GENETIC CHARACTERS IN IMMATURE STAGES OF WILD AND MUTANT HONEYBEES*

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SUMMARY

Wild eye colour can be distinguished from brick, chartreuse and buff mutants: from 4 days before pupation by the presence of a pink rim to the compound eyes, and on the first day after pupation by the presence of pink dots in the compound eyes and by the colour of the ocelli. Brick mutants of both sexes are distinguishable on the third day after pupation, chartreuse on the fourth day in workers and the fifth day in drones: buff mutants start to colour on the sixth day in both sexes. The ocelli of wild bees are almost the same colour as the compound eyes, whereas in the mutant phenotypes they are colourless or white-grey.

Eye colour is thus a good genetic marker in early development stages. On the other hand cordovan body colour can be distinguished from the wild type only one day before emergence from the cell, and is thus not useful.

INTRODUCTION

Genetic proof of the diploid character of drone larvae from eggs laid in worker cells by inbred honeybee queens (Woyke, 1963*a*) has so far been lacking. In the hive these larvae are eaten by the workers shortly after hatching (Woyke, 1963*b*), but they can be reared further if kept apart from bees (Woyke, 1963*c*). Because of the difficulty in rearing any drone from the egg stage to the imago in the laboratory, characteristics were sought by which the bees could be identified genetically in earlier stages of development. Differences in pigment between wild and mutant bees could be useful if they exist in immature stages, but very little has been known about the genetic characters in immature stages of honeybees. Observations (Zander, Löschel & Meier, 1926; Bertholf, 1925; Rhein, 1933; Jay, 1962) have been made on the colour changes of wild honeybees pupae; the microscopical investigations of Phillips (1905) concern the structural development and pigmentation of wild bees only. Colour mutants have been described as imagines only (Mackensen, 1951; Rothenbuhler, Gowen & Park, 1952, 1953; Laidlaw, Green & Kerr, 1953). Myser (1954) has described the structural changes visible in fixed individuals at different stages of development.

MATERIAL AND METHODS

Wild *Apis mellifera ligustica* ~~Pollm~~ Spin. were used, and mutants cordovan body colour (*cd*) and brick (*bk*), chartreuse (*ch*) and buff (*bk*, *ch*) eye colour.

At first larvae from unsealed cells were investigated; later on larvae were always taken from cells just sealed. An area of comb with older unsealed brood was marked, and next day larvae were taken from the cells in it that had been capped. Both drone and

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TABLE 1. Body colour changes in wild and cordovan honeybee pupae

No. days from pupation	*Drones*				*Workers*			
	head and thorax		*abdomen*		*head and thorax*		*abdomen*	
	wild +	cordovan *cd*	wild +	cordovan *cd*	wild +	cordovan *cd*	wild +	cordovan *cd*
1	white	white	white	white	white	white	white	white
2	,,	,,	,,	,,	,,	,,	,,	,,
3	,,	,,	,,	,,	,,	,,	,,	,,
4	,,	,,	,,	,,	,,	,,	,,	,,
5	,,	,,	,,	,,	light yellow	light yellow	,,	,,
6	yellow	yellow	,,	,,	light brown	light brown	light yellow	light yellow
7	light brown	light brown	light yellow	light yellow	brown to light grey with black dots	brown with brown dots	dark yellow with black dots	dark yellow with brown dots
8	brown to light grey with black dots	light brown with brown dots	dark yellow with black dots	dark yellow with brown dots	dark grey with black dots	brown with black dots	light brown with black dots	light brown with brown dots
9	dark grey with black dots	brown with brown dots	light brown black dots	light brown brown dots	IMAGO			
10	IMAGO				black	cordovan	light brown and black	light brown and cordovan
	black	cordovan	light brown and black	light brown and cordovan				

worker larvae were withdrawn from the cells and transferred to pupation dishes; these were petri dishes lined with gauze and divided with cardboard into cell-like compartments. Each dish was covered with filter paper. The dishes were put into a desiccator, kept at 80% relative humidity by a sulphuric acid bath. Larvae, prepupae and pupae were observed through a stereoscopic microscope equipped with a microscope lamp; colour changes were recorded daily. Some individuals were fixed and stored in 10% formalin. Histological investigation of some compound eyes were carried out, from individuals fixed in Bouin's fluid; sections were made parallel and perpendicular to the eye surface. The deposition of pigment in individual cells was studied in sections mounted in Canada balsam without being stained, with a phase-contrast microscope.

Altogether 725 bees at different development stages were investigated.

RESULTS

No distinct genetic differences were found between uncapped larvae carrying wild and mutant genes, so only capped larvae, prepupae and pupae were investigated further.

