MEMBRANES ASSOCIATED WITH
THE DISINTEGRATION OF MYCETOMAL
MICRO-ORGANISMS IN SITOPHILUS ZEA-MAIS
(MOTS.) (COLEOPTERA)

A. J. MUSGRAVE AND I. GRINYER
Department of Zoology and
Department of Avian Pathology and Wildlife Diseases, University of Guelph, Ontario, Canada

SUMMARY

The mycetomal micro-organisms of Sitophilus zea-mais give evidence in electron micrographs of having two peripheral unit membranes, an intermediate layer, intracytoplasmic membranes and DNA-like material. They appear to disintegrate, at times, with the concomitant formation of membrane masses. It is suggested that these are visible with the phase-microscope and that they are probably myelin figures.

INTRODUCTION

A mycetocyte is a cell that harbours micro-organisms that supposedly benefit an insect host. In some insects mycetocytes may form a discrete structure or mycetome. In the genus Sitophilus larval mycetomes disintegrate at metamorphosis. In adults mycetocytes with organisms are found, except in aposymbiotic strains (Musgrave, 1964), in the mesentera, especially of young weevils (Musgrave, Homan & Grinyer, 1964), and in the female gonads.

The morphology (and perhaps the species) of micro-organisms seems to vary with the species of host, so that the symbiotes may be of taxonomic value (Musgrave & Homan, 1962), though there is considerable pleomorphism. In Sitophilus granarius (L.) the presence of 'nuclear equivalents' within, and of cytoplasmic membranes, and sometimes cell walls around, the typical Gram-negative organisms, and the absence of host-provided membranes have been established (Musgrave & Singh, 1965; Singh & Musgrave, 1966; Grinyer & Musgrave, 1966). Rather similar findings have been reported by Malke (1966) and Milburn (1966) regarding the somewhat atypical Gram-positive 'bacteroid' symbiotes in certain cockroaches. Malke, however, found host-provided membranes; and Milburn found 'metamorphosed bacteroids' which were swollen and had developed 'concentric layers of strands' within them.

The mycetomal micro-organisms in Sitophilus zea-mais (Mots.) (Kuschel, 1961) differ from those of S. granarius and frequently assume C-shaped or characteristic tightly spiralled forms. A study of their peripheral membranes and nuclear material...
provided an insight into the origin of masses of concentric, apparently membranous material that had frequently been observed in mycetomal material. These observations are the subject of this paper.

MATERIALS AND METHODS

Weevils were reared at approximately 27 °C and 76 % relative humidity on wheat. Mycetomes and adult mesentera dissected from the insects in drops of distilled or tap water or Bodenstein's Ringer's solution (Buck, 1953), modified Schneider's (1964) and Grace's (1962) media were examined as fresh smears by phase-contrast microscopy. Othersmears were fixed either in the vapour of 2 % osmium tetroxide for 2 h or in Bouin's solution, and then stained in Short's modification of Heidenhain's haematoxylin or Twort's stain (Ollett, 1947, 1951). Mycetomes and adult mesentera were also dissected in Wyatt’s (1956) solution without haemolymph and processed for electron microscopy according to a method already described (Grinyer & Musgrave, 1966, fixation method (b)). This material was embedded in 'Maraglas' epoxy-resin. Some of this material was sectioned at about 1.0 μ and examined with the light microscope after staining in 1 % toluidine blue by the method of Spurlock, Kattine & Freeman (1963).

OBSERVATIONS

In mycetomes from larvae the electron microscope showed the tight spiralling of some of the organisms (Fig. 1). The presence of DNA-like material, and of two trilaminate surface membranes with intermediate material, as well as intracytoplasmic membranes (Fig. 2), were indications that the organisms were Gram-negative. No septa were seen.

Masses (presumably spheroidal) of disintegrating micro-organisms occurred associated with numerous trilaminate membranes (Figs. 3-5). Apparently healthy organisms were located outside these spheroidal masses. There was evidence that as the micro-organisms disintegrated the membranes became more numerous (Fig. 4). That the membranes were a product of degeneration of the micro-organisms was suggested by the appearance of those disintegrating individually (Fig. 6). Some of the membrane masses were larger than the mycetocyte nucleus.

A generally similar situation seemed to exist in mycetocytes in the mesentera of adults.

There was no evidence of proliferation of the Golgi complex in those mycetocytes containing micro-organisms, for the latter seemed to occupy most of the available space.

Phase-microscope studies of unfixed crushed preparations of mycetomes of larvae and prepupae and of mesentera of adults often revealed objects conveniently referred to as 'lamellar masses' (Figs. 7, 8), of a size comparable with that of the spheroidal masses mentioned above, associated with mycetocytes and sometimes within them. These lamellar masses seemed non-birefringent and seemed to grow to a considerable
size (Fig. 11). It seemed that such lamellar masses were not simply the result of damage to the insect cells for, in three adult weevils that were probably more than 20 days old (Musgrave et al. 1964), the mesentera apparently contained neither recognizable micro-organisms nor lamellar masses. It seemed, too, that as the number of lamellar masses increased, the number of spiral forms decreased. The smaller lamellar masses were not disrupted by Bouin’s fixative but they appeared to be undetectable after drying.

In an attempt to discover what configuration the spheroidal and lamellar masses might manifest in fixed and stained smears, these were searched (using the electron micrographs as a guide and the helical organisms as a kind of biological indicator), for evidence of spiral organisms within spheroidal objects. Although some such objects were seen (Fig. 9) in osmium-fixed material, their true nature remained open to doubt. Spheroidal bodies with lamellar masses were clearly seen in 1 µ sections of ‘Maraglas’-embedded material (Fig. 10).

