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EFFECT OF JUVENILE HORMONE III ON THE ULTRASTRUCTURE OF THE *Corpora allata* IN *Melipona quadrifasciata* (HYMENOPTERA, APIDAE, MELIPONINI)

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ABSTRACT

Changes in hormonal levels can produce alternative phenotypes. Juvenile hormone III plays an important role in the regulation of metamorphosis, caste determination and age in bees. In this work, we examined the ultrastructure of *corpora allata* cells from stingless bees (*Melipona quadrifasciata*) treated with juvenile hormone during development. The *corpora allata* cells of *M. quadrifasciata* queens showed greater activity than those of workers. The topical application of juvenile hormone III altered the cellular ultrastructure and either delayed development (as shown by fewer mitochondria and greater chromatin condensation) or enhanced development (looser chromatin and numerous mitochondria) when compared to untreated (control) bees. Our results show that *corpora allata* cells differ in their ultrastructural characteristics and that the cessation of juvenile hormone production by these cells in *M. quadrifasciata* is not synchronous**.**

Key words: Castes, *corpora allata*, juvenil hormone, *Melipona quadrifasciata*, stingless bee

INTRODUCTION

Juvenile hormone, which is synthesized by the endocrine glands of the *corpora allata* and is secreted into the hemolymph, has an important role in insect metamorphosis [7]. The concentration of juvenile hormone in bee hemolymph is an important factor in caste differentiation [4,9] and age polyethism [12]. The topical application of juvenile hormone in *Melipona* larvae in the cocoon-spinning stage, in predefecating larvae and in late larvae of stage 3 (L3) leads to the development of queens from worker larvae by triggering genetic mechanisms that initiate the required differentiation [4]. The ovaries of queens of the stingless bee *Melipona quadrifasciata* produced by treatment with juvenile hormone have the same external morphology as those of natural queens [2]. Similarly, the morphology of the tergal glands of *M. quadrifasciata* queens produced by exposure to juvenile hormone is the same as that of natural queens but different from that of workers [3].

In this work, we examined the ultrastructure of *corpora allata* cells in *M. quadrifasciata* treated and not treated with juvenile hormone (JH) during development to understand the roles of the topic application of JH in *corpora allata* cells during development.

MATERIAL AND METHODS

Melipona quadrifasciata bees were obtained from hives maintained at a meliponary in Uberlândia, in the state of Minas Gerais, Brazil (S 18º55', W-GR 48°17') and in the Department of Genetics, University of São Paulo (USP), Ribeirão Preto, in the state of São Paulo, Brazil. The bees were classified as larvae 2 (L2), larvae 3 (L3), predefecating larvae (PDL), defecating larvae (DL), white-eyed pupae (Pw), pink-eyed pupae (Pp), brown-eyed pupae (Pb), black-eyed pupae (Pd), black-eyed pupae with pigmented body (Pdl), new born (NB) and adult worker (W) based on the criterion of Dias *et al.* [8] and Rossini [S.A. Rossini, Master's dissertation, Paulista State University, Rio Claro, SP, Brazil].

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Hormone treatment

Larvae in the L3 and PDL stages were treated individually with 1 μl of juvenile hormone III (Sigma Chemical Co., St. Louis, MO, USA) dissolved in analytical grade acetone (Merck Industrias, Rio de Janeiro, RJ, Brazil) at a concentration of 0.5 μg/μl. The larvae were subsequently housed in an incubator at 31°C and a relative humidity of 80% (maintained using a saturated solution of KCl in a desiccator). The control group consisted of bees treated with acetone alone and handled in an identical manner.

Preparation of corpora allata for transmission electron microscopy (TEM)

The *corpora allata* from natural queens and workers, and queens induced by treatment with juvenile hormone III were dissected and fixed in freshly prepared 2.5% glutaraldehyde 0.1 M phosphate buffer, pH 7.4. The material was then post-fixed in 2% osmium tetroxide in the same buffer for 1 h at room temperature, dehydrated in a graded acetone and then embedded in a mixture of acetone plus Araldite 6005 (v:v) for 24 h at room temperature. The final embedding and polymerization in Araldite resin was done in an oven at 60°C for 72 h. Sections were subsequently obtained using a Porter-Blum 2B ultramicrotone. Semi-thin sections were stained with toluidine blue to select areas of interest, after which ultrathin sections were stained with 2% aqueous uranyl acetate and lead citrate and then examined and photographed in a Zeiss EM 9 S-2 transmission electron microscope.

