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HAEMOCYTES OF THREE SCALE INSECT SPECIES: *PHENACOCCUS GOSSYPII* TOWNSEND & COCKERELL, *PSEUDOCOCCUS LONGISPINUS* (TARGIONI TOZZETTI) AND *DACTYLOPIUS CONFUSUS* (COCKERELL) (HEMIPTERA: COCCOIDEA).

ABSTRACT

HAEMOCYTES OF THREE SCALE INSECT SPECIES: *PHENACOCCUS GOSSYPII* TOWNSEND & COCKERELL, *PSEUDOCOCCUS LONGISPINUS* (TARGIONI TOZZETTI) AND *DACTYLOPIUS CONFUSUS* (COCKERELL) (HEMIPTERA: COCCOIDEA).

An evaluation of the haemocytes in the cochineal scale, *Dactylopius confusus* (Cockerell), was completed and compared with those found in the mealybugs *Phenacoccus gossypii* Townsend & Cockerell and *Pseudococcus longispinus* (Targioni Tozzetti) to assess the potential sites of the dye pigment source. Four basic cell types were found in the two pseudococcids and five in the cochineal scale. The cell types common to all species included: prohaemocytes, oenocytoids, typical granulocytes and plasmatocytes. In addition, a modified granulocyte (poly-glyco-based granulocyte) was found to be specific to the cochineal scale and this produced rough endoplasmic reticulum derived granules that may be the source for the synthesis of carminic acid.

Key words: function, *Coleus*, *Philodendron*, *Opuntia*, unknown cell type, haemolymph.

INTRODUCTION

The haemolymph in insects not only functions to transport nutrients, hormones and other components throughout the body but, in several species, also functions to protect the individual from parasites or predators. Six primary cell types are recognized in the insect species examined in the past (Gupta, 1991), with the cell types present often depending upon the taxa, stage of development and state of health of the insect. Although substantial research on the haemocytes of insects in different orders exists, only a few studies have been conducted on scale insects (Joshi & Lambdin, 1996: Poisson & Pesson, 1937: Tauber & Yeager, 1935: Yadava, 1967). Historically, the unique red dye derived from the cochineal scale was used in textile, medical and agricultural products in the United States until the mid-1960's. Although the more permanent and more economically produced aniline dyes

that eventually replaced the organic-based dyes are beneficial, concern has arisen regarding their disposal and their accumulation in streams and underground water. Attention is beginning to focus once again on organic-based materials as they might be more environmentally compatible, causing fewer allergic reactions upon contact and possibly breaking down more rapidly in the environment once materials containing the dye are discarded; however, these last two points still need clarification. Our objectives of this study were to assess the haemocytes of three scale insects to determine whether the same haemocytes were common to all three species and to investigate the potential source of the dye in the cochineal scale.

MATERIAL AND METHODS

Populations of three scale insect species (the mealybugs *Phenacoccus gossypii* Townsend and Cockerell and *Pseudococcus longispinus* (Targioni Tozzetti), and the cochineal *Dactylopius confusus* (Cockerell)) were maintained on *Coleus* sp., *Philodendron* sp. and *Opuntia* sp., respectively. Haemocytes were obtained by bleeding adult females (20-24 per species) and processing the haemolymph to obtain sections to view under the transmission electron microscope (TEM) (Joshi & Lambdin, 1996). Histochemical tests for glycogen, mucoproteins and glycoproteins were made on 1µm thick sections and on blood smears by the Periodic acid-Schiff (PAS) reaction, with amylase and dimedone used as control treatments.

RESULTS

We observed four typical haemocyte types (prohaemocytes, typical granulocytes, oenocytoids and plasmatocytes) in all three insects. However, in addition to these, a fifth cell type, a modified granulocyte (poly-glyco-based) was observed in *D. confusus*.