Table 1 shows the changes in body colour of wild and cordovan pupae, drones and workers. The first differences are visible two days before emergence from the cell, but they are too small for a definite identification of the two phenotypes, which, however, become well differentiated one day before emergence, for both workers and drones (Table 1). The *cd* mutation is thus of little use for differentiation of earlier stages.

Changes in eye colour were next investigated. In wild drones (Table 2) a pink rim—the margin of the compound eye—is visible beneath the larval cuticle just behind the head 4 days before pupation; at that time it can be seen only if the first segment is stretched out. The rim is clearly visible the next day, and stretches before the large fold of the first segment (Fig. 1*a*). Two days before pupation the rim is more distinct, and

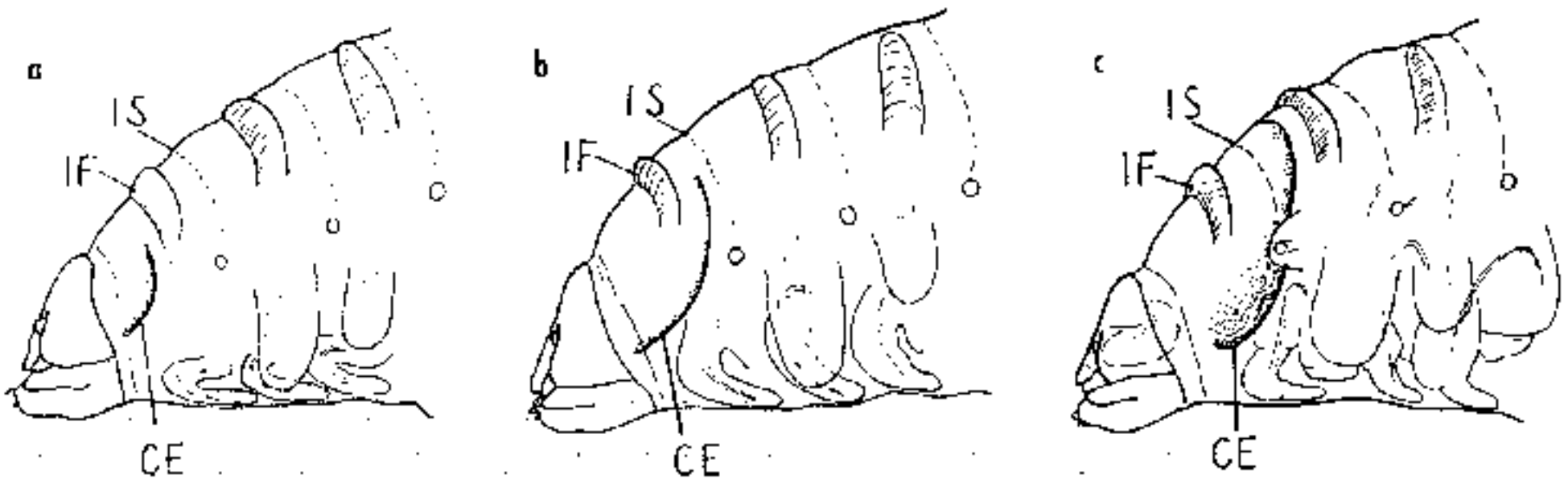

Fig. 1. Head and thorax of live wild drone prepupa, 3, 2 and 1 days before pupation (*a*, *b*, *c*)
CE rim of compound eye
1F first fold of larval cuticle
1S hind margin of first segment

reaches from beyond the first fold near to the posterior margin of the first segment (Fig. 1*b*). One day before pupation the rim is visible in the front half of the second segment, reaching before the second large fold (Fig. 1*c*). The pink colour of the posterior margin of

the compound eyes is now clearly visible; under a stereoscopic microscope the margin is not coloured uniformly, but shows many pink dots. When nearly at pupation the prepupa bends its head to the ventral side, and consequently the dorsal margin of the compound eyes is moved orally in relation to the larval cuticle covering the head. The rim can again reach only slightly beyond the posterior margin of the first segment, as in younger prepupae. But the age of the prepupa can be determined without difficulty by means of the distinct pigmentation of the eye, its relation to the first stigma, and the development of other body parts such as legs (Fig. 1c). Just before pupation the ocelli are slightly marked beneath the larval cuticle, but in some prepupae it is difficult to see them. Thus the position of the rim behind the head makes it possible to determine the age of a prepupa (Table 2).

No coloured rim to the compound eyes is visible in the prepupal stage of the three mutants, and they cannot be distinguished from each other, although the wild phenotype can be distinguished from the mutants four days before pupation. This is very useful for our purpose.

