of workers sealed was significantly less than the number of drones, probably indicating that conditions in drone cells were less satisfactory for rearing workers than for rearing drones.

Table 4 shows that the original diets had little effect on the final percentages sealed, and that workers and diploid drones were found among all groups of larvae kept in the incubator on the two diets for different periods.

Significantly more drone larvae were sealed in the groups transferred after two days than in the one-day and three-day groups. There was no significant difference in percentages sealed between the one-day and three-day groups.

Conclusions

Considerable variation in the results of different experiments prevented a clear assessment of any other factors influencing the survival of the diploid drone larvae, apart from the age of transference and the type of food. It is clear that the optimum

TABLE 4. Percentage of sealed worker and diploid drone larvae; see Table 1.

Exp. no.	*No. days in incubator*											
	1				2				3			
	B		R		B		R		B		R	
	male	*female*	*male*	*female*	*male*	*female*	*male*	*female*	*male*	*female*	*male*	*female*
1	—	—	—	—	—	—	—	—	40·0	10.0	10·0	30·0
2	—	—	—	—	—	—	—	—	28·5	42·8	27·2	9·0
3	—	—	—	—	—	—	—	—	12·4	0·0	55·5	0·0
4	2·6	15·8	4·5	0·0	48·2	17·2	21·0	42·0	3·8	11·5	7·3	21·8
5	9·5	0·0	3·6	0·0	14·2	3·5	12·0	0·0	—(1)	—	—(2)	—
6	3·7	3·7	7·1	0·0	13·3	0·0	33·3	0·0	7·1	0·0	0·0	0·0
7	33·3	33·3	34·7	30·4	28·5	19·0	35·0	15·0	29·3	17·6	18·7	25·0
8	27·2	13·6	14·2	4·6	29·4	5·8	12·4	6·3	20·0	6·6	12·4	6·3
Mean Exp. 4–8	15·3	13·3	12·8	7·0	26·7	9·1	22·7	12·7	15·1	8·3	9·6	13·3
Mean (diet, sex not determined) Exp. 4–8	28·6		19·8		35·8		35·4		24·0		22·9	
Mean (age) Exp. 4–8 all larvae	24·2				35·6				23·5			
Exp. 4–8 drones only	14·0				24·7				12·3			
lsd (0·05) for drones = 10·7												
Overall mean (diet) Exp. 4–8 all larvae	B, 29·8		R, 26·3									
Exp. 4–8 drones only	B, 19·3		R, 15·4									

(1), (2) Missing data are, respectively, 3·1 and 2·1 (see text).

time of transferring larvae to the colony was after two days in the incubator (as found earlier by Woyke, 1969), and also that bee milk was no less suitable as a diet in the incubator than royal jelly.

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