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Multiple bacteroids in the bacteriome of the lantern bug *Pyrops candelaria* Linn. (Homoptera: Fulgoridae)

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Abstract Bacteriome in the lantern bug *Pyrops candelaria* harbored *a*-, *t*-, and companion bacteroids. The *a*- and *t*-bacteroids were irregular bodies, whereas the companion bacteroids were rod-shaped and easily distinguished from the others. The *a*- and *t*-bacteroids were enveloped by three membranes and the companion bacteroids, by two membranes. The cytoplasm of the *a*-bacteroid contained electron-dense bodies.

Introduction

All homopteran insects that feed on plant sap possess symbiotic microorganisms associated with the fat body or in the hemocoel. In most cases the microorganisms are located in specialized insect cells, the mycetocytes, which may be aggregated together to form a discrete organ called the mycetome (Douglas 1988).

Buchner (1965) categorized the homopteran intracellular symbionts by letters of the alphabet as follows: *a*, *b*, *e*, *f*, *g*, *h*, and *t*. The *h* symbionts are yeasts and the remaining ones are prokaryotes. The *a*-symbionts apparently occur in all species. The insects become di- or trisymbiotic when they further acquire *h*- or *t*-symbionts and/or companion symbionts (Chang and Musgrave 1972).

Many workers have studied the ultrastructure of mycetome symbionts of homopteran insects. Most leafhoppers possess two kinds of symbionts, usually including the prokaryotic *a*- and *t*-symbionts (Körner 1969; Douglas 1988), and some leafhoppers have three (Mitsuhashi and Kono 1975) or four kinds of symbionts (Chang and Musgrave 1972). In aphids the inclusions of the mycetocytes have been purported to be either yeast or bacteria (Lamb and Hinde 1967), one kind of symbiont (McLean and Houk 1973), or two kinds of symbionts (Hinde 1971; Griffiths and Beck 1973). In the planthopper, yeast-like symbionts have been reported by Noda (1977). In Pysllidae, two kinds of symbionts, namely, *x* and *y*, have been found (Chang and Musgrave 1969; Waku and Endo 1987). In cicada, primary *a*, auxiliary symbiont *t*, and accessory symbiont KR_E have been demonstrated (Schwemmler 1980).

Preliminary research on the mycetome symbionts of fulgoroid insects was carried out by Müller (1940), but his work was carried out at the light microscopy level. Buchner (1965) has mentioned that *Fulgora europaea* contains an *m*-organ as well as paired *a*- and *x*-organs. To date the ultrastructure of the mycetome (i.e., bacteriome) and its symbionts (i.e., bacteroids) of *Pyrops candelaria* have not yet been investigated.

The objective of the present study was to describe the fine structure of multiple bacteroids in the bacteriome of *P. candelaria* and to understand the relationship between bacteroids and their host.

Materials and methods

Adults of the lantern bug *Pyrops candelaria* Linn. were collected from Lam Chuen and The Chinese University campus in the New Territories of Hong Kong. A red-colored bacteriome was dissected out in 0.2 *M* phosphate buffer (pH 7.2, with 5.4% glucose added), fixed in 2.5% glutaraldehyde in 0.2 *M* phosphate buffer at 4 °C for 1 h, and postfixed in 1% osmium tetroxide in phosphate buffer. Tissues were dehydrated in ascending series of alcohol, infiltrated with acetone and Spurr resin (1:1), and embedded in pure Spurr resin. Sections were cut with a Reichert ultratome and were stained with uranyl acetate and lead citrate. Sections were observed under a JEOL-JEM 1200 EXII electron microscope.

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Results

Histology of the bacteriome

The bacteriome of *Pyrops candelaria* was located laterally in the posterior part of the abdomen. It consisted of bacteriocytes. Three kinds of bacteroids were found in the cytoplasm of bacteriocytes. *a*-Bacteroids were irregular in shape, whereas *t*-bacteroids were slightly oval and companion bacteroids were rod-shaped. A cellular membrane enveloped all of them (Fig. 1).