After pupation, the compound eyes of wild drones become darker every day (Table 2); they are much darker than the eyes of any mutant phenotype. Even on the first day after pupation, when the eyes look white (and are described in the literature as white), many pink dots can be seen under a magnification of 15× or more (Fig. 2). Owing to the

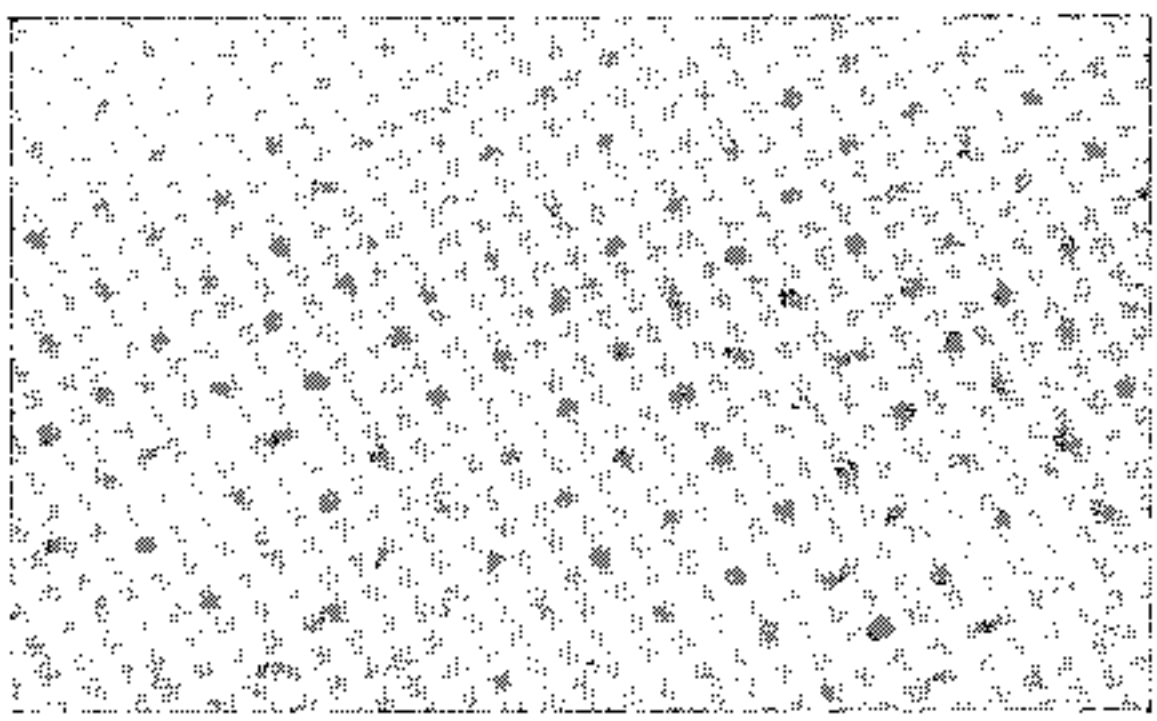

Fig. 2. Surface of compound eye of a wild drone just pupated (145×).

larger size of the compound eye after pupation, the pink dots are dispersed over a greater surface and are no longer visible without a microscope. The dots can be seen still many days after pupation, but not in any of the three mutant phenotypes. These dots enable the pink eyes of younger wild pupae to be distinguished easily from the similar looking pink eyes of older *bk* pupae, which lack the dots.

Observations of different sections showed that these dots are the distal ends of the retinula cells with the first pigment laid down. (Contrary to reports in the literature, the pigment is laid down here several days before the corneal pigment cells become pigmented.) The pink rim of the compound eyes of older larvae and prepupae is also due to the pigment in retinula cells.

TABLE 2. Eye colour changes in wild and mutant prepupae and pupas of drones

No. days from pupation	Wild (black) +		brick *bk*		chartreuse *ch*		buff *bk, ch*	
	compound eyes	*ocelli*	*compound eyes*	*ocelli*	*compound eyes*	*ocelli*	*compound eyes*	*ocelli*
				PREPUPA				
—4	pink rim behind head	invisible	invisible	invisible	invisible	invisible	invisible	invisible
—3	pink rim before 1st fold	,,	,,	,,	,,	,,	,,	,,
—2	pink rim behind 1st fold	,,	marked white	,,	marked white	,,	marked white	,,
—1	pink rim before 2nd fold	slightly marked	marked white	,,	marked white	,,	marked white	,,
				PUPA				
1	white with pink dots	light pink	white	only hills	white	only hills	white	only hills
2	light pink with dots	light pink	white	,,	,,	,,	,,	,,
3	pink	pink	white-pink	,,	,,	,,	,,	,,
4	dark pink	dark pink	light pink	,,	,,	,,	,,	,,
5	pink-purple	pink-purple	light pink	,,	white lower parts, margins yellow	,,	,,	,,
6	dark pink-purple	dark pink-purple	pink	,,	,,	,,	white-buff, lower parts light buff	,,
7	dark purple	dark purple	pink	white	,,	white	,,	white
8	dark purple	dark purple	dark pink	white-grey	,,	white-grey	light buff	white-grey
9	purple-black	purple-black lens transparent	brick	,,	,, or chartreuse	,,	light buff	,,
				IMAGO				
10	brownish-black	transparent pink-purple substratum	brick	white-grey	chartreuse	white-grey	buff	white-grey