RESULTS

The *corpora allata* are endocrine glands located in the head, posterior to the brain and lateral to the esophagus. These glands are smaller in workers than in queens and secrete juvenile hormone. Embryologically, the *corpora allata* are of ectodermal origin and consist of modified epithelial cells arranged in small, spherical organs delimited by an amorphous outer membrane. The *corpora allata* are innervated by nerve endings from the brain and the corpora cardiaca and are ventilated by tracheal branches present in the organ intercellular spaces.

Ultrastructure of the corpora allata during development in untreated bees

Corpora allata cells in L3 larvae had large, regular-shaped nuclei with multiple nucleoli and loosely dispersed chromatin, as well as numerous mitochondria in the cytoplasm. The intercellular space was narrow and contained tracheal tubes and nerve terminals from neurosecretory neurons (Fig. 1).

In workers (Fig. 2C), the nuclei were irregularly-shaped and larger than in L3 larvae, with multiple nucleoli and little chromatin condensation; the cytoplasm contained numerous mitochondria and lysosomes. The *corpora allata* of queen and worker brown-eyed pupae differed in the size and shape of their nuclei, and in their level of chromatin condensation, with queen nuclei showing greater chromatin dispersion (Fig. 2A-C). Queen *corpora allata* cells consistently had more nucleoli than worker cells (Fig. 2A).

In the Pd phase, the *corpora allata* cells of queens and workers had large nuclei with dispersed chromatin and conspicuous nucleoli that were multiple in some queen cells and single in workers. Queen *corpora allata* cells contained electron-dense secretory bodies that were absent in worker cells (Fig. 3A-C).

Analysis of the corpora allata in bees treated with juvenile hormone III

All of the PDL larvae that were treated with juvenile hormone III were transformed into complete females (queens). The *corpora allata* cells of PDL bees had small nuclei with a relatively condensed chromatin and large, multiple nucleoli (Fig. 4A). Queen Pw *corpora allata* cells had large, irregular nuclei, with large, multiple nucleoli that were evident in most of the cells, and loosely dispersed chromatin. The cytoplasm contained numerous, heterogenously shaped mitochondria (Fig. 4B). Lipid droplets surrounded by glycogen granules were seen in the cytoplasm of queen Pb *corpora allata* cells but not in workers (Fig. 4C). In the Pdl phase, the only difference between control and treated bees was the greater intercellular space (Fig. 5A) and the smaller number of mitochondria (Fig. 5B) in bees treated with juvenile hormone III compared to non-treated individuals (Fig. 3A,B).

DISCUSSION

Analysis of the *corpora allata* ultrastructure revealed caste differences, with signs of greater activity in queens than in workers. The topical application of juvenile hormone III altered the ultrastructure, either by decreasing the activity (seen as fewer mitochondria and greater chromatin condensation) or increasing the activity (seen as loosely dispersed chromatin and a greater number of heterogenously-shaped mitochondria) compared to non-treated bees. The

synthesis of juvenile hormone by the *corpora allata* is controlled by a feedback system and the topical application of extra doses of this hormone may have interferred with organ functioning. Hormone-induced cytological changes have also been observed in the *corpora allata* of *Blabera fusca* [5] and *Diplotera punctata* cockroaches [6,10,16], and in the earwig *Labidura riparia* [13].

A qualitative analysis revealed that *M. quadrifasciata* mitochondria were abundant in larvae, pupae and adults. Bees treated with juvenile hormone had fewer mitochondria, possibly indicating that the exogenously applied hormone had a negative feedback effect on the *corpora allata* to reduce the glands' synthetic behavior and energy requirements.

According to Sedlak [14], lysosomes are responsible for the elimination of excess hormone, and of the hormone precursors and enzymes needed for hormone biosynthesis. Our results showed that lysosomes were more evident in the *corpora allata* cells of PDL and Pdl workers than in queens. Lipid droplets surrounded by glycogen granules were seen in the *corpora allata* cells of queens in the Pd and Pdl stages but were absent in worker cells, indicating higher glandular activity in queens than in workers in this developmental phase.

