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ULTRASTRUCTURAL OBSERVATIONS ON SYMBIONT DEGENERATION IN THE MALE LINE OF *PSEUDAULACASPIS PENTAGONA* (TARGIONI TOZZETTI) (HEMIPTERA: COCCOIDEA: DIASPIDIDAE).

ABSTRACT

ULTRASTRUCTURAL OBSERVATIONS ON SYMBIONT DEGENERATION IN THE MALE LINE OF *PSEUDAULACASPIS PENTAGONA* (TARGIONI TOZZETTI) (HEMIPTERA: COCCOIDEA: DIASPIDIDAE).

During the development of the immature stages of male scale insects, the mouthparts become lost at the prepupal stage and this is paralleled by the degeneration of the symbiont micro-organisms inhabiting the mycetocytes. This degenerative process has been studied in the male line of the white peach scale, *Pseudaulacaspis pentagona* (Targioni Tozzetti). In the first two feeding instars, the mycetocytes appear as spherical cells, 30-40µm in diameter, filled with normal micro-organisms, round or oval in shape, 3-5µm long. In the prepupal and pupal stages, some symbionts undergo degeneration by a dissolution of the dense ribosomal granulations which characterize the microorganisms in the two feeding instars. In these symbionts, the fusing of the small vacuoles results in the appearance of larger, more centrally-placed, vacuoles. Other symbionts become loosely reticulated or, alternatively, condensed or contracted, to form regular or irregular bodies. The mycetocytes seem to undergo no reduction in size but their cytoplasm shows signs of dissolution as well. A great number of dense, crystalline-like granulations were found in their proximity. The nature and derivation of these granulations remains to be investigated.

INTRODUCTION

The endosymbionts of Coccoidea have been very little investigated using modern technology, even though this could shed new light on their identity. Results based on the study of 16s rDNA sequences are available only for three pseudococcid species and these indicate that mealybug endosymbionts are members of the ß-subdivision of the Proteobacteria (Munson *et al*., 1992), supporting the idea that endosymbionts of all known mealybugs might be derived from a single ancestral infection, in a manner similar to that which has been demonstrated for the endosymbionts of all aphid species investigated to-date (Baumann *et al*., 1998). The endosymbionts of Diaspididae were first considered to be yeast-like micro-organisms, but subsequent studies have suggested that they might be pleomorphic bacteroids (Tremblay, 1990) and circumstantial evidence indicates that they do not belong to the pseudococcid lineage. In our opinion, this idea is consistent

with the hypothesis that endosymbiont phylogeny may correspond to the phylogeny of the host insect, in small taxon groups at least, due to a single ancestral infection and a lack of endosymbiont exchanges between host lineages, as suggested by Baumann *et al.* (1998).

In diaspidids, the symbionts are localized in specific large cells (mycetocytes or, perhaps, bacteriocytes) dispersed throughout the body of both sexes, especially during the juvenile stages. In an early study by one of the authors (Tremblay, 1960), the mycetocytes of the white peach scale, *Pseudaulacaspis pentagona* (Targioni Tozzetti) were found to decrease in number and to degenerate during development in the male. This phenomenon was found to be correlated with the atrophication of the mouthparts of Coccoidea males. Some data on the ultrastructure of the female *P. pentagona* endosymbionts were given in a paper presented at ISSIS-V in Portici (Tremblay & Ponzi, 1987). Information on the ultrastructure of the degenerating male symbionts of this species is now provided.

MATERIALS AND METHODS

Male nymphs and newly emerged adults of *P. pentagona* were fixed for 5 hours at room temperature in 6% glutaraldehyde I 0.05M sodium phosphate buffer at pH 7.2. Before fixation, the nymphs were punctured on the apex of the head while the adult males were decapitated after dealation. The specimens were then rinsed for 10h in the same buffer and post-fixed for a further 5h in buffered 1% OSO4. After dehydration with a graded ethanol series, the material was treated (3h) with propylene oxide and embedded in consecutive concentrations (3:1; 1:1; 1:3) of a mixture propylene oxide-Epon Araldite. Treatments with each mixture lasted 12 hours and were followed by two other 12h inclusions in pure Epon Araldite and by a final inclusion in this resin in gelatin capsules. Ultrathin and thin (1-2µm) sections were obtained with a LKB Ultratome III and stained in 5% uranyle acetate (1h) and lead citrate (5min). Thin sections were observed and photographed with a Zeiss Axiophot microscope. Ultrathin sections were examined and photographed with a Philips EM 300 electron microscope at 80kV.