**DISCUSSION**

The various kinds of mycetomal symbiotes are generally considered to be supplying a variety of useful metabolites to their insect hosts (see Buchner, 1966; Gilmour, 1965; Henry, 1962; Musgrave, 1964; Steinhaus, 1949; and Trager, 1960). The mechanism of supply is unknown. Presumably, the symbiotes pass the metabolites into the circumambient medium or they may, additionally, perhaps, be lysed and absorbed by the host.

Although some micro-organisms were seen disintegrating in isolation, most of them seemed to disintegrate in presumptively spheroidal masses, suggesting that in mycetocytes there may be organelles from which lysing enzymes diffuse into the surrounding cytoplasm.

The relationship between objects seen by electron microscopy to those seen by phase-contrast and conventional light microscopy can, in the present state of our knowledge, be established only on the basis of circumstantial evidence. The relationship of the membrane masses of the present electron-microscope studies to the lamellar masses of the phase-contrast studies and to spheroidal objects of the fixed and stained smears must therefore be based on this kind of evidence. The electron-microscope studies have shown clearly that the disintegration of the mycetomal micro-organisms is associated with swirls of concentric membranes which often reach a size that should make them visible in the light microscope; and, indeed, lamellar masses of this size were seen by phase-contrast microscopy. It is suggested, therefore, that the membrane masses and lamellar masses may be equated. Moreover, both kinds of masses closely resemble myelin forms or myelin figures (Freeman, 1964; Frey-Wyssling, 1953), supposedly formed as the result of cell degeneration (Cedergren, 1956; Freeman, 1964), phagocytosis (Vickerman, 1962; Freeman, 1964), or as a result of changes in the protein component of the lipoprotein in cellular membranes (Freeman, 1964). Certain kinds of myelin forms grow by swelling and are at times birefringent (Frey-Wyssling, 1953; Mercer, 1962).
It seems justifiable then to speculate that the following is the sequence of events in the mycetome of *S. sea-maia*. In view of Milburn's (1966) findings it is reasonable to start with the assumption that the insect host can time its depredations on its symbiotic organisms to periods to suit its own metabolism, though it is possible that depredations are continuous, even in the larval stage. Disintegration need not proceed in all mycetocytes simultaneously. Perhaps from organelles in mycetocytes lysing enzymes diffuse into the surrounding cytoplasm, causing the disintegration of the microorganisms with consequent formation of innumerable membranes perhaps having the appearance of myelin forms. These masses of membranes perhaps grow as a result of hydration, or continuing microbial disintegration, until they are as big as, or bigger than, the nucleus of the mycetocyte. They thus become easily visible as lamellated bodies in mycetocytes in the phase-contrast microscope. As certain kinds of myelin forms (for example, those from lecithin) can grow by hydration it seems not impossible, on the evidence given in this paper, that the very large myelin figures at times seen in smears from *S. zea-mais* may be a result of symbiote degeneration.

The nature of the metabolites that its symbiotes supply to *Sitophilus* is unknown though various B vitamins may be included (Musgrave, 1964). However that may be, it seems that the myelin forms described represent one way in which the host insects derive some benefit (presumably of some generalized lipid or lipoprotein nature) from their symbiotes.

Grateful acknowledgements are made to Miss Margaret French and Mr J. Webb for technical help, to Professors K. Ronald and J. Schroder, in whose Departments the work was done, and to the National Research Council of Canada for an operating grant to one of us (A. J. M.) and a grant for purchase of an electron microscope.

REFERENCES


Mycetomal micro-organisms


(Received 24 April 1967—Revised 5 July 1967)
Fig. 1. Electron micrograph of most of a spiral organism in section. From a mycetome of *S. zeu-mais*. × 32,000.

Fig. 2. Parts of a similar organism at greater magnification. From a mycetome (cw, cell wall; im, intracytoplasmic membranes; n, nuclear material; pm, plasma membrane). × 150,000.
Fig. 3. Electron micrograph of a mass of disintegrating organisms. Normal organisms are present outside the mass. From a mycetome (dm, disintegrating micro-organism; nm, normal organism; sm, spiral configuration of disintegrating organism). $\times 10000$.

Fig. 4. Electron micrograph of a membrane mass. The organisms are in a more advanced stage of disintegration. From a mycetome (dm, disintegrating micro-organism) $\times 29000$.

Fig. 5. Shows the trilaminate nature of the membranes. From a mycetome. $\times 100000$.

Fig. 6. Membranes apparently arising from an isolated, disintegrating micro-organism. From a mycetome. $\times 52000$. 
Fig. 7. Phase-contrast micrograph showing lamellar masses in an unfixed smear preparation of a prepupal mycetome. No spiral organisms are evident. × 450.

Fig. 8. As in Fig. 7, but from an adult mesenteron. × 450.

Fig. 9. Evidence of spiral organism within a spheroidal object, from osmium-fixed smear of mycetome stained in Short's modification of Heidenhain's haematoxylin. × 1300.

Fig. 10. Thin (1μ) section of part of a mycetome, stained with toluidine blue. In one mycetocyte is a lamellar mass (Im). The nucleus of the mycetocyte is indicated (n). Sections of spiral organisms can be seen. × 1150.

Fig. 11. Relatively large myelin figures in an unfixed crush preparation of adult mesenteron. No spiral forms are evident. Phase contrast. × 350.