Prohaemocytes (Fig. 1a): round to oval stem cells, characterized by the lack of differentiation of sub-cellular material, with a large medial nucleus occupying most of the cell and containing a distinct nucleolus, and a poorly developed rough endoplasmic reticulum (RER) system. From the PAS test, these cells took on a pink colour, indicating a high cytoplasmic density possessing some glycogen components. These cells were few in number and, when found, were amassed in clusters.

Oenocytoids (Fig. 1b): the second most abundant haemocyte type in the haemolymph of all three species examined. Distinguished by their oval to oblong appearance, a small central or exocentric nucleus, well-developed

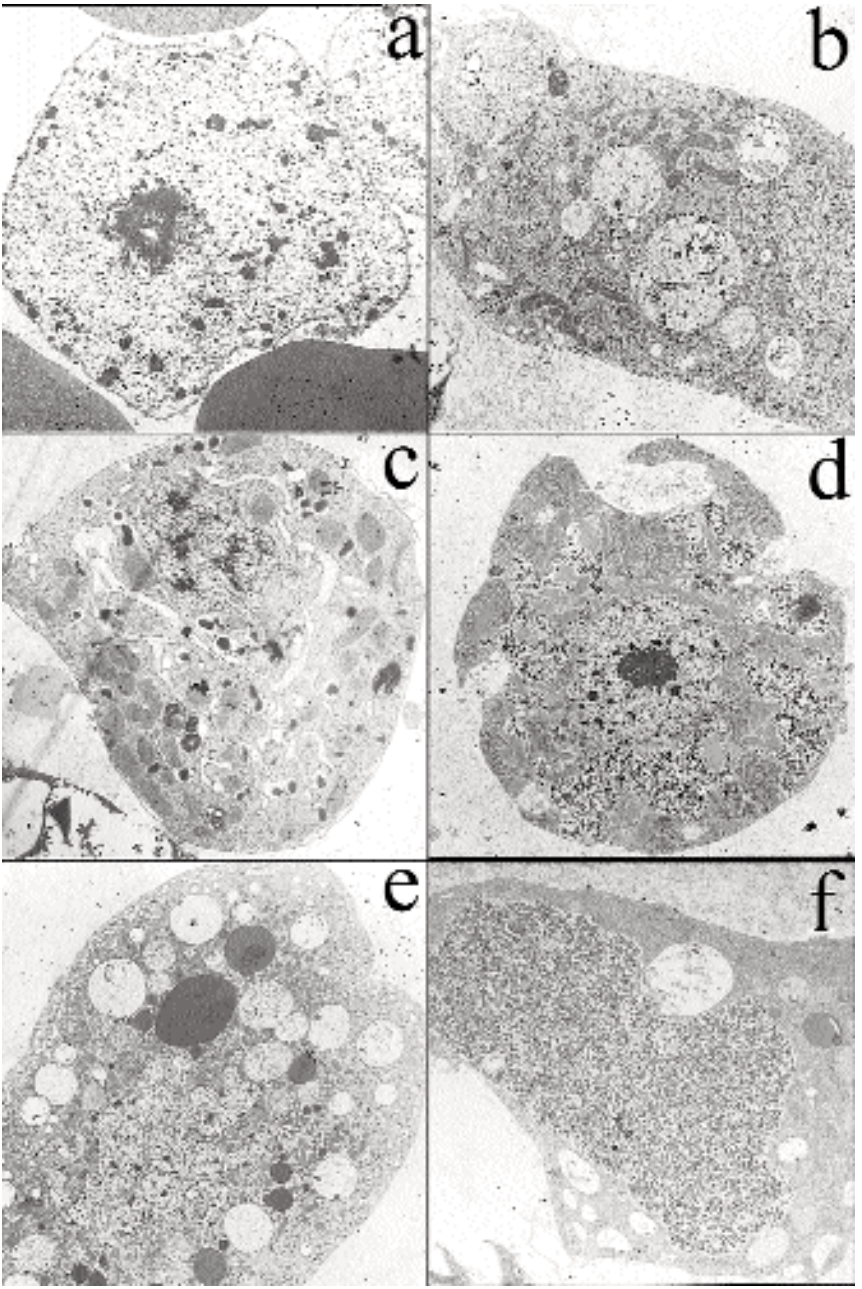

Figure 1 a-f. Haemocytes: a, prohaemocyte; b, oenocytoid; c, typical granulocyte; d, modified granulocyte; e, plasmatocyte; f, undetermined cell type.

