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THE HATCHABILITY OF 'LETHAL' EGGS IN A TWO SEX-ALLELE FRATERNITY OF HONEYBEES*

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SUMMARY

Queens producing scattered brood were obtained by sibling mating for two or three generations. A total of 33 521 individual observations, together with counts of 9634 eggs and larvae, were carried out on the brood these queens produced. Only about 50% of the brood normally survived. But when the eggs were screened and left in normal conditions in the hive, or kept in favourable conditions outside it, nearly all of them hatched. The hatchability of eggs of these queens was in fact similar to that of eggs laid by queens producing normal brood.

Counts made every three hours on brood of low survival rate showed that these eggs also hatched when left unscreened in the hive, but that a high proportion of the *larvae* disappeared within the next few hours.

In a parallel study with brood killed by freezing, dead larvae in the hive disappeared very quickly, but dead eggs could remain up to 5 days. Brood of low survival rate seemed to disappear more quickly than normal brood, so the homozygosity of locus X does not cause the eggs to be lethal.

INTRODUCTION

If a queen is mated to a closely related drone, she is very likely to produce scattered brood. Adults develop only from a proportion of the eggs laid in worker cells. The same thing sometimes occurs when a queen mates with a drone not closely related to her.

It has been believed that a series of alleles exists in the honeybee (*Apis mellifera*) which are viable in heterozygous eggs but lethal in homozygous eggs. For example an $xa/xb \times xb$ mating results in half the fertilized eggs being heterozygous (xa/xb) and viable, and half homozygous (xb/xb) and inviable.

The problem has been studied by Mackensen (1951, 1955), Laidlaw, Gomes and Kerr (1956), Rothenbuhler (1957), and Hachinohe and Jimbu (1958). Daily counts of eggs and larvae showed that the greatest loss occurred between counts on the third and fourth days, just at the time of hatching. It was therefore concluded that the homozygous eggs are lethal and do not hatch.

We had intended to investigate the stage at which the eggs die, and to determine the sex of these eggs. But we found no disturbance in the embryonic development, and an investigation was therefore started on the hatching of these eggs.

MATERIAL AND METHODS

Five queens mated with closely related drones, and one naturally mated, were used in the present investigations, which included 33 521 individual observations and counts of 9634 eggs and larvae. Detailed information is given in Table 1. Besides the test on survival rate of brood, hatchability was investigated altogether on 4971 eggs. The largest number of observations (16 281) were those on hatchability and the time at which the brood disappeared.

Virgin queens were inseminated with the semen of one drone on a brother-sister or uncle-niece system. When they were laying, a test for survival rate of brood—

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corresponding to the viability or hatchability test of Mackensen—was carried out as follows. After the queen, free or caged under a queen-excluder cover, had laid eggs in a comb for one day, she was caged under the same cover on another comb. On the same day a piece of cardboard, in which a rectangular hole had been cut, was placed over the comb, and all normally deposited eggs within the area so marked were counted. The counts were repeated in the same manner on the fifth day, when the youngest larvae were one day old.

TABLE 1. Numbers of observations on eggs and larvae

Type of observation	*No. individual brood*	*No. observations*
Two-allele fraternity	3 258	5 152
Hatchability only	3 297	6 594
Hatchability and time of disappearance	1 674	16 281
Disappearance of brood previously killed	1 405	5 494
Total	9 634	33 521

To find out how many eggs hatched, the queen was caged on a comb with a queen-excluder cover for 12 hours. Then she was moved to a new area, and the eggs covered with a screen that prevented the bees from touching them. In the first experiments the eggs were covered immediately after the queen was moved, but in others 72 hours afterwards. In further experiments the comb with the eggs was taken from the hive and put in an incubator at 34·5°; some pieces were also cut out and placed in the incubator in a Petri dish with a little water at the bottom. The emerged larvae were counted on the 4th day after egg laying started.

The proportion of larvae which hatched out was determined by counting individual larvae under a dissecting microscope with a magnification of 15–40×. Larvae completely hatched, or covered with small patches of chorion, were classified as emerged. Larvae covered with chorion on the head, ventral side and tip of the abdomen were classified as partly hatched.

To find out at what stage the brood of low survival rate disappeared from the comb in the hive, the following observations were made. The queen was allowed to lay eggs as described above, and after she had been moved to a new area, the comb was left in the hive for 72 hours (from the beginning of egg laying). The comb, together with the bees covering it, was then removed from the hive every 3 or 6 hours and the number of remaining eggs and larvae counted. At first the number of eggs and larvae in each row of cells was counted; later the contents of each cell was recorded. The impression left in the comb by the queen-excluder cover made it possible to find the same row and cell each time.