According to Silva de Moraes *et al.* [15] and Akahira *et al.* [1], a lower degree of chromatin condensation indicates higher nuclear activity. As shown here, different levels of chromatin condensation were seen in *M. quadrifasciata*. Chromatin condensation gradually increased from Pb queens (juvenile hormone-induced queens and untreated individuals) and workers (untreated individuals) to maximum condensation in adult workers and queens. However, the degree of chromatin condensation in natural queen bees (not induced with juvenile hormone) did not correlate

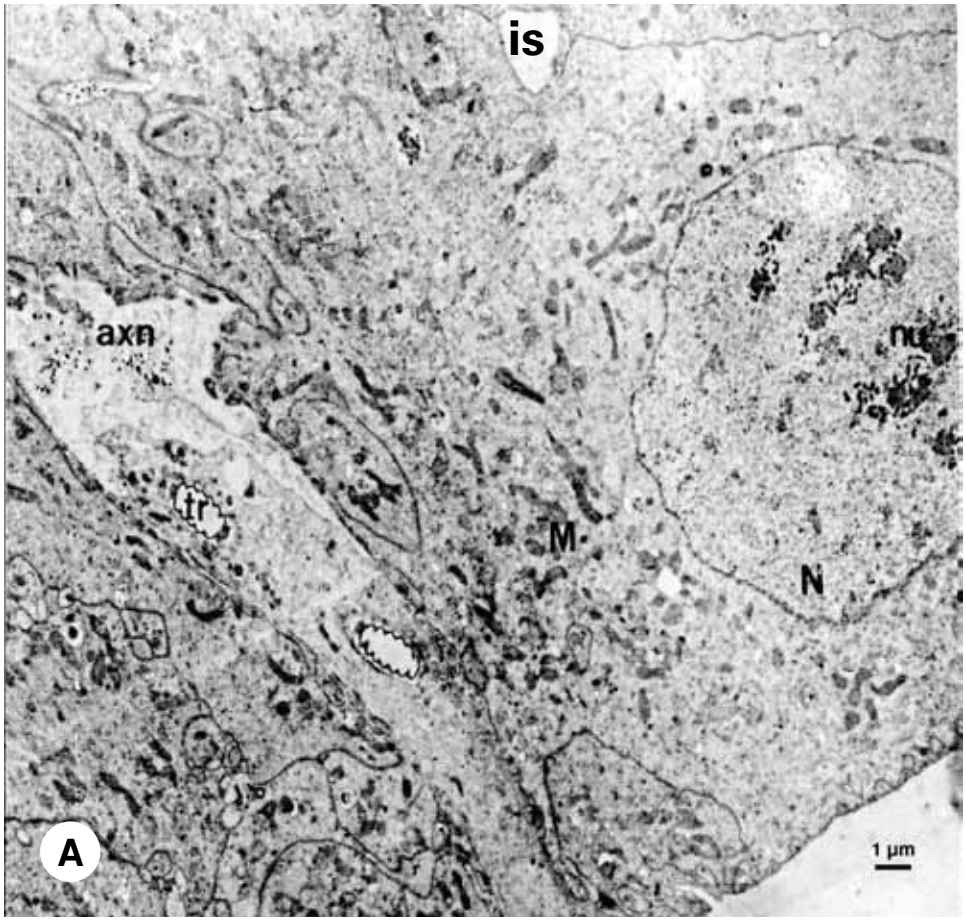

Figure 1. Transmission electron micrograph of the *corpora allata* in late larval stage 3 (L3) of *M. quadrifasciata* (control). Note the large nucleus (**N**) with several nucleoli (**nu**) and the wide intercellular spaces (**is**), some of them containing tracheoles (**tr**) and neurosecretory axons (**axn**). **M** = mitochondria.

