

## THE BIPARENTAL ORIGIN OF ADULT HONEYBEE DRONES PROVED BY MUTANT GENES\*

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### Summary

Mutant queens were mated instrumentally with their brothers of wild or mutant genotype, and 20 queens producing scattered brood were chosen for further investigation.

Phenotypes of haploid drones, and of workers originating from those queens, were determined. Using the method for rearing diploid drones, 691 drones were reared from eggs laid in worker cells. Participation of the father, as well as the exclusion of androgenesis or parthenogenesis, was demonstrated in some of these drones. Mating of 5 queens homozygous for one mutant gene with brothers mutant for another gene allowed 302 drones of wild phenotype to be reared, proving genetically their biparental origin.

### Introduction

Woyke (1963a) showed that drone larvae hatched from one half of all the eggs laid in worker cells by a sibling-mated queen honeybee (*Apis mellifera*). Spermatozoa were found in almost all these eggs (Woyke, Knytel & Bergandy, 1966), and normal fertilization occurred in them (Woyke & Knytel, 1966). These findings suggested that drone larvae hatching from eggs laid in worker cells by sibling-mated queens are diploid. Nevertheless, there was no direct proof of the diploid character of the drones, nor was evidence obtained of their biparental origin.

The presence of spermatozoa in an egg is not a sufficient proof of the zygotic origin of the individual which develops from that egg, because of the androgenetic origin of certain male tissues in the honeybee (Rothenbuhler, Gowen & Park, 1952).

Rothenbuhler (1957), and Drescher and Rothenbuhler (1964), presented genetic evidence for the biparental origin of some patches of tissue in the eyes of mosaic drones. But these drones could not have been of completely zygotic origin because they would have been eaten by the worker bees as very young larvae (Woyke, 1963b). Genetic evidence of the participation of a father in the origin of the latter zygotic drones was obtained by Woyke (1965a) in larvae and pupae reared outside the colony (in an incubator). But the possibility of androgenesis was not excluded.

After the method of rearing diploid drones in the colony had been described (Woyke, 1965b, 1969), genetic evidence of their completely zygotic origin was presented as a preliminary report (Woyke & Adamska, 1966) of the present paper. The main part of the investigation described here is the rearing of drones in which parthenogenesis, as well as androgenesis, can be excluded; other individuals are also described.

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TABLE 1. Participation of the father in the origin of drones reared by the technique for diploids, shown by characteristics of the progeny of queens homozygous for one mutant gene, mated with drones of a wild type.  
 $cd$  = cordovan mutant  $cd^+$  = wild type  $ch$  = chartreuse mutant  $ch^+$  = wild type

Queen no.	Genotype of parents	Haploid drones				Drones reared by the method for diploids				Workers
		No. tested	Genotypes	Phenotypes	No. tested	Genotypes	Phenotypes	No. tested	Genotypes	
91	$cd/cd \times cd^+$	all	$cd$	cordovan	37	$cd/cd^+$	black	all	$cd/cd^+$	black
121	$cd/cd \times cd^+$	all	$cd$	cordovan	4	$cd/cd^+$	black	all	$cd/cd^+$	black
273	$cd/ch \times ch^+$	248	$ch$	chartreuse	86	$ch/ch^+$	black	575	$ch/ch^+$	black
875	$ch/ch \times ch^+$	all	$ch$	chartreuse	21	$ch/ch^+$	black	all	$ch/ch^+$	black
880	$ch/ch \times ch^+$	all	$ch$	chartreuse	13	$ch/ch^+$	black	all	$ch/ch^+$	black
Total		248 + others			161			575 + others		

TABLE 2. Exclusion of androgenesis in certain drones reared from brood of low survival rate, shown by characteristics of the progeny of heterozygous queens mated with mutant drones.  
 $cd$  = cordovan mutant  $cd^+$  = wild type

