Rev. Brasil. Genet. III, 2, 115-122 (1980)
(Brazil. J. Genetics)

COMPARATIVE STUDIES ON PIGMENT GRANULES AND PIGMENT DISTRIBUTION IN THE COMPOUND EYES OF WILD TYPE AND MUTANT WORKER HONEYBEES: AN ULTRASTRUCTURAL ANALYSIS. II.*

Carminda da Cruz-Landim[1], José Chaud-Netto[1] and Lionel Segui Gonçalves[2]

ABSTRACT

Differences in pigment morphology, amount and distribution in the compound eyes of wild honeybee workers and mutants were studied in four different genotypes of worker honeybees. Five different types of pigment granules were observed: compact, vesicular, fibrous, partially crystalline, and polymorphous. The crystalline granules were observed exclusively in the "chartreuse red" mutants, and polymorphous granules only in mutant eyes ("chartreuse red" and "laranja").

INTRODUCTION

In view of the extraordinary ability of honeybee workers to orient themselves by polarized light, the morphology of the compound eye has been

---

*Research supported by CNPq (PIG 04-007)

[1] Departamento de Biologia, Instituto de Biociências, UNESP, 13.500 Rio Claro, SP, Brasil.
[2] Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras, USP, 14.100 Ribeirão Preto, S.P., Brasil.

examined in several light and electron microscopy studies (Bäumgartner, 1928; Portillo, 1936; Stockhammer, 1956; Goldsmith, 1962; Varela and Porter, 1969; Varela, 1970; Grundler, 1974; Sommer and Wehner, 1975). The study of behavioral alterations in eye-color mutant honeybees represents a new challenge in this field. Langer *et al.* (1972) have investigated the screening pigment system in the compound eyes of wild drones and workers and white-eyed mutants (cream and ivory), finding differences in pigment distribution.

Genetic control in eye pigmentation in *Drosophila melanogaster* has been extensively analyzed and much is known about the biochemistry of pigment synthesis. A well-characterized system such as *Drosophila's* offers an ideal starting point for the study of the developmental consequences of gene action at the ultrastructural level. Shoup (1966) studied eye pigmentation in wild type *Drosophila* flies and in three eye-color mutants, finding four types of morphologically distinct pigment granules whose presence in a given eye-color is not exclusive.

Cruz-Landim *et al.* (1979), in paper I of this series, reported differences in the amount and distribution of pigment in wild and mutant types of eye-color in *Apis mellifera*. The objective of this investigation was to examine the pigment pattern in the eyes of wild type honeybee workers, as well as of the "laranja" and "chartreuse" mutants, and to attempt to interpret these structural differences as the result of specific genetic mutations.

MATERIAL AND METHODS

The insects used in this study were adult honeybee workers with wild homozygous (Ch/Ch; ${}_{S}La/{}_{S}La$), wild heterozygous (Ch/ch^r; ${}_{S}La/{}_{S}La$), "laranja" (Ch/Ch; ${}_{S}la/{}_{S}la$), and "chartreuse red" (ch^r/ch^r; ${}_{S}La/{}_{S}La$) genotypes obtained from laboratory hives. Young (2-5 days old) and middle aged (10-15 days old) bees were used. All bees were decapitated, and the dorsal and lateral teguments of the heads, as well as the glands and air sacs around the optic lobe were removed to facilitate penetration of the fixing solution. The heads were immersed in a fixing solution of 3% glutaraldehyde in 0.2 M cacodylate, pH 7.2, for 2 hours at room temperature. The optic lobes were dissected from the heads, cut into small fragments, rinsed in buffer, and post-fixed in 1%

osmium tetroxide in the same buffer for 1 hour. The material was dehydrated in ethanol, stained with 1 % uranyl acetate and embedded in epon-araldyte. Thin sections were cut with a Porter-Blum Mt 2 ultramicrotome using glass knives, stained with lead citrate, and examined with a Zeiss EM 9 S2 electron microscope. A tentative evaluation of quantitative differences was made by estimating the number of pigment granules in the regions identified in Table II in three electron micrographs magnified 10,000 times.