To find out when eggs and larvae *known to be dead* disappear from their cells, a comb with hatching eggs, containing both eggs and larvae, was chosen. All this brood was killed in a deep freezer at −5°C., the comb being alternately frozen and defrozen for 3 days; in the last experiment it was frozen at −20°C. for a day. The combs were defrozen in an incubator at 34°C. and then inserted into the brood nest of a hive. (The

When the combs containing eggs were put in an incubator, the hatchability of the eggs was low. This was found to be due to the low humidity in the incubator. When a small section of the same comb was put in an incubator in a Petri dish with a little water at the bottom, almost all the eggs hatched.

These data show that when the eggs of a queen producing brood of low survival rate are in normal conditions in the hive, or in favourable conditions outside it, all or

TABLE 3. Hatchability of 3297 eggs laid by different queens, producing brood of low or high survival rate

Queen no.	*Mating system*	*Survival rate*	*No. eggs laid**	*Four days after egg laying*			*Where on comb eggs were screened*
				% emerged larvae	*% partly hatched eggs*	*% unhatched eggs*	
Eggs left	**in hive, screened**	**immediately after being laid**					
264	brother-sister	low	204	98·3	—	1·7	centre
			143	27·1	43·7	29·2	side
265	,,	low	185	100	—	—	centre
			213		many unhatched		side
266	,,	low	369	100	—	—	centre
			323	95·1	3·1	1·8	centre
			352	—	—	100	side
			118	51·6	48·4	—	side
273	uncle-niece	high	79	93·7	—	6·3	centre
			29	31·0	—	69·0	side
Eggs left	**in hive, screened**	**on the third day**					
264	brother-sister	low	77	100	—	—	centre
			53	86·7	3·8	9·5	side
			38	93·2	2·6	5·2	side
266	,,	low	113	96·5	0·9	2·6	centre
76	natural mating	high	117	90·6	9·4	—	centre
			158	90·5	3·8	5·7	side
Comb with eggs transferred to incubator on the third day							
266	brother-sister	low	93	50·5	46·3	3·2	
			112	—	—	100	
Piece of comb containing eggs put in a moist Petri dish in the incubator on the third day							
266	brother-sister	low	116	96·5	3·5	—	
			94	99·0	—	1·0	
			77	93·5	1·3	5·2	
			86	100	—	—	
264	brother-sister	low	35	100	—	—	
580	brother-sister	low	113	99·1	—	0·9	

*Different entries in this and subsequent columns relate to different combs

queen was caged, or kept away by a queen excluder). The dead eggs and larvae remaining in each cell of a determined area of the comb were then recorded every few hours.

RESULTS

The inbred crosses were made for two and three generations; many queens of these matings were unfortunately superseded, so that at the end of this experiment only five queens remained, four in 1961 and a new one in 1962.

To establish the number of alleles, observations were made on the survival rate of the brood. The results are given in Table 2. The survival rate of the brood of the three

TABLE 2. Survival rate of worker brood produced by different queens

Queen no.	*Mating system*	*No. eggs laid*	*No. larvae after 5 days*	*Percentage survival*
264	brother-sister	290	124	42·8
		334	163	48·8
265	brother-sister	168	77	45·8
		96	59	61·5
		318	168	52·8
		307	143	46·6
266	brother-sister	444	123	27·7
		141	58	41·1
		139	78	56·1
273	uncle-niece	124	84	67·7
		137	113	82·5
		212	211	99·5
		297	261	87·5
76	natural	75	73	97·3
		176	159	90·3
Total		3258	1894	

queens from brother-sister matings was about 50%, indicating a two-allele fraternity. The survival rate of brood of the queen mated on the uncle-niece system, and of the queen mated naturally, was much higher. This indicates three or more allele fraternities. The 1962 queen no. 580 was a daughter of queen no. 266. She also produced brood of low survival rate.

Do low-survival eggs hatch or not?

The next experiment was carried out in order to discover whether the eggs of a queen producing brood of a low survival rate hatch or not, the cells containing the eggs being covered with a screen. Table 3 shows that almost all eggs laid in the centre of the comb hatched, irrespective of whether the queen laying them produced brood of low or high survival rate. Eggs near an edge of the comb did not hatch, because the temperature there was too low.

When the eggs were left in the hive until the third day after being laid, and were then screened only a few hours before hatching, they hatched well, whether laid in the centre or at the side of a comb, and whether the queen laying them produced brood of low or high survival rate.

almost all hatch. Their hatchability is in fact similar to that of eggs laid by queens producing brood of high survival rate.