Queen no.	Genotype of parents	Haploid drones				Drones reared by the method for diploids				
		No. tested	Genotypes	Phenotypes	No. tested	Genotypes	Phenotypes	No. tested	Genotypes	Phenotypes
63	$cd/cd^+ \times cd$	10	$cd$	cordovan	13	$cd/cd$	cordovan	13	$cd/cd$	cordovan
173	$cd/cd^+ \times cd$	25	$cd^+$	black	17	$cd^+/cd$	black	17	$cd^+/cd$	black
		17	$cd$	cordovan	15	$cd/cd$	cordovan	15	$cd/cd$	cordovan
175	$cd/cd^+ \times cd$	14	$cd^+$	black	16	$cd^+/cd$	black	16	$cd^+/cd$	black
		4	$cd$	cordovan	1	$cd/cd$	cordovan	1	$cd/cd$	cordovan
177	$cd/cd^+ \times cd$	5	$cd^+$	black	1	$cd^+/cd$	black	1	$cd^+/cd$	black
		5	$cd$	cordovan	23	$cd/cd$	cordovan	23	$cd/cd$	cordovan
					11	$cd^+/cd$	black	11	$cd^+/cd$	black
Total		107			97			97		

## Materials and Methods

Queens and drones carrying mutant genes for cordovan body colour (*cd*), and brick (*bk*) and chartreuse (*ch* with or without the *m* modifier) eye colour, were used. All the mutant genes are recessive to the wild type, and they all occupy different loci. Interaction of *bk* and *ch* results in a buff (*bk, ch*) phenotype. The *ch*<sup>1</sup> gene is affected by an *m* modifier gene, and *ch*<sup>1</sup>,*m* produces a brown body colour.

Genetic investigations were conducted on the offspring of 23 queens, of which 20 were sibling-mated and produced scattered brood. Altogether 691 drones reared by the diploid-producing method were individually examined. Phenotypes of 3082 haploid drones and 3638 workers originating from the same queens were determined.

In order to obtain the diploid drones, their parents should have one sex allele in common (sibling mating), and to detect their origin the parents should differ in certain mutant genes.

The virgin queens were the progeny of queens heterozygous for one or two mutant genes, and mutant drones. The virgins were, therefore, homo- or heterozygous for these genes. Each virgin was mated instrumentally with one mutant brother or one wild-type brother. Thus parthenogenesis or androgenesis could be excluded in some of the offspring. The biparental origin of diploid drones was proved by mating queens homozygous for one mutant locus with their brothers which were mutant for another locus. Any mutant offspring would then indicate parthenogenesis or androgenesis, and the wild-type offspring would indicate a bi-parental origin.

The inseminated queens were introduced into hives with entrances covered with queen excluder. After the queens started to lay eggs, the survival rate of brood in worker cells was checked. Diploid drones were reared from queens producing brood of 50% survival rate, using the method described by Woyke (1969).

The brood reared was covered with a screen cage a few days before emergence of the imagines. The phenotype of the drones reared was examined on the day of emergence. Haploid drones and workers of particular queens were obtained from brood combs placed in the isolators in an incubator a few days before the emergence of the imagines. The genotype of the queens was checked by examination of their haploid drone progeny.

## Results

### *Participation of the father in the origin of drones reared by the diploid drone method*

Queens homozygous for one mutant gene were mated with a brother of a wild type gene for the same locus. Table 1 shows that mutant (haploid) drones developed from unfertilized eggs laid in drone cells, as was expected. All the workers were of the wild phenotype.

Drones were also reared, by the diploid drone method, from eggs laid by 5 sibling-mated queens in worker cells. Table 1 shows that all the 161 individuals were of the wild phenotype, like the father and the workers. This is, therefore, confirmation of the participation of the father in the origin of all the drones reared.

### *Exclusion of androgenesis in some drones reared from brood of low survival rate*

Queens heterozygous for the *cd* gene were each mated with one cordovan brother; they produced (in drone cells) haploid drones of *cd* and wild type (Table 2). (Drones from queens no 63 and no. 175 were used for other purposes before the phenotype

TABLE 3. Participation of the father and the exclusion of androgenesis in certain drones reared by the technique for diploids, shown by characteristics of progeny of a queen heterozygous for two mutant genes mated with a drone mutant for one gene.  
 $bk =$  brick mutant  $bk^+ =$  wild type  $ch =$  chartreuse mutant  $ch^+ =$  wild type  $bk, ch =$  buff

Queen no.	Genotype of parents	Drones reared by the method for diploids					
		No. tested	Genotypes	Phenotypes	No. tested	Genotypes	Phenotypes
863	$bk/bk^+, ch/ch^+ \times bk^+, ch$	45	$bk, ch$	buff	5	$\{ bk/bk^+, ch/ch$	chartreuse
		25	$bk, ch^+$	brick		$\{ bk^+/bk^+, ch/ch$	chartreuse
		38	$bk^+, ch$	chartreuse	6	$\{ bk/bk^+, ch^+/ch$	black
		42	$bk^+, ch^+$	black		$\{ bk^+/bk^+, ch^+/ch$	black
Total		150			11		

