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GENETIC PROOF OF THE ORIGIN OF DRONES FROM FERTILIZED EGGS OF THE HONEYBEE*

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SUMMARY

Homozygous cordovan queens and homozygous chartreuse queens (from crosses of heterozygous mutant mothers with mutant fathers) were each inseminated from a single wild-black brother. After tests for survival rate of brood had been carried out, four queens producing low-survival brood were chosen, and the eggs they laid in both worker and drone cells were hatched in an incubator. The 321 young larvae were reared further in the incubator.

Drone pupae grafted as larvae from worker cells and originating from the cordovan queen showed some characters of the father. Stronger evidence is supplied by brood produced by the chartreuse queens. Drone prepupae and pupae reared in the incubator from eggs they laid in drone cells showed only the genetic character of the mother, as expected. But drone prepupae and pupa from eggs in worker cells laid by the same queens, and reared from the egg stage in an incubator, showed the genetic character of the father, as did the female offspring of these queens. This proves that drone larvae from eggs laid in worker cells by inbred queens producing low-survival brood develop from fertilized eggs.

INTRODUCTION

It has been shown that the so-called 'lethal' eggs laid in worker cells by sibling mated queens of *Apis mellifera* are viable (Woyke, 1962), and that larvae hatching from them are males (Woyke, 1963*a*, *b*). But in the colony they are eaten by the workers shortly after hatching (Woyke, 1963*c*). Whereas females can be reared in incubators to the imaginal stage without great difficulty, this is not true for either haploid or diploid drones (Woyke, 1965). Only occasional drones were reared to this stage from a large number of grafted larvae (Woyke, 1963*e*, *f*). Although the origin of these drones showed that they must have developed from fertilized eggs, the parental genetic complex did not allow phenotypical proof of their diploidy.

The difficulties in laboratory rearing to imago stimulated investigations into possible methods of establishing the diploid character of drones in the developmental stages. Pigment development in immature stages of wild and mutant bees has now been described (Woyke, 1964). Wild and cordovan body colour can be determined only shortly before the bee emerges from the cell. The wild eye colour can, however, be distinguished from the chartreuse, brick or buff eye colour even four days before pupation; this makes it possible to establish the origin of drones in the pupal or even prepupal stage.

MATERIAL AND METHODS

Cordovan mutant and wild bees were used in 1962, and chartreuse mutant and wild bees in 1963. One or two days before emergence the body colour of cordovan pupae is

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brown with brown dots; wild pupae show in addition a grey colour with black dots. The wild eye phenotype has a pink rim to the compound eyes in the prepupa, and pink or purple eyes in the pupa. The chartreuse phenotype has no such rim, and the compound eyes of pupae are white or yellow.

All queens were inseminated artificially, and the hive entrances then covered with queen excluders until the queens started to lay eggs. First, heterozygous mutant queens inseminated with mutant drones were chosen; they produced mutant and wild virgins and drones. The drones emerged in isolators and were maintained in the colony in them, or in special cages, until used for insemination. Next, each homozygous mutant virgin was inseminated with one wild-black brother only. Since wild is dominant to the mutant used, the experimental queens should produce mutant haploids and phenotypical wild diploids.

The six 1962 cordovan virgins were bred from queen 266, producing brood of low survival rate. After sibling mating, all the young queens should produce brood of 50% survival rate; of these, queen 652 was chosen to produce the experimental eggs.

The twenty 1963 chartreuse virgins were bred from queens producing brood of high survival rate. As a result of sibling mating, only about half the young queens should produce in worker cells brood of low survival rate; three were chosen (748, 752, 753) to produce the experimental material.

The phenotype of workers and drones produced in the colony by all the queens was tested either by examining the young and emerging bees in the hive, or putting the brood combs of older pupae in a special isolator (left in the colony or put in an incubator) and examining these emerging workers and drones. The survival test was conducted as described in previous papers (Woyke, 1962, 1963*b*).

Larvae of the 4 queens producing brood of low survival rate were reared in the incubator from the egg: combs with older eggs were placed in moist isolators and checked every 3 hours; the hatched larvae were transferred on to royal jelly and reared as described earlier (Woyke, 1963*e*,*f*). The results described below relate to 321 grafted larvae. Of these, 99 were reared in 1962, all from larvae hatched in worker cells; in 1963 174 larvae were taken from the worker cells where they were laid (fertilized eggs) and 48 from drone cells (unfertilized eggs). The sex of the larvae was determined on the 5th day of rearing (Woyke, 1963*d*, *e*) and the sexes separated. After transferring the larvae to pupation dishes, they were examined for genetic characters each day.

RESULTS

Obtaining experimental queens

All 6 cordovan queens mated to a black brother produced low-survival brood, as was expected. Workers developing from fertilized eggs were wild-black, and drones produced in the hive in drone cells from unfertilized eggs were cordovan. Owing to lack of semen, queen 651 also produced drones among the scattered brood in worker cells; they were cordovan, showing that they developed from unfertilized eggs. Queen 652 (chosen to supply the experimental material) produced brood of which 55% survived from 196 counted eggs (Table 1). Many larvae were also reared from eggs of this queen for other purposes; at 5 days old half the larvae were females and half males (Woyke, 1963*e*). This shows that the sex-alleles had been reduced in this colony to two only.