TABLE 3. Eye colour changes in wild and mutant larvae, prepupae and pupae of worker honeybees

No. days from pupation	Wild (black) +		brick *bk*		chartreuse *ch*		buff *bk*, *ch*	
	compound eyes	*ocelli*	*compound eyes*	*ocelli*	*compound eyes*	*ocelli*	*compound eyes*	*ocelli*
				LARVA AND PREPUPA				
—4	pink rim behind head	invisible	invisible	invisible	invisible	invisible	invisible	invisible
—3	pink rim before 1st fold	,,	,,	,,	,,	,,	,,	,,
—2	pink rim behind 1st fold	,,	marked white	,,	marked white	,,	marked white	,,
—1	pink rim before 2nd fold	,,	marked white	,,	marked white	,,	marked white	,,
				PUPA				
1	white with pink dots	slightly marked	white	only hills	white	only hills	white	only hills
2	light pink with dots	pink	white	,,	white	,,	,,	,,
3	pink, dark pink	dark pink, pink-purple	white-pink	,,	white	,,	,,	,,
4	pink-purple, dark pink-purple	dark pink-purple	light pink	,,	white, white-yellow	,,	,,	,,
5	dark purple	dark purple	light pink	,,	light citrine	,,	,,	,,
6	very dark purple	very dark purple	pink	white	light citrine	white	white-buff	white
7	purple-black	purple-black	pink-brick	white-grey	light yellow, lower parts and margin yellow	white-grey	light buff	white-grey
8	purple-brown black	brownish-black, lens transparent	brick	white-grey	chartreuse	white-grey	light buff	white-grey
				IMAGO				
9	brownish-black	transparent, pink-purple substratum	brick	transparent, pink substratum	chartreuse	transparent, white substratum	buff	transparent, white substratum

The compound eyes of mutant drones begin to colour on the third (*bk*, pink), fifth (*ch*, yellow), and sixth (*bk*, *ch*, white-buff) day after pupation. The buff phenotype, resulting from the action of brick and chartreuse, is lighter in the pupa and can be determined later than the two mutants. The chartreuse and buff mutations can be determined easily only 1–2 days before emergence.

By observing the dots in the compound eyes, and their colour, the wild phenotype of pupae can thus be distinguished from the three mutations at any time from the first day after pupation.

The colour of the ocelli is also a very good genetic marker. In the wild phenotype it is almost the same colour as the compound eyes, whereas ocelli of the three mutant phenotypes are colourless or white, and for the first 6 days after pupation they are visible only as three small hills of pupal skin. The true ocelli of these mutants are visible in a pupa 7 days old, when the pupal cuticle around the ocelli begins to colour yellow or brown. One or two days before emergence the ocelli of the mutations *look* brick, chartreuse or buff, but it is due to reflection from the large compound eyes: if these are covered, the ocelli appear white-grey. The imaginal lenses are transparent in wild drones and turbid in mutants.

Colour changes in the different development stages of workers (Table 3) are similar to those described for the drones; they enable wild worker pupae to be distinguished from the mutants from the first day after pupation. The pink rim of the compound eyes can be seen beneath the larval cuticle 4 days before pupation, although the worker is still in the larval stage, spinning its cocoon. The pink rim is less visible in the worker than in the drone throughout the period before pupation. The chartreuse colour is visible in workers one day earlier, and is more distinct than in drone pupae; it can be distinguished more easily from the buff colour. The ocelli changes are similar to those in drones, except that in mutants the imaginal lenses are not turbid but transparent, with a substratum that is pink in *bk* and white in *ch* and *bk*, *ch*.

CONCLUSIONS

The colour of the compound eyes and ocelli of wild honeybees and of three mutant phenotypes can be distinguished much earlier than other characters, in both workers and drones. It is therefore a useful genetic marker for these stages. The wild or cordovan body colour is not useful as a genetic marker in immature stages.

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