RESULTS AND DISCUSSION

The compound eye of the honeybee is composed of ommatidia with a dioptic apparatus consisting of a cuticular lens and a crystalline cone, and a sensory apparatus made up of elongated nerve cells, called retinula. At its edge, closest to the ommatidial axis, the retinula differentiates to form a rhabdomere. The rhabdomeres of each receptor are fused to form a central circular rhabdom. A layer of pigment cells separates the ommatidia from one another.

The pigment of the compound eye is found in the retinular and pigment cells. Five different types of pigment granules could be morphologically distinguished in the adult bees of the genotypes studied here: compact (Fig. 1 a), vesicular (Fig. 1 b), fibrous (Fig. 1 c), partially crystalline (Fig. 1 d), and polymorphous (Fig. 1 e). Some of these granules may possibly represent only different phases of maturation. The vesicular granule consists of a membrane-bound vesicle containing a very dense granule, usually excentrically located (Fig. 1 b). According to Shoup (1966), similar granules arise from the Golgi in *Drosophila*, containing a brown pigment believed to be ommochrome. In vertebrates, the brown pigment, melanin, is synthetized within vesicles formed on the Golgi, going through a phase similar to that of the vesicle described here, before reaching the compact form known as a melanin granule. Shoup also found that the fibrous granule in *Drosophila* may reach a compact form by fiber worling and condensation. Some of the compact granules in our material show a few fine fibrils at their periphery (Fig. 1 a), thus suggesting a similar origin. It was often difficult to observe the membrane enveloping the granule.

The granules with crystals are usually elliptical and variable in size. They are characterized by an elongated and electron-transparent crystal in the center, surrounded by dense material. They were found only in the "chartreuse red" mutants.

The polymorphous granules are present only in the mutants, regardless of genotype and are characterized by contents of concentrically arranged membranes, and thus considered to derive from lysosomes.

The amount of each type of pigment differs in the different genotypes (Table I), although they all show increased pigmentation with age. Distribution of pigment granules also varies in different regions of the ommatidia (cf. Table II). In an attempt at quantification, the ommatidium was divided into three regions: I, crystalline cone; II: retinula; III: basal zone. The pigmentation in the crystalline cone region is due exclusively to the pigments contained in the pigment cells around the cone, while in the other two regions it is found in both the pigment and retinular cells (Fig. 2). Wild heterozygotes (Fig. 2 b) show less pigment than wild homozygotes (Fig. 2 a) in the retinular cells, but pigment granules are compact in both. In the mutants, pigmentation is practically absent from the retinular cells. The pigment cells in the "laranja" mutants have mostly vesicular granules, while the "chartreuse red" mutants mainly have compact granules.

In the basal zone also, the pigment granules are located inside the pigment cells. The amount of pigment in wild homozygotes (Fig. 3 a) differs from that in wild heterozygotes (Fig. 3 b) although both show mostly compact granules. The "laranja" mutants (Fig. 3 c) have mostly fibrous granules, and the "chartreuse red", vesicular granules.

If we postulate that the fibrous and vesicular granules are precursors of the compact granules, then the granules observed in the mutants should be considered to be immature. A feature commonly observed in the mutants is the clustering together of various granules forming large pigment complexes (Fig. 4 a and b) held together by a "cement" of fibrous nature.

Table I - Pigment types in the compound eyes of different genotypes for eye-color in *Apis mellifera*

	Compact granule	Vesicular granule	Fibrous granule	Granule with crystals	Polymorphous granule
Wild Homozygous Ch/Ch ; ${}_{S}La/{}_{S}La$	++++	+++	+	–	–
Wild Heterozygous Ch/ch^r ; ${}_{S}La/{}_{S}La$	+++	+++	+	–	–
"Laranja" ${}_{S}la/{}_{S}la$; Ch/Ch	–	+++	+++	–	++
Chartreuse-red ch^r/ch^r ; ${}_{S}La/{}_{S}La$	–	+	++	++	+++

Table II - Pigment granules distribution in the ommatidia of the compound eyes of *Apis mellifera* workers. Average in 144 cm^2 of micrographs magnified 10,000 times

