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Oogenesis of *Nicoletia phytophila* (Zygentoma, Nicoletiidae). Preliminary Studies

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Synopsis

The ovaries of *Nicoletia phytophila* are of panoistic type. Each ovary is composed of three ovarioles. The ovariole has terminal filament, germarium and vitellarium. The vitellogenesis is accompanied by the development of microvilli on the oocyte surface and by changes in the ultrastructure of follicular cells.

Introduction

Zygentoma and Microcoryphia, which used to be combined in one order Thysanura, are Apterygota most closely related to Pterygota. As histological and ultrastructural studies have shown, both Zygentoma and Microcoryphia have panoistic ovaries (Bitsch, 1980a, b; Bitsch and Bitsch, 1982). Vitellogenesis, of heterosynthetic or mixed type with heterosynthetic kind dominating, is typical of these insects (Bitsch, 1980b; Bitsch and Bitsch, 1982). *Nicoletia phytophila* is a representative of Zygentoma of the family Nicoletiidae. Oogenesis has not yet been studied in this family and seems to be worth a closer examination, because of the unusual life style of this species (see Material and Methods). Besides of it, the analysis of the oogenesis of *N. phytophila* might lead to interesting phylogenetic conclusions. This is all more likely, since studies of oogenesis are a good basis for consideration of the relationships of groups of insects and other invertebrates (Eckelbarger, 1983).

Material and Methods

Nicoletiaphytophila is a species of Cuban origin, where it lives in heaps of termites of the *Prorhinotormes simplex*. It is a parthenogenetic species, and breeds easily in the laboratory.