RESULTS

In the 1st- and 2nd-nymphal instars of the male, the mycetocytes were mostly localized in the peripheral part of the body and appeared as large spherical cells, 30-40µm in diameter, with a large nucleus and filled with round or oval 3-5 µm long microorganisms, characterized by dense ribosomal granulations (Fig. 1). These cells were surrounded by adipocytes (ad) and

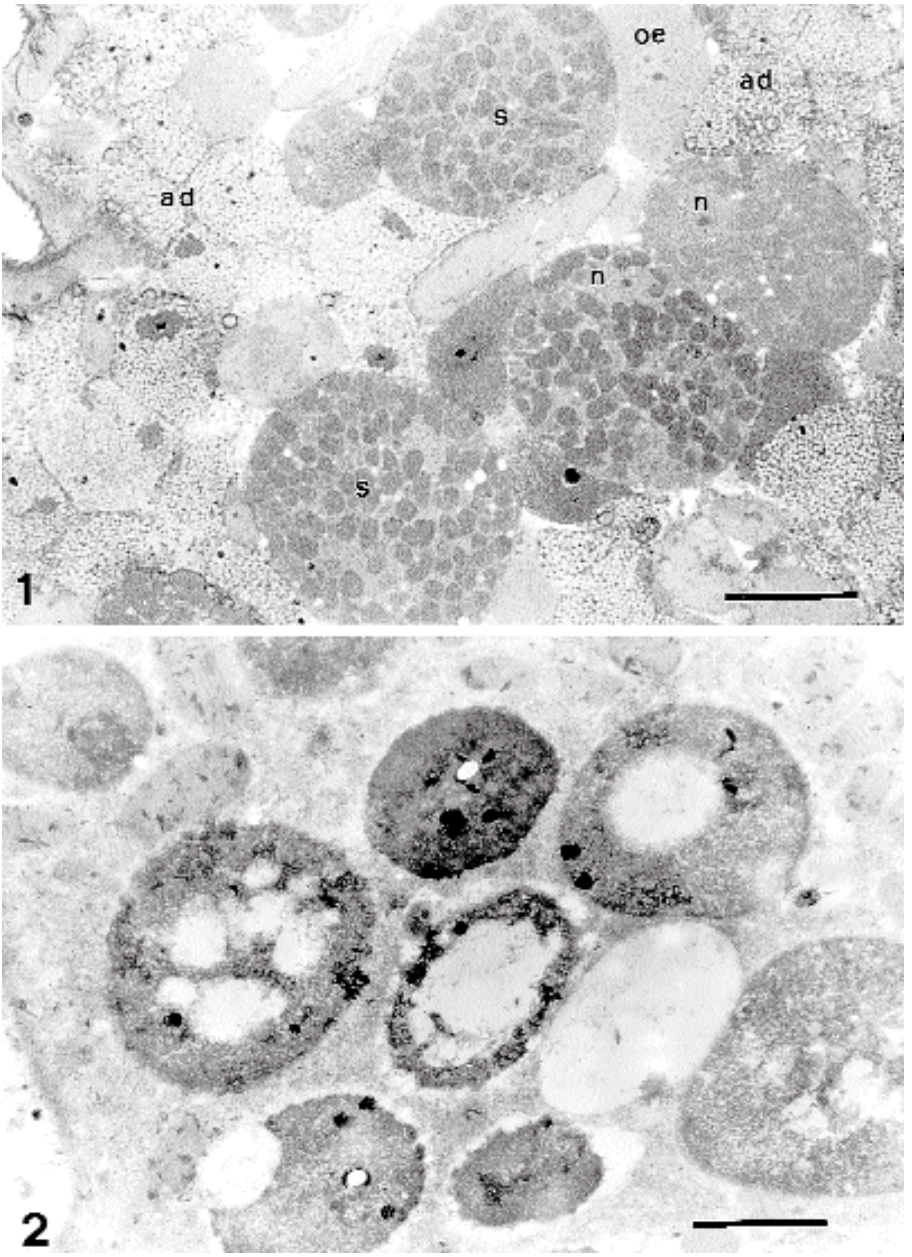
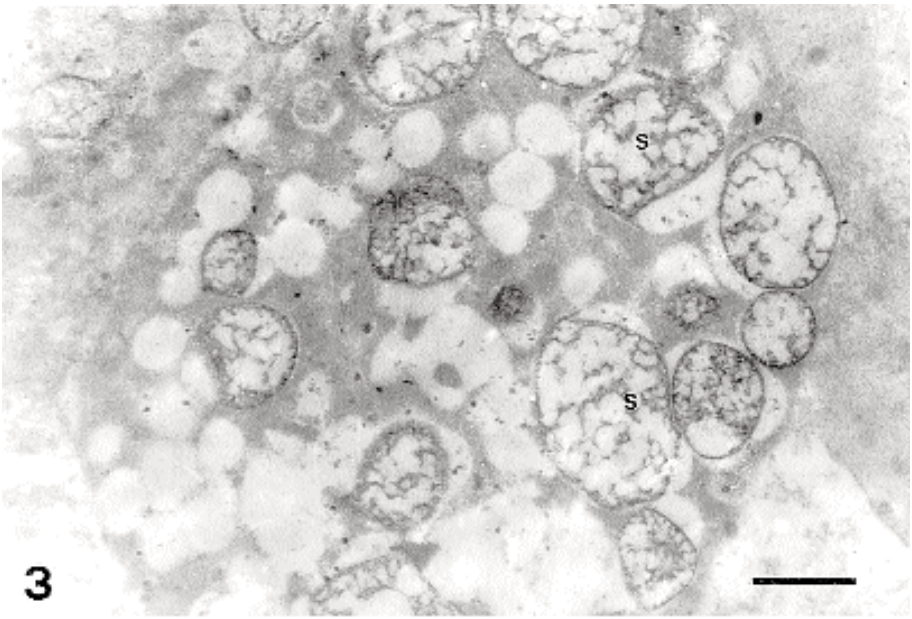