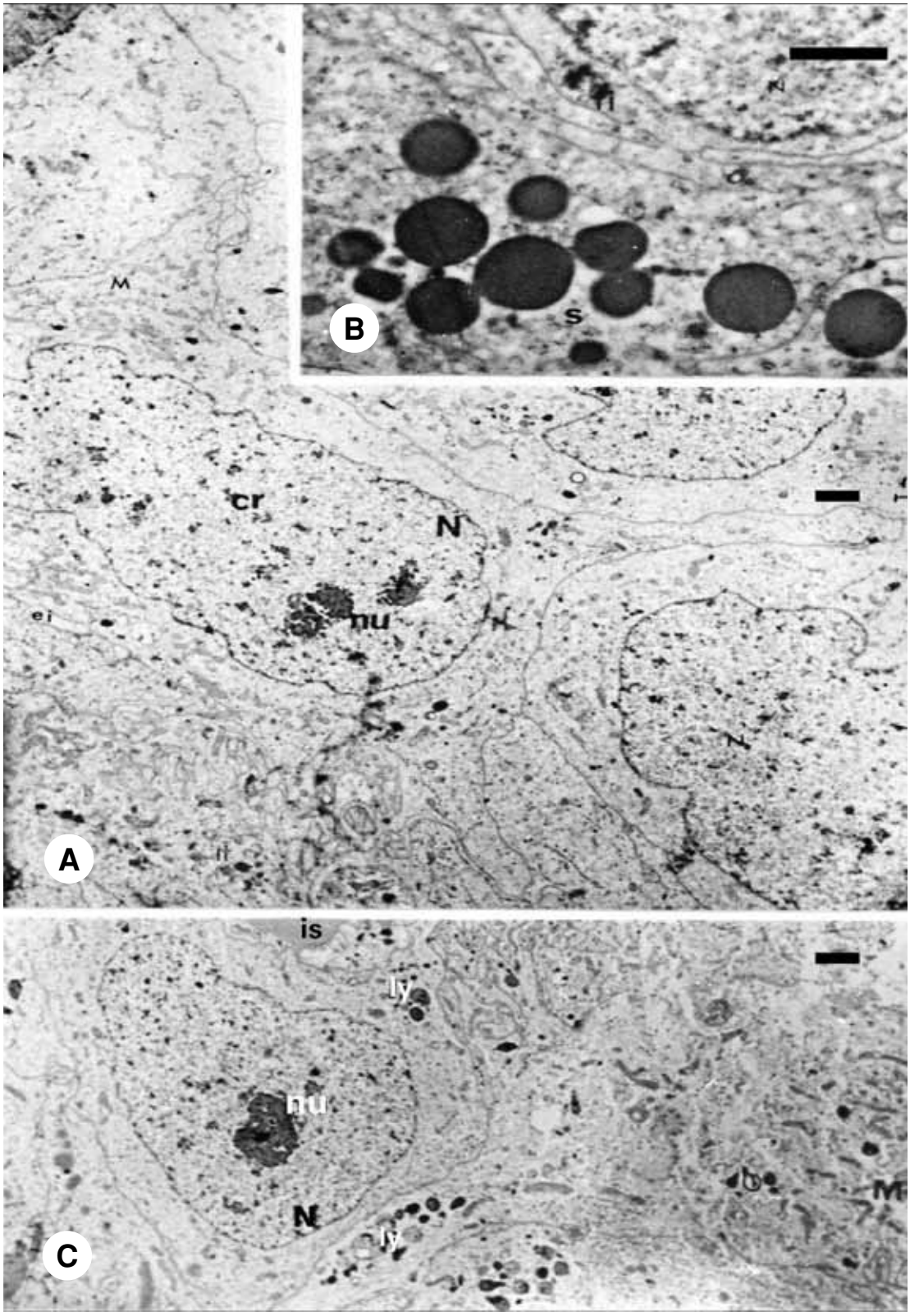

Figure 2. Transmission electron micrographs of *corpora allata* cells in *M. quadrifasciata* (control). **A.** Brown-eyed queen pupae (Pb) showing the general appearance of the cells. **B.** Detail of the secretory granules (**s)** present in some queen cells. **C.** Brown-eyed worker pupae (Pb) showing the presence of lysosomes (**ly**) in the cells. **cr** = chromatin, **is** = intercellular space, **N** = nucleus.

with the typical occurrence of organelles described above for active cells since chromatin condensation would mean less active cells.

Based on the cellular ultrastructure described here, the *corpora allata* was apparently more active in L3 and predefecating larvae, in brown-eyed, black-eyed, and black-eyed pupae with a pigmented body, and in adults. This conclusion is supported by the presence of organelles normally associated with greater cellular activity, e.g. larger nuclei, multiple nucleoli, numerous mitochondria of various shapes and large intercellular spaces.

In conclusion, our results show that *corpora allata* cells differ in their ultrastructural characteristics and that the cessation of juvenile hormone production by these cells in *M. quadrifasciata* is not synchronous, as also suggested by Johnson *et al*. [11] for *Diploptera punctata*.