TABLE 4. Participation of the father in the origin of certain drones reared, and the exclusion of androgenesis in all of them, shown by characteristics of the progeny of a queen heterozygous for one mutant gene mated with a drone mutant for another gene.  
 $ch =$  chartreuse mutant  $ch^+ =$  wild type  $bk =$  brick mutant  $bk^+ =$  wild type

Queen no.	Genotype of parents	Drones reared by the method for diploids					
		No. tested	Genotypes	Phenotypes	No. tested	Genotypes	Phenotypes
212	$ch/ch^+, bk^+/bk^+ \times ch^+, bk$	23	$ch, bk^+$	chartreuse	28	$\{ ch/ch^+, bk^+/bk$	black
		26	$ch^+, bk^+$	black		$\{ ch^+/ch^+, bk^+/bk$	black
Total		49		28		77	

TABLE 5. Participation of the father in the origin of all drones reared, and the exclusion of androgenesis in certain of them, shown by characteristics of the progeny of queens homozygous for one mutant gene and heterozygous for others, mated with mutant drones.  
*bk* = brick mutant *ch* = chartreuse mutant *cd* = cordovan mutant *ch<sup>+</sup>* = *ch* mutant affected by *m* *m* modifier affecting *ch<sup>+</sup>* gene (interacts brown)  
*bk<sup>+</sup>* = wild type *ch<sup>+</sup>* = wild type *cd<sup>+</sup>* = wild type *cd<sup>+</sup>* = wild type *ch<sup>+</sup>* = *ch* mutant affected by *m* *m* modifier affecting *ch<sup>+</sup>* gene (interacts brown)  
*bk, ch* = buff

Queen no.	Haploid drones			Drones reared by the method for diploids			Workers		
	Genotype of parents	No. tested	Phenotypes of eyes and body	Genotypes	No. tested	Phenotypes of eyes and body	Genotypes	No. tested	Phenotypes of eyes and body
94	<i>bk/bk<sup>+</sup>, ch/ch<sup>+</sup>, cd/cd</i>	82	buff, cordovan	<i>bk/bk<sup>+</sup>, ch/ch,</i> <i>cd/cd<sup>+</sup></i>	7	chartreuse, black	<i>bk/bk<sup>+</sup>, ch/ch,</i> <i>cd/cd<sup>+</sup></i>	89	chartreuse, black
	$\times$ <i>bk<sup>+</sup>, ch, cd<sup>+</sup></i>	159	chartreuse, cordovan	<i>bk<sup>+</sup>/bk<sup>+</sup>, ch/ch,</i> <i>cd/cd<sup>+</sup></i>	6	chartreuse, black	<i>bk<sup>+</sup>/bk<sup>+</sup>, ch/ch,</i> <i>cd/cd<sup>+</sup></i>		chartreuse, black
		105	brick, cordovan	<i>bk/bk<sup>+</sup>, ch<sup>+</sup>/ch,</i> <i>cd/cd<sup>+</sup></i>		black, black	<i>bk/bk<sup>+</sup>, ch<sup>+</sup>/ch,</i> <i>cd/cd<sup>+</sup></i>		black, black
		211	black, cordovan	<i>bk<sup>+</sup>/bk<sup>+</sup>, ch<sup>+</sup>/ch,</i> <i>cd/cd<sup>+</sup></i>		black, black	<i>bk<sup>+</sup>/bk<sup>+</sup>, ch<sup>+</sup>/ch,</i> <i>cd/cd<sup>+</sup></i>		black, black
455	<i>ch<sup>+</sup>/ch<sup>+</sup>, m/m, cd/cd</i>	22	brown, cordovan	<i>ch<sup>+</sup>/ch<sup>+</sup>, m/m, cd/cd<sup>+</sup></i>	8	brown, black	<i>ch<sup>+</sup>/ch<sup>+</sup>, m/m, cd/cd<sup>+</sup></i>	92	brown, black
	$\times$ <i>ch<sup>+</sup>, m, cd<sup>+</sup></i>	32	black, cordovan	<i>ch<sup>+</sup>/ch<sup>+</sup>, m/m,</i> <i>cd/cd<sup>+</sup></i>	8	black, black	<i>ch<sup>+</sup>/ch<sup>+</sup>, m/m,</i> <i>cd/cd<sup>+</sup></i>	126	black, black
459	<i>ch<sup>+</sup>/ch<sup>+</sup>, m/m, cd/cd</i>	36	brown, cordovan	<i>ch<sup>+</sup>/ch<sup>+</sup>, m/m, cd/cd<sup>+</sup></i>	7	brown, black	<i>ch<sup>+</sup>/ch<sup>+</sup>, m/m, cd/cd<sup>+</sup></i>	618	brown, black
	$\times$ <i>ch<sup>+</sup>, m, cd<sup>+</sup></i>	35	black, cordovan	<i>ch<sup>+</sup>, ch, m/m,</i> <i>cd/cd<sup>+</sup></i>	7	black, black	<i>ch<sup>+</sup>, ch, m/m,</i> <i>cd/cd<sup>+</sup></i>	561	black, black
206	<i>bk/bk<sup>+</sup>, ch/ch, cd/cd<sup>+</sup></i>	5	buff, cordovan	<i>bk/bk, ch/ch<sup>+</sup>, cd/cd</i>	11	brick, cordovan	<i>bk/bk, ch/ch<sup>+</sup>, cd/cd</i>		brick, cordovan
	$\times$ <i>bk, ch<sup>+</sup>, cd</i>	6	buff, black	<i>bk/bk, ch/ch<sup>+</sup>,</i> <i>cd<sup>+</sup>/cd</i>	11	brick, black	<i>bk/bk, ch/ch<sup>+</sup>,</i> <i>cd<sup>+</sup>/cd</i>		brick, black
		8	chartreuse, cordovan	<i>bk<sup>+</sup>/bk, ch/ch<sup>+</sup>,</i> <i>cd/cd</i>	14	black, cordovan	<i>bk<sup>+</sup>/bk, ch/ch<sup>+</sup>,</i> <i>cd/cd</i>		black, cordovan
		4	chartreuse, black	<i>bk<sup>+</sup>/bk, ch/ch<sup>+</sup>,</i> <i>cd<sup>+</sup>/cd</i>	13	black, black	<i>bk<sup>+</sup>/bk, ch/ch<sup>+</sup>,</i> <i>cd<sup>+</sup>/cd</i>		black, black
Total		705			92			1571	