Ultrastructure of the bacteroids

a-Bacteroids

These had an oval or amorphous granular appearance. Their size ranged from 5 to 8 μm in length and from 0.94 to 3.53 μm in width. The cytoplasm contained ribosome-like particles and electron-dense bodies. In the cytoplasm of the bacteriocyte a nucleus with scattered chromatin material, mitochondria, and rough endoplasmic reticula were present (Fig. 2). An *a*-bacteroid was surrounded by three membranes comprising (1) a membrane envelope on the outside, (2) the cell wall, and (3) an internal plasma membrane of unit membrane construction (Fig. 3). The *a*-bacteroid reproduced by binary fission. Two daughter cells might share the same membrane envelope immediately after cell division. Occasionally, residual bodies were found in the cytoplasm of the bacteriocyte (Fig. 4). They were presumably formed from the breakdown of bacteroids.

t-Bacteroids

These had large irregular or spherical bodies. Their size ranged from 4.94 to 9.65 μm in length and from 0.47 to 4.94 μm in width. Ribosome-like particles typically massed on the periphery of the cytoplasm and were less dense in the central part. A *t*-bacteroid was also enveloped by three membranes. The membrane envelope was tightly bound to the cell wall (Fig. 5). *t*-Bacteroids probably propagated by binary fission, and in all sections, various stages of this process could be seen.

Companion bacteroids

Under the electron microscope the rod-shaped companion bacteroids were easily distinguished from *a*- and *t*-bacteroids. A cellular membrane clearly surrounded the bacteriome. The size of companion bacteroids ranged from 8.33 to 21.67 μm in length and from 2.5 to 4.17 μm in width. The cytoplasm was more uniform in appearance than that of the *a*- and *t*-bacteroids. Ribosome-like particles were distributed throughout the cytoplasm. The bacteroid was enclosed by only two membranes. An electron-transparent space was seen between them (Fig. 6). Companion bacteroids also divided by binary fission.

Myelin figures were present in the cytoplasm. These structures were related to the lysosomal breakdown of bacteroids. Bodies resembling lysosomes occurred in the cytoplasm of the bacteriocyte. These bodies were attached to the membrane envelope and presumably discharged enzymes into the space around the bacteroid (Fig. 7). The bacteroid had shrunk away firstly from the membrane envelope and was very irregular in outline. The cytoplasm was more electron-dense. Sometimes, several degradative bacteroids were found inside one membrane envelope (Fig. 8). Associated with this, some of the cytoplasm was vacuolated and several rings of membrane appeared. This was a primary residual body. With the breakdown of plasma membrane, the bacteroid finally formed a residual body, which condensed to form a myelin figure.

Discussion

Homopteran insects are often multisymbiotic (Chang and Musgrave 1972). As many as five different symbionts have been described in one host of *Oliarus* (Müller 1940). The bacteriome of *Pyrops candelaria* possesses three kinds of bacteroids: *a*-, *t*-, and companion bacteroids.

The *a*-symbionts in *Helochara communis* are irregular, dark granular bodies. Each is enclosed by three peripheral membranes. The symbionts contain ribosome-like particles and amorphous dense materials. Occasionally, dense bodies or spherical inclusions are present. They have never seen any evidence of DNA (Chang and Musgrave 1972). The *a*-bacteroids of *P. candelaria* are also irregular in shape and enclosed by three membranes; dense bodies are present in the cytoplasm. The *a*-symbiont of *Nephotettix cincticeps* is surrounded by two thin membranes and contains a small electron-dense inclusion in its cytoplasm (Mitsuhashi and Kono 1975). While a bigger amorphous dense body is present in the matrix of the *a*-bacteroid of *P. candelaria*, a smaller one is sometimes found. In embryos of the leafhopper *Euscelis plebejas* (Körner 1969), *a*-symbionts contain crystalline bodies or tubular membrane bodies. The *a*-symbiont in cabbage aphids *Brevicoryne brassicae* is surrounded by a pair of unit membranes (Lamb and Hinde 1967). In the center of the cytoplasm a large osmiophilic area has been found. In most cases, homopteran symbionts reproduce by a process of binary fission, usually without formation of cross-walls (Houk and Griffiths 1980). *a*-Bacteroids of *P. candelaria* also divide by binary fission.