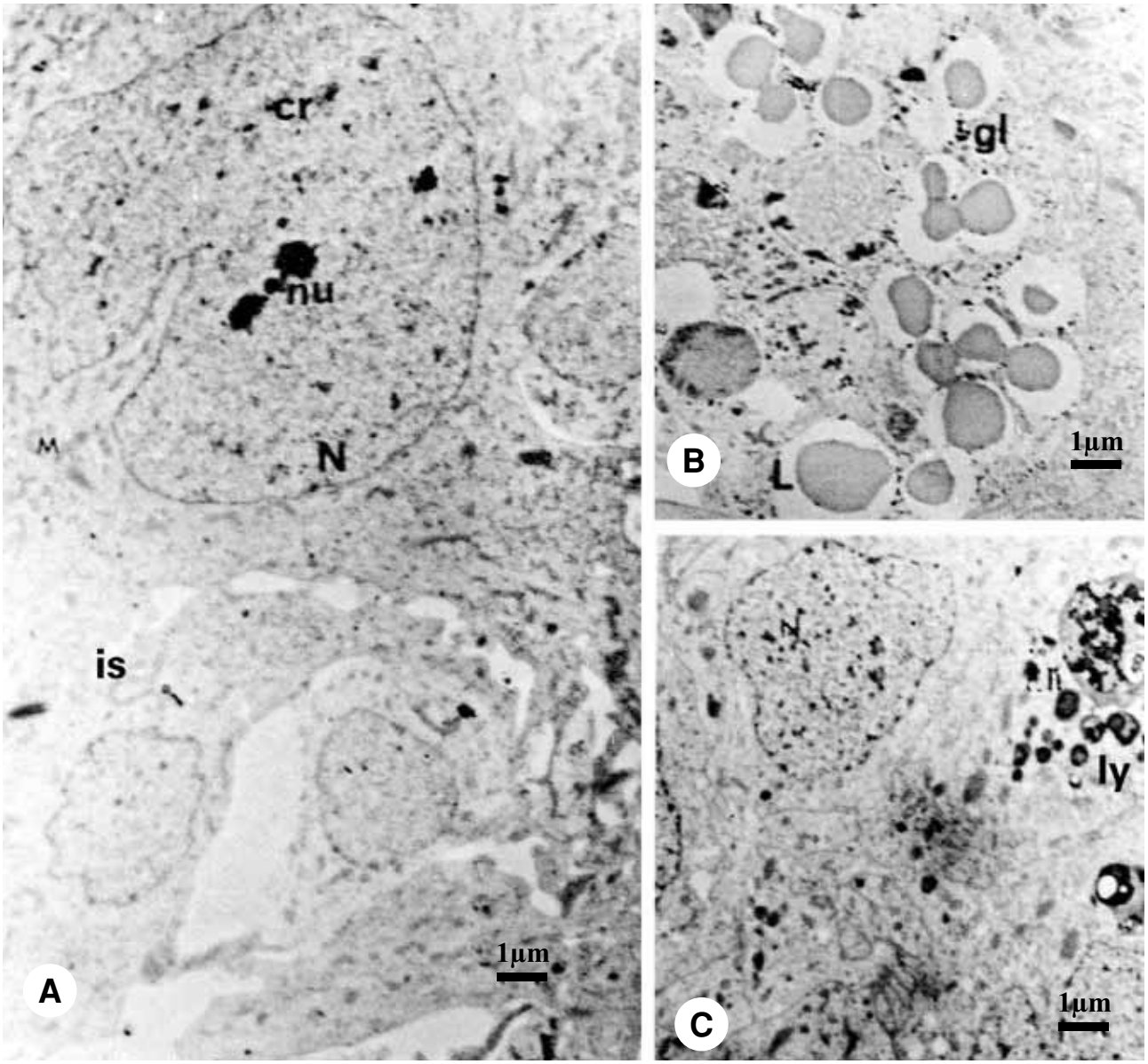

Figure 3. Transmission electron micrographs of *corpora allata* cells in *M. quadrifasciata* pupae (control). **A.** Black-eyed queen pupae (Pd) showing the large nuclei with dispersed chromatin and wide intercellular spaces (**is**). **B.** Black-eyed queen pupae (Pd) showing glycogen granules (**gl**) and lipid droplets (**L**). **C.** Black-eyed worker pupae (Pd) showing lysosomes (**ly**). **M** = mitochondria, **N** = nucleus, **nu** = nucleoli.