TABLE 6. Biparental origin of all the drones reared by the method for diploids, shown by characteristics of the progeny of queens homozygous for one mutant gene, mated with drones mutant for another gene. For key see Table 5.

Queen no.	Genotype of parents	Haploid drones			Drones reared by the method for diploids			Workers		
		No. tested	Genotypes	Phenotypes of eyes, body	No. tested	Genotypes	Phenotypes of eyes, body	No. tested	Genotypes	Phenotypes of eyes, body
204	$bk^+/bk^+, ch/ch,$ $\times bk, ch^+$	11	$bk^+, ch$	chartreuse	9	$bk^+/bk, ch/ch^+$	black	223	$bk^+/bk, ch/ch^+$	black
439	$bk/bk, cd^+/ch^+$ $\times bk^+, ch$	245	$bk, ch^+$	brick	157	$bk/bk^+, ch^+/ch$	black	621	$bk/bk^+, ch^+/ch$	black
272	$ch/ch^+, m^+/m, cd^+/cd^+$ $\times ch^+, m^+, cd$	142	$\left\{ \begin{array}{l} ch, m^+, cd^+ \\ ch, m, cd^+ \end{array} \right.$	chartreuse, black	75	$\left\{ \begin{array}{l} ch/ch^+, m^+/m^+, \\ cd^+/cd \\ ch/ch^+, m/m^+, \\ cd^+/cd \\ ch^+/ch^+, m^+/m^+, \\ cd^+/cd \\ ch^+/ch^+, m/m^+, \\ cd^+/cd \end{array} \right.$	black, black	500	$\left\{ \begin{array}{l} ch/ch^+, m^+/m^+, \\ cd^+/cd \\ ch/ch^+, m/m^+, \\ cd^+/cd \\ ch^+/ch^+, m^+/m^+, \\ cd^+/cd \\ ch^+/ch^+, m/m^+, \\ cd^+/cd \end{array} \right.$	black, black, black, black, black, black
55	$ch^+, m^+, cd^+$	chartreuse, black	black, black							
53	$ch^+, m, cd^+$	chartreuse, brown, black	black, black							
458	$ch^+/ch^+, m^+/m^+, cd$ $cd/cd \times ch^+, m, cd^+$	353	$ch^+, m^+, cd$	black, cordovan	39	$ch^+/ch^+, m^+/m^+, \\ cd/cd^+$	black, black	148	$ch^+/ch^+, m^+/m^+, \\ cd/cd^+$	black, black
753	$ch/ch, cd^+/cd^+$ $\times ch^+, cd$	all	$ch, cd^+$	chartreuse, black	22	$ch/ch^+, cd^+/cd$	black, black	all	$ch/ch^+, cd^+/cd$	black, black
Total		859 + others			302			1492 + others		