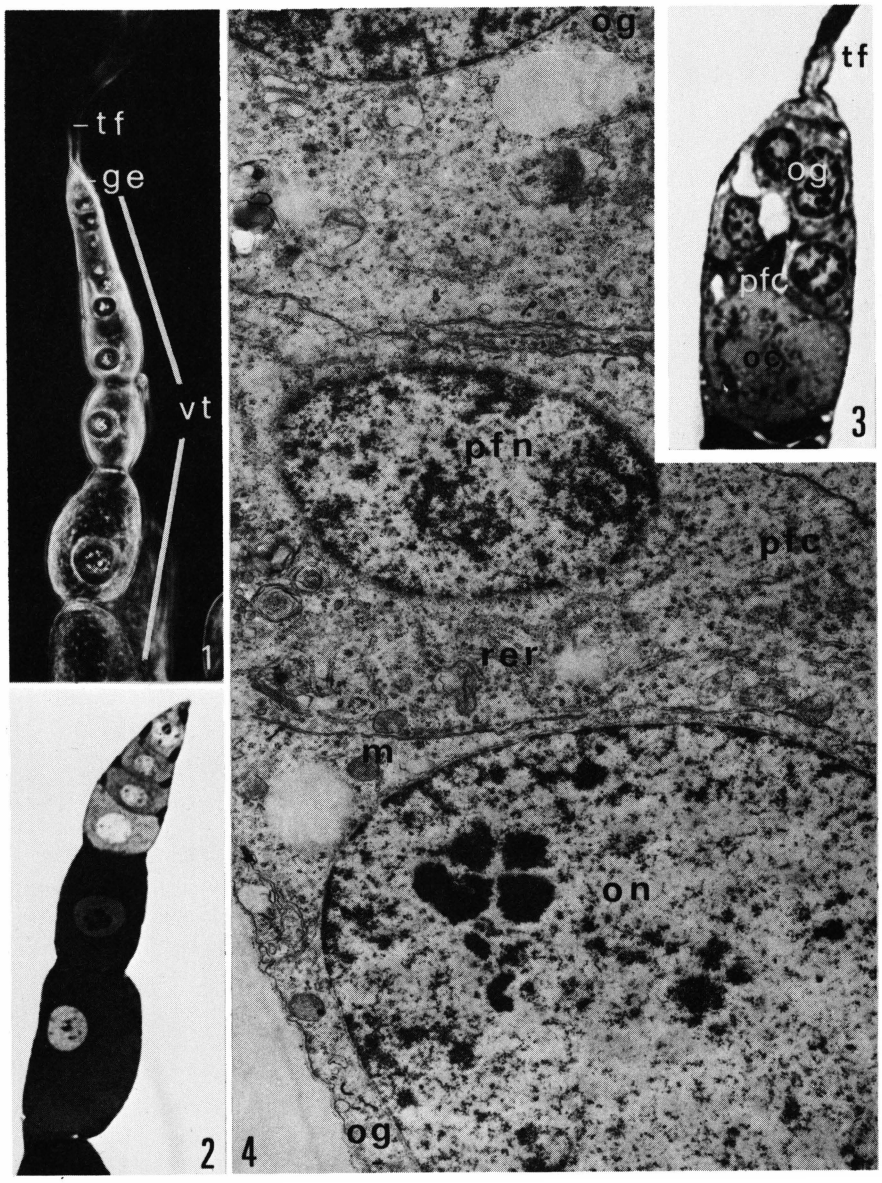

Fig. 1. Phase-contrast micrograph of an ovariole. × 180.
Fig. 2. Photomicrograph of an ovariole. Methylene blue. × 240.
Fig. 3. Photomicrograph of a germarium. Methylene blue. × 1,000.
Fig. 4. Electron micrograph of a germarium. × 15,000.
ge, germarium; m, mitochondria; oc, young oocyte; og, oogonia; on, oogonial nucleus; pfc, prefollicular cells; pfn, prefollicular cell nucleus; rer, rough endoplasmic reticulum; tf, terminal filament; vt, vitellarium.

The insects were placed in a container filled with wood, taken from their natural environment. After ovaries dissected out, they were placed in Hoyle's medium for insects, then photographed in the Peraval Interphaco Carl Zeiss Jena light microscope in negative phase contrast. Ovaries destined for ultrastructural studies were fixed in buffered 3% glutaraldehyde and 2% osmium tetroxide and embedded in epoxy resin Epon 812.

Semithin sections were stained with 1% methylene blue. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined with a Tesla BS 500 electron microscope.

Results

The ovaries of *N. phytophila* are composed of three ovarioles of the panoistic type. In each ovariole, the terminal filament, germarium and vitellarium may be distinguished (Figs. 1, 2). The germarium is fairly small, and oogonia, prefollicular cells and young oocytes are found in it (Fig. 3).

The oogonial cells are isodiametric (Fig. 4), have small, regularly spherical nuclei and a small amount of cytoplasm. Part of the chromatin is in condensed form. Numerous elements of the rough endoplasmic reticulum (RER) can be found in the cytoplasm, as well as small, spherical mitochondria, lamellar bodies and free ribosomes. The prefollicular cells are cone-shaped. Their nuclei are ovoid, the chromatin forms irregular aggregations inside the nucleus and along the nuclear envelope. The cytoplasm of prefollicular cells contains mainly elements of the RER and free ribosomes. Small spherical mitochondria have also been observed. Young oocytes are larger than oogonia, their size increases distally in the ovariole. The nucleus of young oocytes is spherical, with a diameter of 1.6 μm. The chromatin is diffuse, with several irregularly spaced aggregations of electron-dense material. A large amount of mitochondria is found in the cytoplasm of young oocytes (Fig. 5). Oocytes are accompanied by occasional prefollicular cells. The cell membranes of young oocytes and prefollicular cells adhere closely to one another (Fig. 8).

In the vitellarium, all the oocytes are arranged linearly. The oocytes located apically are in the stage of previtellogenesis. At this stage the oocyte cytoplasm is richer in mitochondria than before and has RER. The nuclei are spherical, and the chromatin despiralized. Single follicular cells may be found both on the surface of oocytes, and also between them. These cells are elongated, possess a large ovoid nucleus which occupies the greater part of the cell. The cytoplasm which surrounds the nucleus is filled mainly by mitochondria and free ribosomes. In the stage of previtellogenesis, the oolemma begins to form a few microvilli (Fig. 9).