Only the *a*-symbionts can be present alone in the host, and the type *t*-symbionts, when present, are always associated with it (Schwemmler 1980). *t*-Symbionts normally appear as large irregular or spherical bodies, and each consists of a granular matrix, a nuclear area, and an enclosing membrane. They are surrounded by

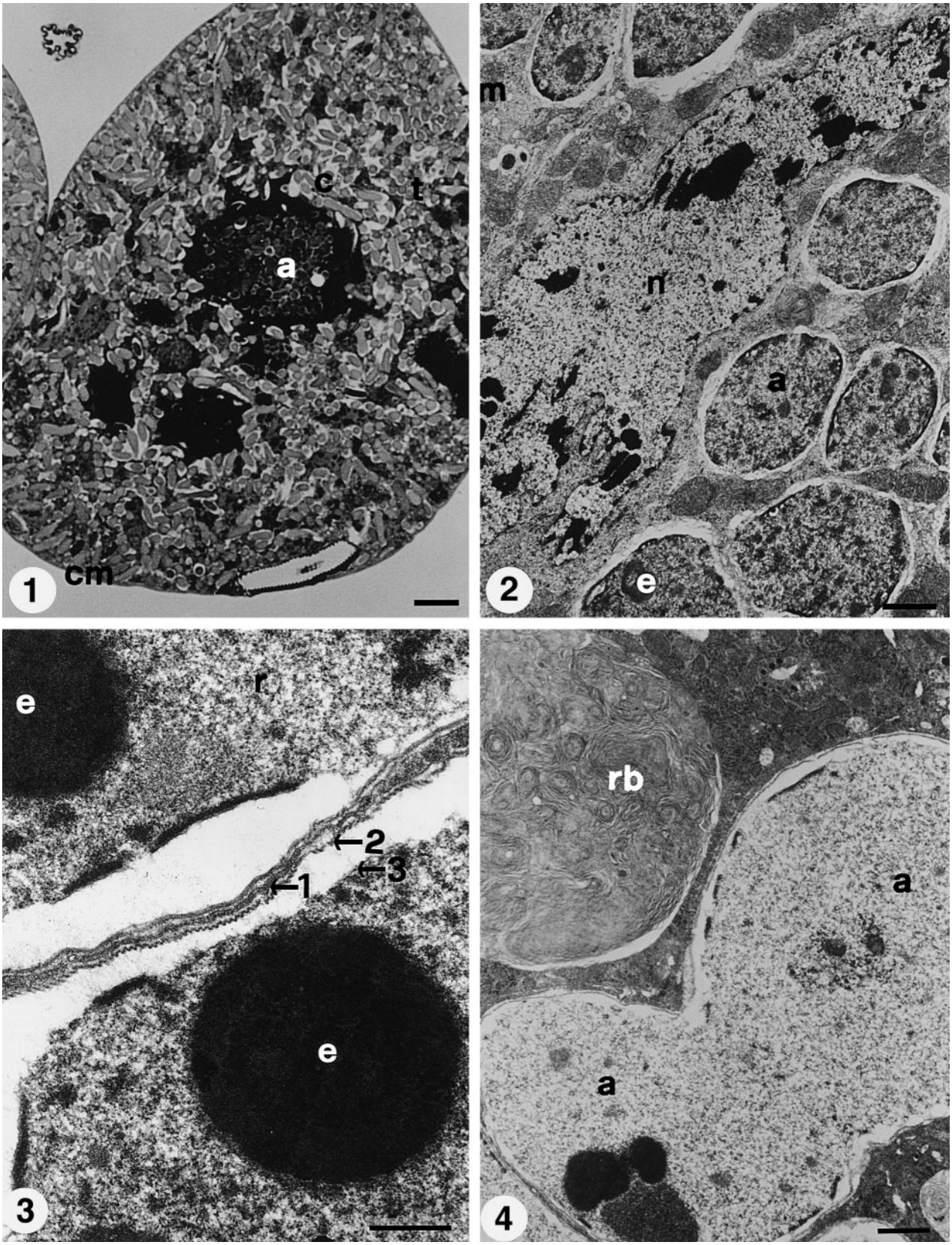
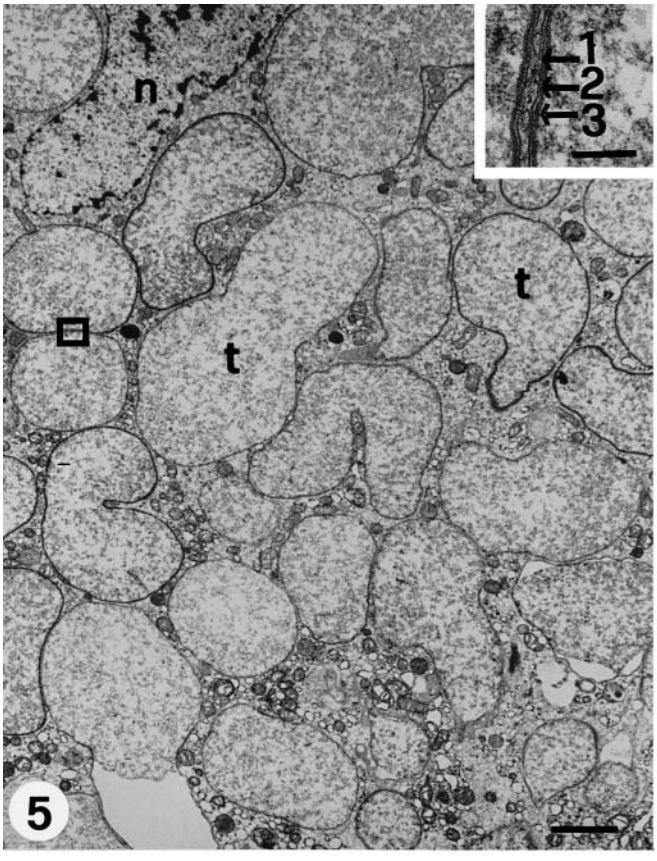