TABLE 1. Survival rate of brood produced by the cordovan and chartreuse queens chosen to supply the experimental material

Queen and series no.	*No. eggs counted*	*Percentage of larvae surviving*
652*a*	196	55
748*a*	359	36
b	297	43
752*a*	207	48
b	145	47
753*a*	164	35
b	295	50

Out of the 20 homozygous chartreuse virgins mated to their black brothers, 16 started to lay eggs. All produced chartreuse-eyed drones from unfertilized eggs laid in drone cells; all females from fertilized eggs in worker cells had black eyes (wild). Nine queens produced brood of high survival rate (about 90%) and 7 brood of low survival rate (below 50%). Table 1 shows the survival rate of brood produced by the three chartreuse queens chosen to supply the experimental eggs. Roughly 50% of each survived in the worker cells. The sex-alleles were presumably reduced in these colonies to two.

Genetic characters of brood reared ·

Table 2 gives results for drone pupae reared far enough to allow differentiation between wild and cordovan body colour. The sex ratio of larvae 5 days old (42 : 30) was similar to the survival rate of brood in this colony. Only about half the drone larvae alive on the 5th day were still alive at the time of transference to pupation. Unfortunately only three drones reached the stage of older pupae, and none emerged as an imago. The thorax of the drone pupae became grey. The wild phenotype (like the females produced by this queen) demonstrated the origin of these drones from fertilized eggs. But it was

TABLE 2. Results of rearing low-survival brood originating from a sibling-mated cordovan queen and a black drone (*cd* / *cd* × *cd*+) 1962

Queen and series no.		*No. grafted larvae*	*No. larvae alive after 5 days of rearing*			*No. male larvae transferred to pupation*	*No. older male pupae*
			total	*females*	*males*		
652	4	36	31	21	10	6	1
	9	29	25	15	10	6	1
	10	34	17*	6	10	4	1
Total		99	73	42	30	16	3

* sex of one larva not distinguished.

observed that the colour of other bees which died instead of developing normally also changed to grey or black. Further and more convincing evidence for diploid drones was therefore necessary.

The results of laboratory rearing of brood originating from the three chartreuse queens are given in Table 3. Female and male larvae were found in every series of all three queens. The sex ratio of all larvae 5 days old originating from these queens and taken from worker cells was roughly unity (44: 47). This indicates a reduction of the sex-alleles in these colonies to two only, and shows that the choice of these queens for the experiments was reasonable.

TABLE 3. Results of rearing low-survival brood originating from sibling-mated chartreuse queens and wild drones (*ch* / *ch* × *ch*+) 1963

Queen and series no.	*Eggs*†	*No. grafted larvae*	*No. larvae alive after 5 days of rearing*			*No. male larvae transferred to pupation*	*No. male prepupae with characters:*	
			total	*females*	*males*		*(ch) mother only*	*(+) father*
752 3–4	*w*	52	30	13	17	10	0	1
5	*w*	52	16	7	9	2	0	1
6*a*	*w*	21	9	6	3	2	0	1
6*b*	*d*	14	12	—	12	6	3	0
748 7*a*	*w*	30	23	11	12	7	0	4
7*b*	*d*	15	13	—	13	2	2	0
7*c*	*d*	—	—	—	—	10*	8*	0
753 10*a*	*w*	19	13	7	6	2	0	2
10*b*	*d*	19	8	—	8	3	2	0
Total	*w*	174	91	44	47	23	0	9
	d	48	33	—	33	11 + 10*	7 + 8*	0
	w + *d*	222	124	44	80	34 + 10*	7 + 8*	9

* Transferred to pupation dish from sealed cells.
† In column 2 *w* denotes that the eggs came from worker cells, and *d* from drone cells.

The female larvae developed further into prepupae whose compound eyes had a pink rim and then into pupae and imagines with the wild (black-eyed) character.

Many of the male larvae in Table 3 died, and only 23 drone larvae from worker cells and 11 from drone cells were transferred to pupation dishes. To enlarge the control group of brood from unfertilized eggs, 10 larvae of series 7*c* were transferred to the pupation dish directly from sealed drone cells in the colony; 8 developed normally.

All (haploid) drone prepupae from drone cells had compound eyes which showed no sign of wild character. The eyes were white, as chartreuse eyes are in that development stage; as expected, they showed only the character of their mothers. On the other hand, a distinct pink rim, typical of wild black eyes (Woyke, 1964), was visible in the compound eyes of all drone preupae from worker cells, showing the same genetic character as the female prepupae, and as the male parent.

Only one of the haploid prepupae that hatched in the incubator in drone cells pupated, but 8 larvae tranferred to the pupation dish directly from sealed drone cells in the hive did so. Compound eyes of all these drone pupae were white in the first few days after pupation, and then chartreuse. The ocelli were invisible at first except as three small hills of the pupal cuticle. These changes are typical for chartreuse-eyed drones, and show that the pupae inherited only the characters (chartreuse) of the female parent.

Only one prepupa pupated completely out of all the drone larvae that hatched in the incubator in worker cells. The compound eyes of this pupa were white with pink dots on the first day; during the following days they turned gradually pink, pink-purple and dark purple. The ocelli were light pink the first day after pupation, and the colour then underwent changes similar to those of the compound eyes; these changes are typical for black-eyed drones and similar to those in females produced by the same queen. Thus the drone pupa inherited the character of its father.

CONCLUSION

The results described show that drones hatched in worker cells from eggs laid by sibling-mated queens producing brood of low survival rate inherit the character of the father. It can therefore be concluded that these drones develop from fertilized eggs, and should thus be diploid in their origin.

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