During vitellogenesis, the ultrastructure of the oocyte and follicular cells differs from that described for the stages previously studied. At the beginning of vitellogenesis the follicular epithelium surrounds the whole oocyte (Fig. 6). The follicular cells are usually cubical and adhere closely to one another. The onset of vitellogenesis is indicated by the appearance of pinocytotic vesicles in their cytoplasm (Fig. 10). Numerous mitochondria, ribosomes and cysterns of RER may also be found in the ooplasm. The oolemma forms microvilli, which branch out. These microvilli lie very close to each

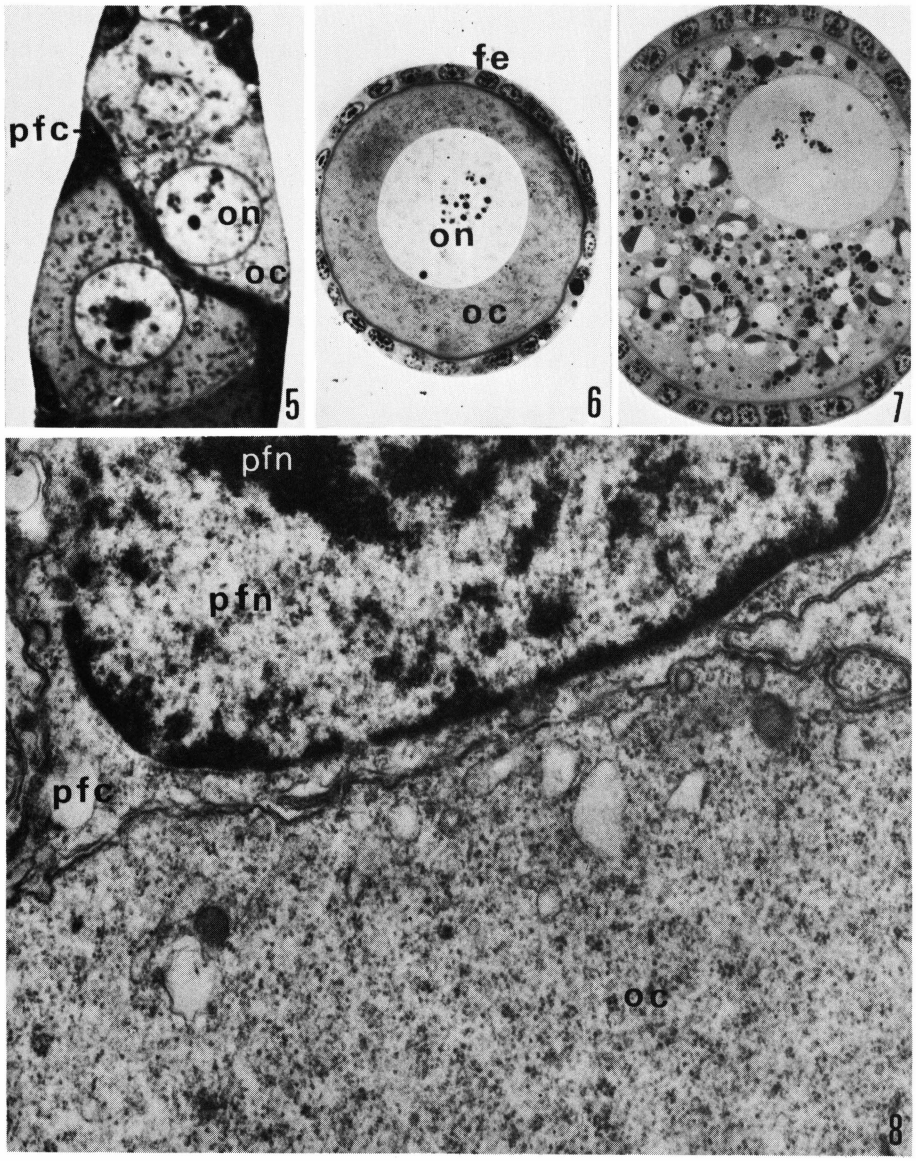

Fig. 5. Photomicrograph of the apical part of an ovariole. Methylene blue. × 800.
Fig. 6. Photomicrograph of early vitellogenic oocyte. Methylene blue. × 560.
Fig. 7. Photomicrograph of vitellogenic oocyte. Methylene blue. × 540.
Fig. 8. Electron micrograph of interface between young oocyte and prefollicular cell. × 26,000.
fe, follicular epithelium; oc, oocyte; on, oocyte nucleus; pfc, prefollicular cells; pfn, prefollicular cell nucleus.