Fig. 1 Light micrograph of a bacteriome, showing *a*-bacteroids (*a*), *t*-bacteroids (*t*), companion bacteroids (*c*), and the covering cellular membrane (*cm*). *Bar* = 5 μm. **Fig. 2** Transmission electron micrograph showing *a*-bacteroids (*a*), nucleus (*n*), mitochondria (*m*), and electron-dense bodies (*e*). *Bar* = 1 μm. **Fig. 3** Transmission electron micrograph showing the bacteroid membrane envelope (*1*), cell wall (*2*), plasma membrane (*3*), ribosome-like particles (*r*), and electron-dense bodies (*e*). *Bar* = 0.5 μm. **Fig. 4** Transmission electron micrograph showing dividing bacteroids (*a*) and a residual body (*rb*). *Bar* = 1 μm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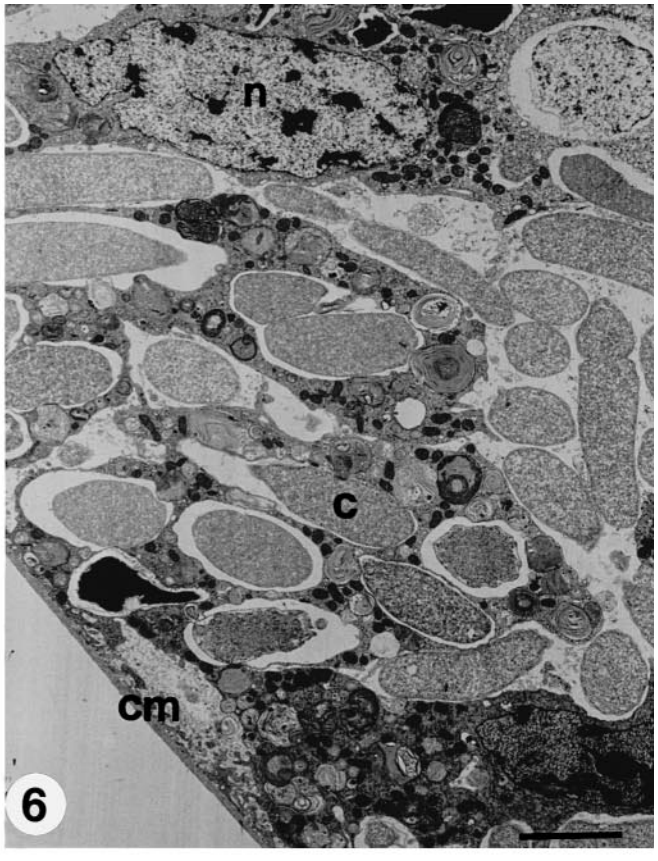
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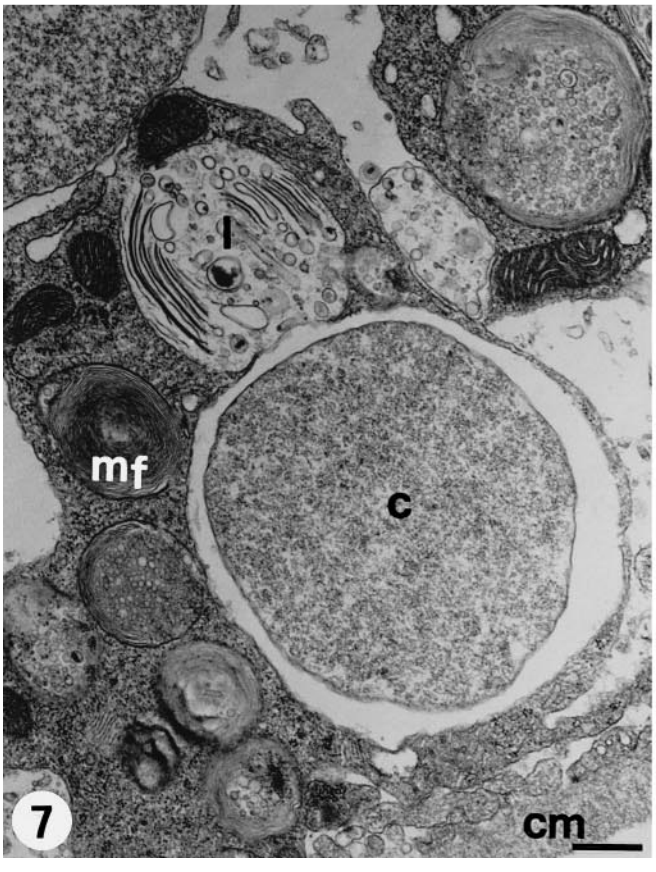