workers). The wild body colour of all the drones reared indicated their paternal origin, and the wild phenotype of the eyes excluded androgenesis in half the drones reared (paternal eye mutant).

Queen no. 206 was homozygous for the chartreuse mutant gene (*ch*) and heterozygous for two additional genes (*bk* and *cd*). She was mated with a brother mutant for both these last genes. Lack of chartreuse phenotype indicated paternal participation in the origin of all the drones reared by the technique for diploids. The presence of the paternal phenotype in a quarter of the males reared (*bk* and *cd* in the same individual) excluded androgenesis in three-quarters of the remaining individuals.

*Participation of the father and exclusion of androgenesis in all the drones reared (i.e. biparental origin of all the drones)*

Queens homozygous for one mutant gene were each mated with one brother mutant for another gene. (In some parents, modifier *m* of the *ch*<sup>1</sup> gene was present in addition.) Phenotypes of the haploids presented in Table 6 confirmed homozygosity of queens for one mutant gene. All the workers produced by those queens were of the wild type. Table 6 shows also that all the 302 drones reared by the new method and originating from 5 queens were of the wild phenotype. This absence of the mutant phenotypes expressed by the genes carried by the mother excluded parthenogenesis in all the drones, while lack of the phenotypes expressed by the genes of the father excluded androgenesis in all the individuals. The wild phenotype of the drones reared by the new method could be obtained only after the two gametes carrying mutant genes of two different loci united. This indicates participation of both parents in the origin of the drones reared by the new method.

An additional test was carried out to ascertain that no error had been made, and that the given mutant genes were actually present in the cross produced. For this purpose daughter queens were reared from the brood of queen no. 439 (*bk/bk*, *ch*<sup>+</sup>/*ch*<sup>+</sup> × *bk*<sup>+</sup>, *ch*), which produced diploid drones. The daughter queens (*bk/bk*<sup>+</sup>, *ch*/*ch*<sup>+</sup>) were black-eyed (wild type). Table 7 shows that they produced haploids of four phenotypes: those expressed by the maternal gene *bk* and by the paternal gene *ch* and the other two phenotypes as expected. This proves that the genotypes of parents and of the crosses had been correctly determined. Thus drones reared from that queen by the method for producing diploids were undoubtedly of biparental origin.

TABLE 7. Proof of the presence of mutant genes reported for queen no. 439 (*bk/bk*, *ch*<sup>+</sup>/*ch*<sup>+</sup> × *bk*<sup>+</sup>, *ch*) which produced diploid drones, from the phenotypes of haploid drones produced by her daughters.

*bk* = brick mutant                      *ch* = chartreuse mutant  
*bk*<sup>+</sup> = wild type                         *ch*<sup>+</sup> = wild type

Daughter queen no.	Genotype of queen	No. of haploid drones of phenotype			
		<i>buff</i>	<i>brick</i>	<i>chartreuse</i>	<i>black</i>
544	<i>bk/bk</i> <sup>+</sup> , <i>ch/ch</i> <sup>+</sup>	21	17	35	34
687	<i>bk/bk</i> <sup>+</sup> , <i>ch/ch</i> <sup>+</sup>	62	63	50	47
688	<i>bk/bk</i> <sup>+</sup> , <i>ch/ch</i> <sup>+</sup>	151	160	169	155
Total		234	240	254	236

## Conclusions

Our data (Tables 1–6) exclude both parthenogenesis and androgenesis in the production of diploid males, and also prove genetically the biparental origin of diploid drones that were obtained by the method described by Woyke (1969).

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