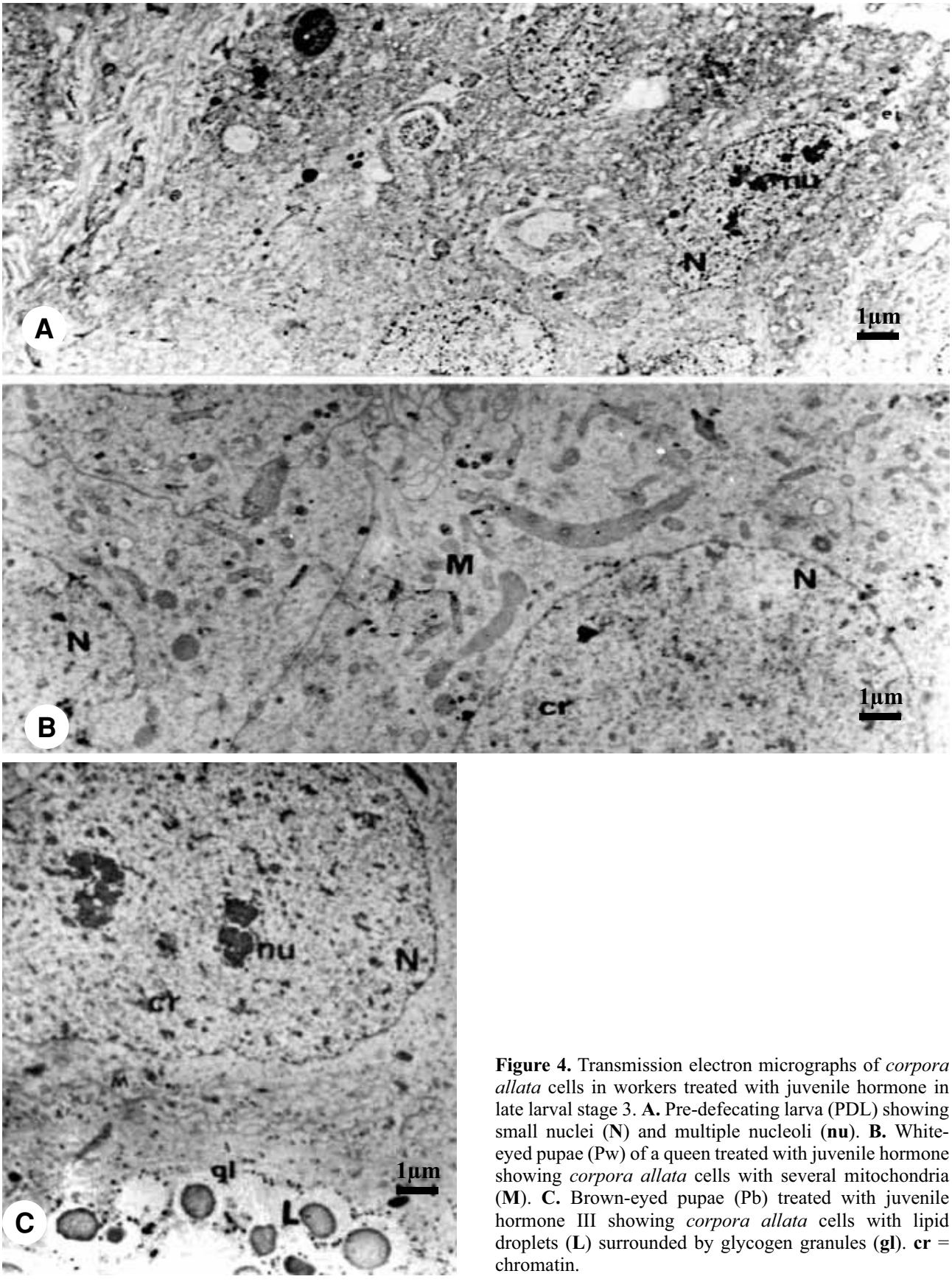

Figure 4. Transmission electron micrographs of *corpora allata* cells in workers treated with juvenile hormone in late larval stage 3. **A.** Pre-defecating larva (PDL) showing small nuclei (**N**) and multiple nucleoli (**nu**). **B.** White-eyed pupae (Pw) of a queen treated with juvenile hormone showing *corpora allata* cells with several mitochondria (**M**). **C.** Brown-eyed pupae (Pb) treated with juvenile hormone III showing *corpora allata* cells with lipid droplets (**L**) surrounded by glycogen granules (**gl**). **cr** = chromatin.