		Types of pigment granules				
Ommatidia Regions	Genotype	Compact	Vesicular	Fibrous	Crystalline	Polymorphous
Crystalline Cone	$Ch/Ch;{}_{s}La/{}_{s}La$	50	10	–	–	–
	$Ch/ch^r;{}_{s}La/{}_{s}La$	35	10	–	–	–
	$Ch/Ch;{}_{s}la/{}_{s}la$	15	20	–	–	1.5
	$ch^r/ch^r;{}_{s}La/{}_{s}La$	–	–	20	–	2
Retinulae	$Ch/Ch;{}_{s}La/{}_{s}La$	40	15	–	–	–
	$Ch/ch^r;{}_{s}La/{}_{s}La$	35	13,5	–	–	–
	$Ch/Ch;{}_{s}la/{}_{s}la$	–	15	30	–	5
	$ch^r/ch^r;{}_{s}La/{}_{s}La$	–	–	20	20	5
Basal Zone	$Ch/Ch;{}_{s}La/{}_{s}La$	30	5	2	–	–
	$Ch/ch^r;{}_{s}La/{}_{s}La$	20	5	5	–	–
	$Ch/Ch;{}_{s}la/{}_{s}la$	–	5	30	–	5
	$ch^r/ch^r;{}_{s}La/{}_{s}La$	–	5	10	3	2

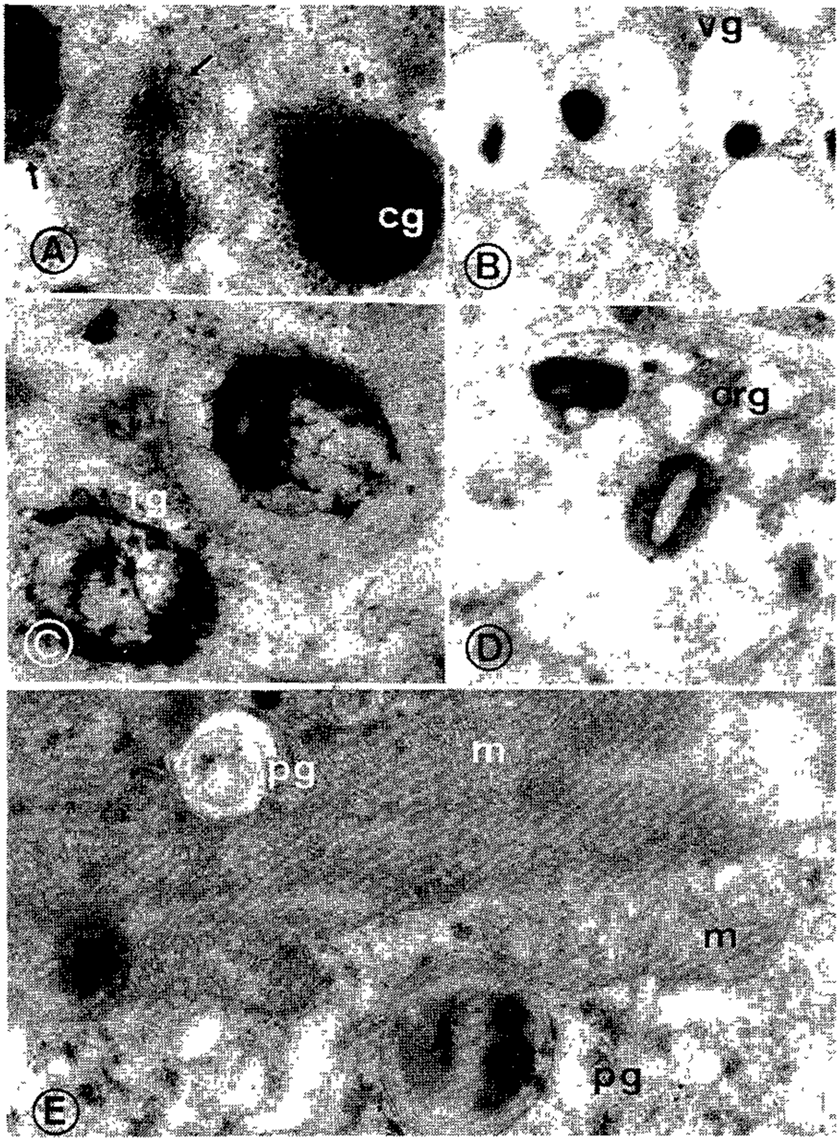

Figure 1 - Types of pigment present in the compound eyes of worker honeybees. A, compact granule (cg). The arrows point to the fibrous material which probably forms the granule. B, vesicular granule (vg). C, fibrous granule (fg). D, partially crystalline granule (crg). E, polymorphous granule (pg). m, mitochondria. 27,000 X.