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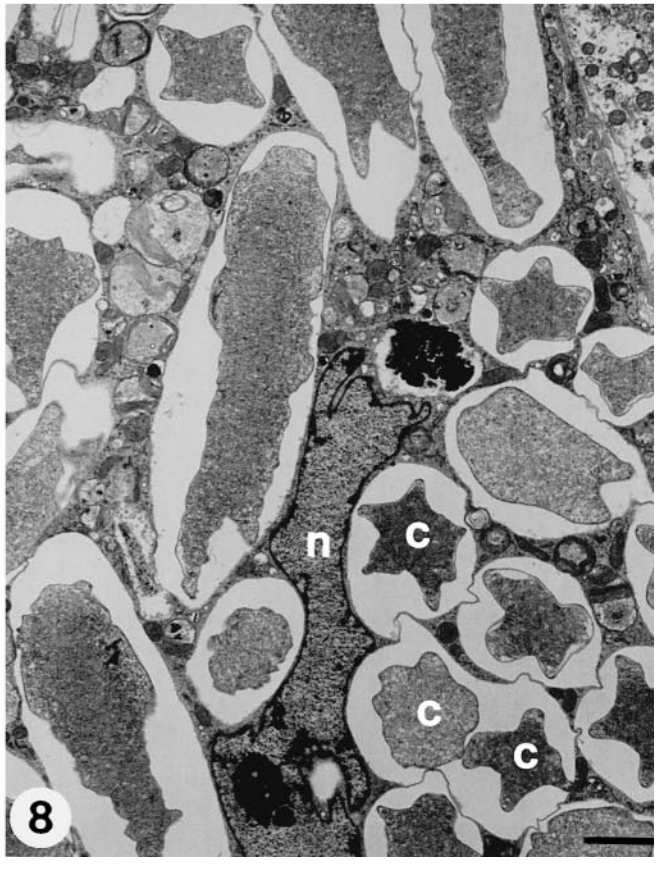
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Fig. 5 Transmission electron micrograph of *t*-bacteroids (*t*) and the nucleus (*n*). *Bar* = 2 μm. The area marked with the *rectangle* is magnified in the inset. *Inset*: Bacteroid membrane envelope (*1*), cell wall (*2*), and plasma membrane (*3*). *Bar* = 0.5 μm. **Fig. 6** Transmission electron micrograph of companion bacteroids (*c*), showing the nucleus (*n*) and covering cellular membrane (*cm*). *Bar* = 5 μm. **Fig. 7** Transmission electron micrograph showing bacteroids (*c*), a lysosome (*l*), a myelin figure (*mf*), and the covering cellular membrane (*cm*). *Bar* = 0.5 μm. **Fig. 8** Transmission electron micrograph showing shrunken bacteroids (*c*) and nucleus (*n*). *Bar* = 2 μm