It can thus be seen that sibling mating does not result in a decrease of the viability of the eggs. Eggs of such queens are viable, and do hatch. So far as this problem is concerned, therefore, it is incorrect to speak of eggs of high or low hatchability, or of lethal eggs.

At what stage does the brood disappear?

To determine what kind of brood disappears from the comb, and at what age, more frequent counts (every 3-6 hours) were made on brood of three queens producing brood of low survival rate. The results, given in Tables 4 and 5, show that on many occasions most of the eggs hatched, and that the larvae then disappeared from the cells. The detailed records showed that often all eggs in a row of cells would hatch before the larvae started to disappear. Only for 7–21% of brood (average 14·9%) was it impossible to say whether the brood disappeared as egg or larva. The rest of the disappearances (about 30% of the brood) certainly occurred in the larval stage.

To obtain more detailed data, individual records for each cell were taken every 3 hours; these results are given in Table 6. An average of 6·4% of the brood disappeared in the 3 hours before the emerged larvae were seen, and it cannot be said for certain whether the egg or the larva disappeared. But larval food was found in a proportion of the cells for which the kind of brood that disappeared was unknown (1·7% of all brood), and a residue of larval food in a cell indicates that the larva probably emerged in it. This would reduce the percentage of unclassified disappearances to 4·7%: the remainder were disappearances *after the emerged larva was seen*. The most common period of disappearance was the first six hours after hatching.

These results show that, contrary to current belief, it is not the eggs, but the larvae, that disappear.

The frequent removal of combs from the hive did not increase the rate of disappearance of the brood, but rather decreased it, suggesting that this disturbance of the colony interfered with the bees' activities in removing the larvae of low survival rate from their cells.

Can nurse bees tell whether an egg is dead or alive?

The questions arise whether the worker bees are able to distinguish dead eggs from live ones, and whether they quickly remove dead eggs. To answer these questions, combs from the previous experiments containing eggs that did not hatch were put in the centre of the brood nest. The queen was caged on another comb under a queen-excluder cover. The comb containing the dead eggs was examined 2–3 times a day. In one experiment the dead eggs disappeared after a few hours. In three other experiments some eggs were found to remain after 1, 2 and 4 days respectively. These experiments show that bees do not remove dead eggs very quickly.

To obtain more detailed data, and to compare the time of disappearance of dead eggs and dead young larvae, individual records were made for each cell containing an egg or larva killed in a deep freezer. Table 7 shows that the greatest number of dead larvae disappeared during the first 3-6 hours after being put into the hive. The periods of disappearance of dead larvae and larvae of a queen producing brood of low survival rate were similar. But killed eggs took a long time to disappear, and some dead eggs

TABLE 4. Stage at which brood of low survival rate disappeared (each row of cells recorded individually)

Queen no., and egg laying period	No. 266, 12 hr.		No. 266, 6 hr.		No. 265, 6 hr.		No. 265, 6 hr.		No. 266, 6 hr.		No. 266, 6 hr.	
No. hours after beginning of egg laying	*No. brood remaining (eggs + larvae)*	*No. brood disappeared*	*No. brood remaining (eggs + larvae)*	*No. brood disappeared*	*No. brood remaining (eggs + larvae)*	*No. brood disappeared*	*No. brood remaining (eggs + larvae)*	*No. brood disappeared*	*No. brood remaining (eggs + larvae)*	*No. brood disappeared*	*No. brood remaining (eggs + larvae)*	*No. brood disappeared*
6 (once 12)	351		162		67		44		75		78	
72	339+ 1	11	160	2	45+ 16	6	29+ 13	2	64	11	66	12
78	180+157	3	95+64	1	11+ 44	6	5+ 37	0	61+ 3	0	66	0
81	—		—	—	—	—	35	7	—	—	—	—
84	51+271	15	3+142	14	2+ 37	16	27	8	46+ 18	0	45+ 19	2
87	—		—		—		—		—		25+ 37	2
90	4+276	44	127	18	1+ 31	7	26	1	21+ 41	0	12+ 50	0
93	—		—		—		—		9+ 49	6	6+ 56	0
96	1+244	35	116	11	1+ 31	0	25	1	5+ 51	2		
99	—	—	—		—		—		3+ 52	1	3+ 57	2
102	240	5	106	11	—		23	2	52	3	1+ 59	0
108	231	9	97	9	31	1	23	0	49	3	59	1
114	220	11	84	13	—		23	0	46	3	57	2
120	204	16	70	14	—		23	0	46	0	57	0

remained in the cells even after five days. Killed brood of low survival rate disappeared from the cells more quickly than killed brood of a naturally mated queen. It may be that the bees could distinguish brood of low survival rate from normal brood even after it had been killed. Killed brood of low survival rate disappeared most quickly when it was put into the brood nest of a colony already having such brood. But the comb in question had been frozen for 2 days at −5°C. and for 3 days at −20°C., and this long freezing period may have caused fundamental changes in the structure of eggs and larvae. Nevertheless, it is possible that the bees in a colony having brood of low survival rate are more sensitive than those in normal colonies to the presence of abnormal brood in the hive.