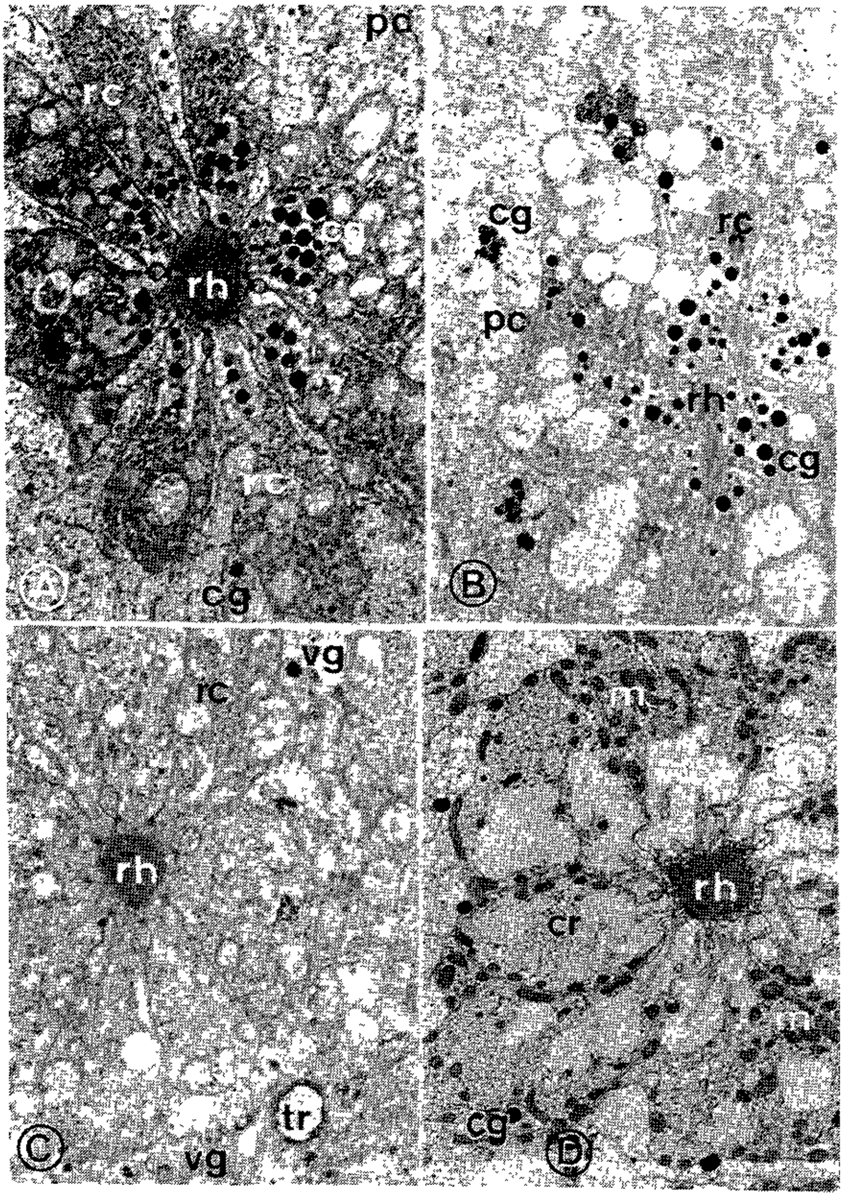

Figure 2 - Cross-sections of the retinula. A, wild homozygote. B, wild heterozygote. C, "laranja" mutant. D, "chartreuse red" mutant. rc, retinular cell; rh, rhabdom; cg, compact granule; pc, pigment cell; vg, vesicular granule; tr, tracheole. 10,000 X.