three membranes and possess a large, centrally located nucleoid filled with condensed fine fibrils. It contains rod-shaped crystalline inclusions (Chang and Musgrave 1972). The *t*-bacteroids of *P. candelaria* resemble those of *H. communis* (Chang and Musgrave 1972). However, the cytoplasm does not contain inclusions except for ribosome-like particles. The *t*-symbiont of the green rice leafhopper *N. cincticeps* is enveloped by only two membranes, and electron-dense inclusion bodies have been seen in the matrix (Mitsuhashi and Kono 1975). The *t*-symbionts might be L-phase bacteria, but the *a*-symbionts probably derive from *t*-symbionts (Chang and Musgrave 1972).

Companion symbionts in the leafhopper *H. communis* are elongated bodies located in the mycetomal epithelium. They are enveloped by three parts: an inner membrane, an intermediate zone, and an outer membrane. In their cytoplasm, centrally located nucleoids and parallel striations have often been observed (Chang and Musgrave 1972). Companion bacteroids in the bacteriome of *P. candelaria* are slightly different from those in *H. communis*. They are surrounded by two membranes. An electron-transparent space, not an intermediate zone, is present between the two membranes. The cytoplasm contains neither nucleoids nor parallel striations.

The companion symbionts show certain similarities with rickettsiae (Chang and Musgrave 1972), such as rickettsia-like organisms in the green rice leafhopper *N. cincticeps* (Mitsuhashi and Kono 1975). They are sausage-shaped, measure 0.8–1.2 μm in length, and are enclosed by three membrane layers. Generally, rickettsiae or rickettsia-like organisms measure about 2 μm in length (Anderson et al. 1965). Companion bacteroids of *P. candelaria* are larger than rickettsiae.

Buchner (1965) concluded from extensive observations on insect symbiosis that there was a host-controlled balance between the rate of symbiont reproduction and the rate of breakdown. In any insect-endosymbiont relationship the host always appeared to be in control of the partnership. Several mechanisms have been proposed to explain the insect-endosymbiont relationship. The most clearly documented mechanism by which the mycetocytes selectively remove some symbionts is lysosomal breakdown (Houk and Griffiths 1980). If the mycetocytes were to become too crowded with symbionts, lysosomal activity could eliminate the excess (Hinde 1971). This is also true for the breakdown of bacteroids in *P. candelaria*.

The microorganisms investigated in this study are apparently harmless to their hosts, and the hosts contain these microorganisms without exception. This obligatory relationship has been proven by many authors in different insect species. Symbionts play a useful, perhaps obligatory function for the host. However, different types of symbionts may perform different functions (Griffiths and Beck 1973). This could explain the ultrastructural differences observed among *a*-, *t*-, and companion symbionts.

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