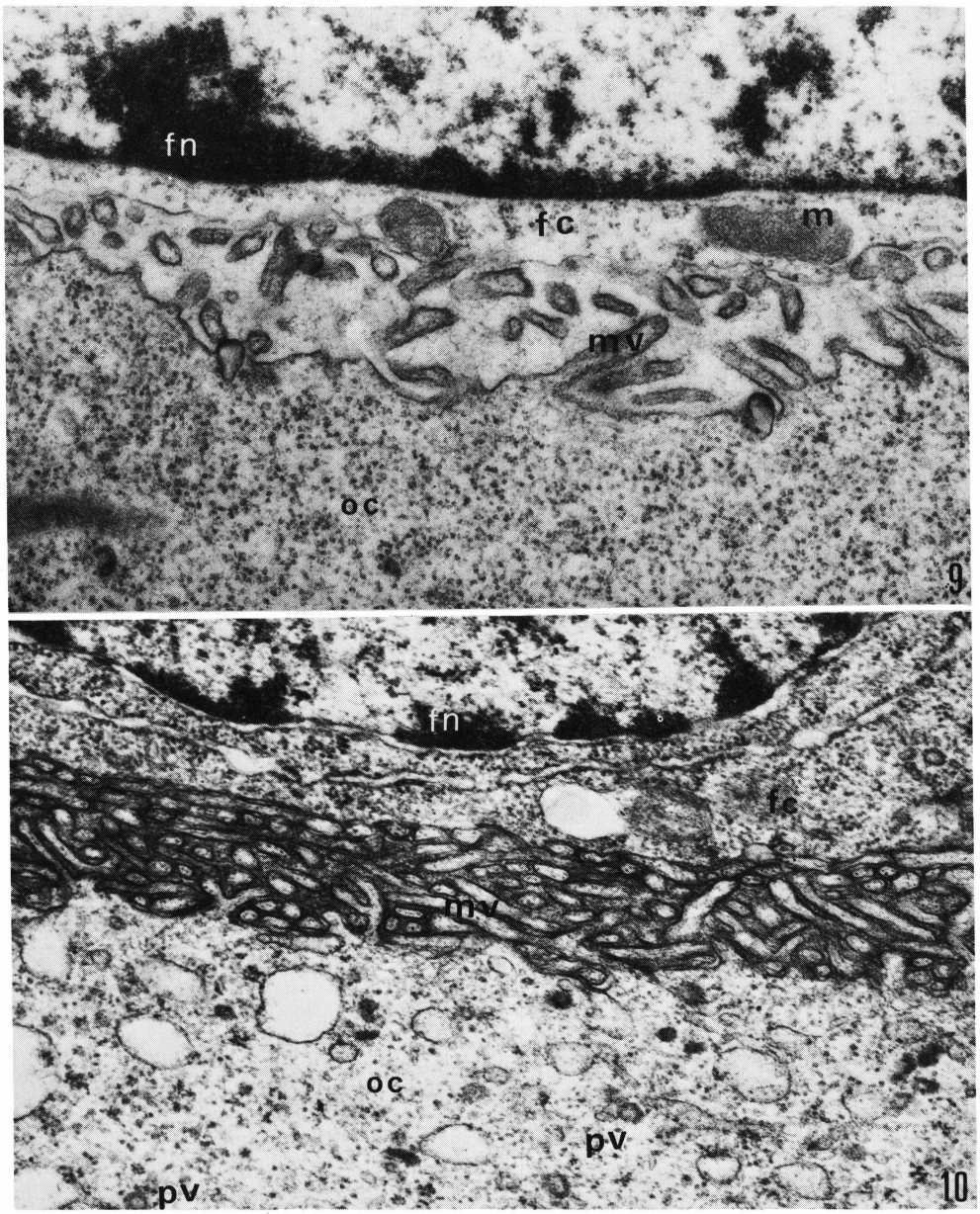

Fig. 9. Electron micrograph of interface between oocyte and follicular cell. × 32,000.
Fig. 10. Electron micrograph of interface between early vitellogenic oocyte and follicular cell. × 16,000.
oc, oocyte; fc, follicular cell; fn, follicular cell nucleus; m, mitochondria; mv, microvilli; pv, pinocytotic vesicles.

other and are often intertwined with each other. In this stage the ultrastructure of the oocyte nucleus does not change. The follicular cells have more cytoplasm, and also a greater number of cell organelles. Mitotic figures have frequently been observed in follicular cells in the beginning of vitellogenesis (not shown).