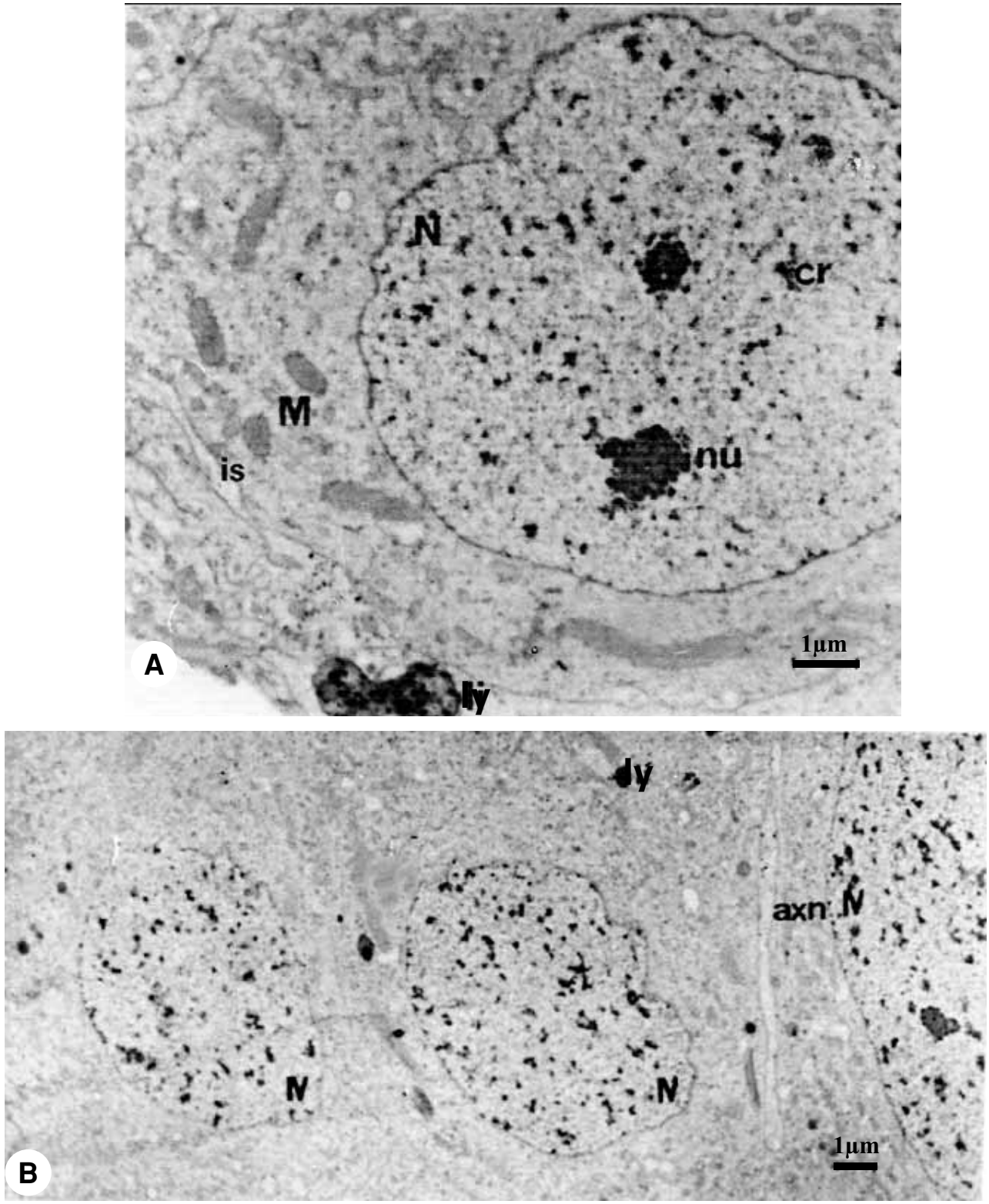

Figure 5. Transmission electron micrographs of *corpora allata* cells in *M. quadrifasciata* black-eyed queen pupae with a pigmented body (Pdl) treated with juvenile hormone. Panel **A** shows a detail of the intercellular space (**is**) and panel **B** shows the absence of mitochondria. **axn** = neurosecretory axon, **cr** = chromatin, **M** = mitochondria, **N** = nucleus, **nu** = nucleol, **is** = intercellular space, **ly** = lysosome.

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