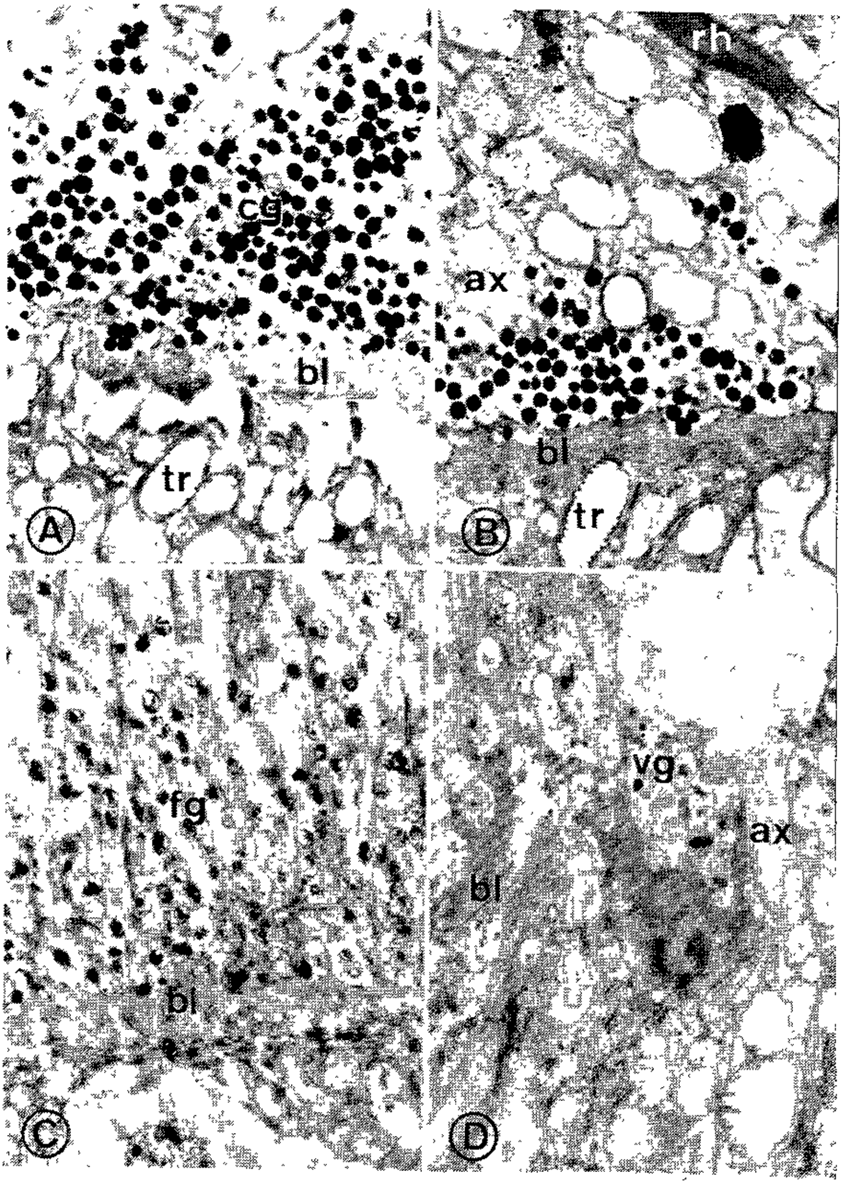

Figure 3 - Basal zone of the retinula. A, wild homozygote. B, wild heterozygote. C, "laranja" mutant. D, "chartreuse red" mutant. cg, compact granule; fg, fibrous granule; vg, vesicular granule; bl, basal lamina; ax, axon; tr, tracheole.