In advanced stages of vitellogenesis, large amounts of reserve substances, such as lipid and protein droplets appear; these eventually fill almost all the cytoplasm. The structure of the oocyte nucleus remains unaltered in this phase of oogenesis. The nuclei of follicular cells are branched and lobated (Fig. 7).

Discussion

The studies carried out on the oogenesis of *N. phytophila*have shown that females of this species have typically developed panoistic ovaries. An ovary of similar structure is characteristic of all the Zygentoma and Microcoryphia (Bitsch, 1980a, b; Bitsch and Bitsch, 1982) and also some primitive pterygote insects belonging to the following orders: Odonata, Ephemeroptera, Plecoptera, Phasmida, Orthoptera, Grylloblattodea, Thysanoptera (reviewed by King and Büning, 1985) and in megalopterans belonging to the family Corydalidae (Matsuzaki *et al.*, 1985). The ovary in *Nicoletia* is composed of three ovarioles. As in other Zygentoma and Microcoryphia the number of ovarioles in the ovary is small, for example 7 in *Lepismachilis* (Bitsch, 1980b), 5 in *Thermobia* (Bitsch and Bitsch, 1982). Germ line cells are present in the ovary: these are the oogonia and oocytes. Prefollicular and follicular cells are somatic cells. The process of vitellogenesis is accompanied by changes in the organization of the follicular epithelium. In the stage of previtellogenesis, the follicular cells are few and elongated in shape. Their nuclei are fairly large, the cytoplasm contains mainly mitochondria and elements of the RER. At the moment of transition from previtellogenesis to vitellogenesis, the number of follicular cells grows considerably. As a result, a single layer of continuous, thin epithelium appears on the oocyte surface. Cell shapes change simultaneously, and they become cubical. In the stage of early vitellogenesis, endomitotic division of the follicular cell nuclei has been observed. A change in the shape of the nucleus is the result of these endomitoses, it becomes branched and lobated at this stage. Similar changes in the follicular cell nuclei have been observed in a representative of the Microcoryphia, *Lepismachilis targionii* (Bitsch, 1980a).

As the pictures obtained indicate, the chromatin despiralizes in the beginning of the period of previtellogenesis. This state is maintained until the end of vitellogenesis. The above observations show that intensive RNA synthesis takes place in the previtellogenic and vitellogenic oocytes. The studies, which have been made to date on RNA synthesis during the oogenesis of insects, have shown that during the duration of this process rRNA and long living mRNA are accumulated in the ooplasm (reviewed by Telfer, 1975). Thus it would seem that in *Nicoletia*, as in other insects, it is these kinds of RNA which accumulate. Further studies on the structure of the oocyte nucleus, using the electron microscope and histochemical methods will enable us to gain a better understanding of this phenomenon.

The growth of the oocyte in *Nicoletia* is accompanied by the complication of its cell membrane. The oolemma forms microvilli, which are relatively few in previtellogenesis,

but during the previtellogenesis-vitellogenesis transition their number increases considerably. This brings about an increase in the surface of the cell membrane of the oocyte. The formation of microvilli in the beginning of vitellogenesis is a typical phenomenon and has been frequently decribed for various insect species (Biliński, 1976, 1979; Biliński *et al.*, 1985; Bitsch, 1980b; Bitsch and Bitsch, 1982; Książkiewicz-Ilijewa, 1977; Matsuzaki, 1971; Matsuzaki *et al.*, 1979, 1985). In early vitellogenesis, pinocytotic vesicles appear in the cortical cytoplasm of the oocyte, and grow in numbers as the process of yolk accumulation progresses. Thus the heterosynthetic type of vitellogenesis occurs in *Nicoletia*, as is the case for other Zygentoma and Microcoryphia (Bitsch, 1980b; Bitsch and Bitsch, 1982). This would indicate the close relationship of this group with pterygote insects. Moreover, the relation of Zygentoma and Microcoryphia to Pterygota seems to be closer than with the remaining apterygote insects, in which, as has already been shown, vitellogenesis is of an autosynthetic or mixed type (Biliński, 1976, 1977, 1979).

It is reasonable to suppose that further studies of the oogenesis of *Nicoletia phytophila* ought to yield interesting data on the relationship of the Zygentoma and Microcoryphia with the Pterygota on the one hand, and the Apterygota on the other.

Acknowledgements

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