TABLE 5. Percentage survival and disappearance of the brood whose history is given in Table 4.

Queen no., and egg-laying period	*Percentage survival of brood*	*Percentage disappearance of brood*	
		in larval stage	*stage unknown*
no. 266, 12 hr.	58	26	16
no. 266, 6 hr.	43	49	8
no. 265, 6 hr.	46·3	34·3	19·4
no. 265, 6 hr.	52	41	7
no. 266, 6 hr.	61	19	20
no. 266, 6 hr.	73	6	21
Average	55·5%	29·6%	14·9%

TABLE 6. Percentages of brood of low survival rate that disappeared at different times (each cell containing brood recorded individually)

No. hours after hatching of eggs	*No. eggs laid*							*Total*
	191	172	173	88	187	22	64	897
	Queen no. 266			Queen no. 265		Queen no. 264		*Average*
0–3	—	2·9	1·2	12·5	10·7	9·1	10·5	6·4
0–6	5·2	—	—	—	—	—	—	—
3	—	7·6	14·5	13·6	24·1	0	7·8	11·2
6	14·1	7·6	12·7	9·1	12·3	0	1·6	10·5
9	—	5·8	8·1	6·8	4·3	9·1	1·6	4·6
12	3·7	5·2	4·0	0	1·6	0	1·6	3·0
15	—	3·5	1·7	0	1·6	4·5	0	1·5
18	0·5	1·2	0·6	1·2	1·1	4·5	0	0·9
21	—	0·6	0·6	0	0·5	0	1·6	0·5
24	0·5	0·6	0·6	0	0·5	0	0	0·5
24–48	0·5	2·9	2·3	2·3	1·6	18·2	3·1	2·3
Total (% disappeared within 48 hr.)	24·5	37·8	46·2	45·5	58·3	45·5	28·1	41·1

TABLE 7. Percentages of dead eggs and larvae that disappeared from the comb at different times

No. hrs. after insertion of comb in hive	*Interval between two counts in hrs.*	*In colony with normal brood*										*In colony with low survival brood*	
		Brood of naturally mated queens						*Brood of low survival rate*					
		1		2		3		4		5		6	
		eggs	*larvae*	*eggs*	*larvae*	*eggs*	*larvae*	*eggs*	*larvae*	*eggs*	*larvae*	*eggs*	*larvae*
3	3	4·8	36·1	14·3	83·2	13·0	12·7	1·9	79·0	27·9	30·6	70·0	81·4
6	3	9·6	12·4	5·5	6·3	3·2	21·3	23·8	4·8	25·3	38·8	17·0	10·3
9	3	7·2	8·2	2·2	4·2	4·5	15·3	0·9	3·8	27·9	14·4	7·5	5·1
15	6	9·6	23·7	9·9	4·2	9·8	32·0	41·9	8·6	3·6	11·7	3·4	1·9
21	6	9·6	15·4	1·1	2·1	7·2	12·0	12·4	0·0	3·6	2·7	0·7	1·3
33	12	2·4	2·1	19·7	—	6·5	6·0	2·9	3·8	4·5	1·8	1·4	—
45	12	13·3	2·1	7·7	—	7·2	0·7	4·8	—	0·9	—	—	—
57	12	10·9	—	4·4	—	7·8	—	2·9	—	1·8	—	—	—
69	12	2·4	—	6·6	—	12·3	—	7·6	—	4·5	—	—	—
83	12	2·4	—	0·0	—	7·8	—	0·9	—	—	—	—	—
95	12	2·4	—	9·9	—	11·7	—	—	—	—	—	—	—
107	12	3·6	—	3·3	—	4·5	—	—	—	—	—	—	—
119	12	3·6	—	7·7	—	2·6	—	—	—	—	—	—	—
survived		18·2	—	7·7	—	1·9	—	—	—	—	—	—	—
Total no. brood observed		83	97	91	95	154	150	105	105	111	111	147	156
		Total no. eggs = 691			Total no. larvae = 714				Total no. brood = 1405				

CONCLUSION

These results show that if the eggs of a queen producing brood of low survival rate did not hatch, they would not be removed so quickly from the cells. When a queen producing brood of low survival rate lays eggs, the eggs hatch, and it is the very young larvae which disappear from the cells of the comb. Thus the homozygosity at locus X does not cause the eggs to be lethal.

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