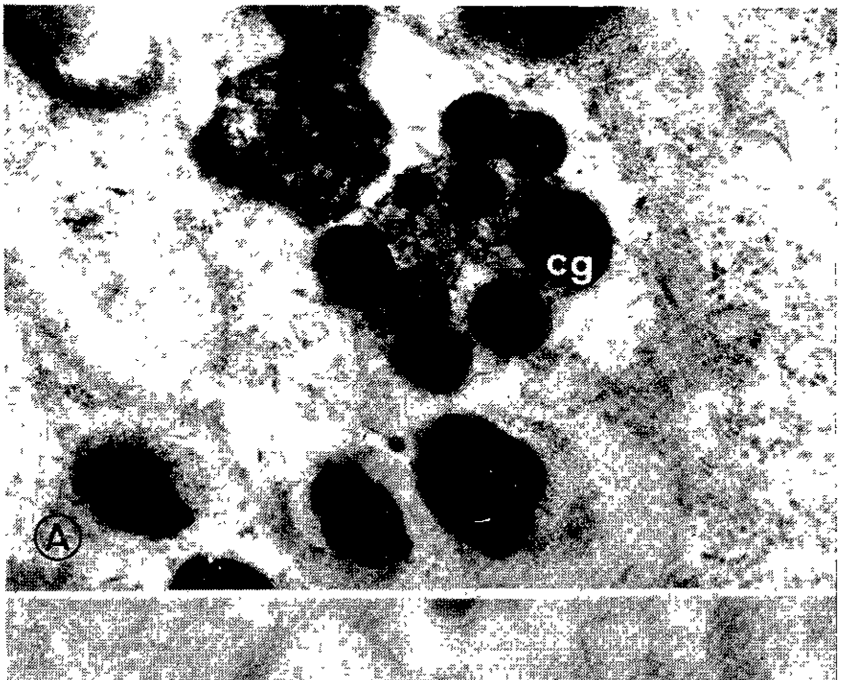

Figure 4 - Pigment granule coalescence. A, fusion of compact granules (cg). B, fusion of partially crystalline granules (crg).

CONCLUSIONS

As shown in Tables I and II, the genotypes studied show differences in the amount and type of pigment. These differences may be due to gene action during synthesis and maturation of pigment granules, and may be responsible, at least in part, for the behavioral differences observed in certain mutants

RESUMO

O presente estudo refere-se a diferenças na morfologia, quantidade e distribuição do pigmento em 4 genótipos diferentes de operárias de *Apis mellifera* Cinco tipos diferentes de grânulos de pigmento estão presentes nas abelhas estudadas: compactos, vesiculares, fibrosos, parcialmente cristalinos e polimorfos. Os grânulos cristalinos foram encontrados exclusivamente nos mutantes "chartreuse red" e os polimorfos apenas nos olhos de mutantes ("chartreuse red" e "laranja").

REFERENCES

Baumgartner, H. (1978). Der formensinn und die Schcharfe der Bienen *Z. Vergl. Physiol. 7*: 56-143.

Cruz Landim, C., Chaud-Netto, J. and Gonçalves, L.S. (1979). Morphological alterations in the compound eyes of eye-color mutants of *Apis mellifera* L. (Hymenoptera – Apidade). *Rev. Bras. Genet. II*: 223-231.

Goldsmith, T.H. (1962). Fine structure of the retinulae in the compound eye of the honey bee. *J. Cell. Biol. 14*: 489-494.

Grundler, O.J. (1974). Elektronenmikroskopische untersuchungen am Auge der Honigbiene (*Apis mellifica*). I. Untersuchungen zur morphologie und Anordnung der neum retinilazellen in ommatidien verschiedener augenbereiche und zur perzeption linear polarisierten lichtes. *Cytobiologie 9*: 203-220.

Langer, H., Schneider, L. and Tauber, K. (1972). Vergleichende untersuchungen an Augenpigmentsystem von wildform und weissaügigen mutanten der honigbiene (*Apis mellifica*). *Cytobiologie 4* : 427-438.

Portillo, J. del (1936). Beziehungen zwischen den Öffnungswinkeln der Ommatidien, Krümmung und Gestalt der Insektenaugen und ihrer funktionellen Aufgabe. *Z. Vergl. Physiol. 23* : 100-145.

Shoup, J.R. (1966). The development of pigment granules in the eyes of wild type and mutant *Drosophila melanogaster. J. Cell Biol. 29*: 223-249.

Sommer, E.W. and Wehner, R. (1975). The retinalamina projection in the visual system of bee, *Apis mellifera. Cell. Tiss. Res. 163*: 45-61.

Stockhammer, K. (1956). Zur wahrnehmung der schwingungsrichtung linear polirisierten Lichtes bei Insekten. *Z. Vergl. Physiol. 38*: 30-83.

Varela, F.G and Porter, K.R. (1969). Fine structure of the visual system of the honeybee (*Apis mellifera*). I. The retina. *J. Ultrastruct. Res. 29*: 236-259.

Varela, F.G (1970). Fine structure of the visual system of the bee (*Apis mellifera)*. II. The Lamina. *J. Ultrastruct. Res. 31*: 178-194.

(Received August 31, 1979)