mitochondria, a moderate smooth endoplasmic reticulum (SER) and RER systems, and the presence of large crystals within the vacuoles. Like the typical granulocytes, these crystals stained metachromatically with toluidine blue and are PAS-negative. As such, they have a different chemical nature and functional role to the granules observed in the modified granulocytes (i.e. storage of excretory products). Release of crystals into the haemolymph also indicates an additional role for these crystals in this species.

Histochemical tests with PAS provided a positive colour reaction within the cytoplasm of oenocytoids. The colour reaction of these granules was less than that for the modified granulocytes. The granules appeared to be synthesized on the polyribosomes on the RER membrane. The crystals aggregated within the vascular spaces of the more mature cells and were eventually deposited into the haemolymph. Numerous free crystals, which did not have a colour reaction with the PAS test, were found within the haemolymph.

Typical Granulocytes (Fig. 1c): observed in the two mealybug species. Subcircular, with a central nucleus, well-developed RER and SER systems, numerous mitochondria and SER-originated membrane-bounded granules. These granules stained metachromatically with toluidine blue inferring that the mode of origin and structure of the large granules was different from that of the rosette-shaped granules common to the modified granulocytes.

Modified granulocytes (Figs. 1d): absent in the mealybug species but by far the most abundant haemocyte population in adult female *D. confusus*. Distinguished by the numerous mitochondria and well-developed RER, producing numerous, rosette-like, fine granules within the cytoplasm. Based on histochemical data from the PAS test, a positive colour reaction occurred, ranging from a light pink in the cytoplasmic area to a high intensity pink found in the dense granules. As such, these fine granules appeared to be glycogen or glycoprotein in nature. No colour reaction occurred in the control treatment when subjected to the PAS histochemical test.

Plasmatocytes (Fig. 1e): the largest haemocytes observed, distinguished by their ovoid to amoeboid shape. Plasmatocytes possess a large, elongated and lobed nucleus and numerous small vesicles along the periphery of the plasma membrane. Histochemical tests with PAS did not provide a colour reaction either in the cytoplasm or vascular content, while toluidine blue heavily stained these structures. Also, the cytoplasm and vacuoles did not stain metachromatically with toluidine blue. These cells were capable of surrounding or engulfing foreign particles and probably function as one of the first lines of defense against potential disease-causing agents.

An **undetermined cell** (Fig. 1f) was observed in the haemolymph of the cochineal insect. The large nucleus occupying much of the cell and the numerous mitochondria present in the cytoplasm were the most pronounced traits of this cell and were unlike that found in the prohaemocytes.

DISCUSSION

Based on histochemical tests and observations at the TEM level, four types of haemocyte were identified in adult female *Phenacoccus gossypii* and *Pseudococcus longispinus* and five types in the cochineal scale. It would appear that the cell types present may differ depending on the species. It is unclear why these differences exist. The selected genetic trait for this modified granulocyte may have originally developed as a result of feeding activity by species of *Dactylopius* on *Opuntia* and may now aid in protection against natural enemies and invading foreign agents. The special, modified granulocytes are believed to perform a synthetic and secretory function in the cochineal scale. That this modified granulocyte is the source of the proteins responsible for the colour of the haemolymph is supported by the production of fine particles, which are synthesized along the length of, and secreted from, the RER into vacuoles, and by the exocytosis of these particles in a mass into the haemolymph.

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