Fig.1. Section of a 2nd-instar male nymph showing some mycetocytes; ad, adipocytes; oe, oenocytes; n, mycetocyte nucleus; s, symbionts. Bar length: 20µm.
Fig. 2. Degenerating symbionts in a male pupa. Bar length: 2µm.

oenocytes (oe) in most sections. At this stage of development, their shape and localization did not differ from those observed in females. However, in the prepupae and pupae, clear signs of degeneration appeared, both in the endosymbionts and in the mycetocyte structure. Some microorganisms initially showed some dissolution of their dense ribosomal granulation followed by the formation of large, central vacuoles (Fig. 2). Other vacuoles became apparent in the mycetocyte cytoplasm as well. In addition, other symbionts became loosely reticulated or, alternatively, condensed or contracted, to form regular or irregular bodies (Fig. 3). In the adult male, the mycetocytes showed no reduction in size when compared with those of the nymphs or the adult female but their cytoplasm did show some signs of dissolving (Fig. 4). The final stage of these degenerative processes seems to be the dissolution of the internal mycetocyte structure. In the sections, a great number of dense, crystalline-like granulations (cl) were found in the proximity of the degenerating mycetocytes.

DISCUSSION

Recently Prameelarani Kantheti *et al.* (1996), while studying the expression of an endosymbiont 16S rRNA gene in the mealybug *Planococcus lilacinus* (Cockerell), discovered that male adults of this species were free or nearly free of endosymbionts. These conclusions were confirmed by microscopic examination of adult males, which were devoid of mycetome-like structures. A study of the embryos, 1st- and 2nd-nymphal instars of both sexes, and the adult female using the same gene probe, revealed the presence of endosymbionts. In the scale insect genus *Stictococcus* Cockerell, males are dwarf, lack mouthparts and are symbiont-free from their earliest embryonic stages (Buchner, 1955) because, in the mother's ovaries, the symbionts infect only female eggs. Buchner (1955) considered that the symbionts were responsible for sex determination in this scale. In the gall-forming aphids of the genus *Pemphigus* Hartig, both the sexuales (female and male) are dwarf and without mouthparts but only the males lack symbionts. Immunodetection of symbionin, a symbiont specific protein, showed that in a group of eusocial aphids, males and soldiers lacked symbionts, which were, in contrast, regularly present in the reproductive females (Fukatsu & Ishikawa, 1992). On the other hand, the soldiers of aphids which suck phloem possess symbionts. One explanation for all these observations is the "host's selection hypothesis", which assumes that the host rejects the symbionts when these are no longer necessary because they cost energy and resources (Fukatsu & Ishikawa,



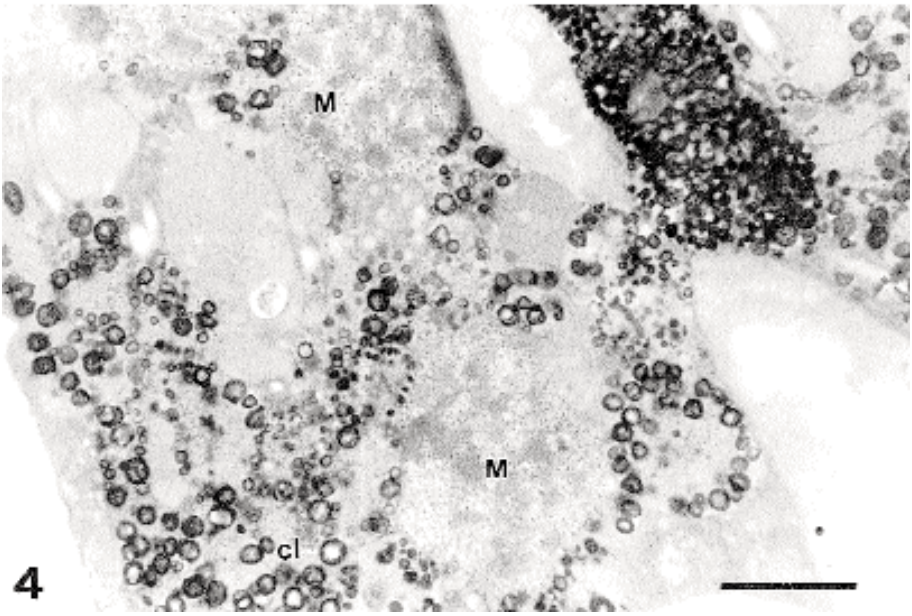


Fig. 3. Degenerating symbionts in a male adult. Bar length: 3µm.
Fig. 4. Mycetocytes (M) of an adult male; cl, crystalline-like inclusions. Bar length: 20µm.

1992). Evidence for symbiont degradation apparently controlled by the host insect was obtained by Hinde (1971) for some aphids and their symbionts. The breakdown of symbionts was attributed by Hinde to the action of insect lysosomes and is considered to be a form of regulation of symbiont number and activity by the host insect. Residual bodies in the form of dense and small myelin figures were also reported by Hinde (1971) as a result of lysosome action. These bodies showed some similarity to the crystalline-like elements found by us in *P. pentagona* males (Fig. 4). In addition, similar crystalline granulations, of a possible excretory nature, were found in the alimentary canal and malpighian tubules of both sexes of *Quadraspidiotus ostreaeformis* (Curtis) by Bielenin & Weglarska (1967). Other speculative ideas on the nature of the crystalline-like inclusions were presented by Tremblay (1960). However, at present their origin is still obscure and deserves further investigation.

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