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TOPOGRAPHICAL RELATIONS BETWEEN OOCYTES AND OTHER OVARIAN CELLS IN THREE MITE SPECIES (ACARI)

by Wojciech WITALIŃSKI

SUMMARY: Ultrastructural observations of oocyte-ovary attachment in three mite species have been presented. In *Euryparasitus emarginatus* (Gamasida), the broad stalk is composed of two cell types: central and parietal. The oocyte adheres to central cells via the micropylar orifice in the vitelline envelope. At the site of contact, numerous spherical protrusions of oocyte interdigitate with invaginations of the central cells. In *Erythraeus phalangoides* (Actinedida) the oocytes are placed on distinct stalks composed of four cellular elements: (1) pear-shaped main stalk cells which surround (2) the centrally running microtubule containing probably nutritive protrusion, (3) border cells which separate the stalk from the rest of ovary, and (4) scarce muscle cells twining around the stalk. Through the micropylar orifice passes a nutritive protrusion which connects oocyte with multinucleate nutritive cell embedded in the ovarian wall. In *Hafenrefferia gilvipes* (Oribatida) only oogonia and previtellogenic oocytes possess microtubule-rich protrusions which join each other to form anucleate, microtubule-rich central mass.

Possible functions of stalks and attachment sites are discussed.

RÉSUMÉ: Ce travail présente les observations ultrastructurales de l'union de l'ovocyte et de l'ovaire chez trois espèces d'acariens. Chez *Euryparasitus emarginatus* (Gamasida) un large pédoncule est formé par des cellules de deux types : centrales et parietales. L'ovocyte est lié aux cellules centrales par le micropyle dans l'enveloppe vitelline. À cet endroit les prolongements sphériques de l'ovocyte s'engrènent avec les invaginations des cellules centrales. Chez *Erythraeus phalangoides* (Actinedida) les oocytes sont placés sur les pédoncules bien formés constitués des quatre éléments suivants : (1) des cellules centrales, pyriformes, qui entourent (2) un prolongement nutritif, possédant des microtubules, qui passe au centre (3) des cellules liminaires, qui séparent le pédoncule du reste de l'ovaire, (4) des cellules musculaires peu nombreuses, qui entourent le pédoncule. Par le micropyle un prolongement nutritif passe et relie l'ovocyte à une cellule polynucléaire nutritive, logée dans la paroi de l'ovaire. Chez *Hafenrefferia gilvipes* (Oribatida), seules les oogonies et les oocytes prévitellogènes possèdent des prolongements riches en microtubules. Ces prolongements s'associent en formant une structure centrale, anucleaire et riche en microtubules.

Les fonctions possibles exercées par le pédoncule et l'union de l'ovocyte et de l'ovaire sont discutées.
INTRODUCTION

The ovary is composed of germ cells and somatic cells, constituting two compartments which remain in a close relationship both morphologically and functionally. However, the available pool of data on oogenesis and vitellogenesis is much larger than that on somatic cell structure and on contacts between oocytes and somatic cells. This gap is considerable in invertebrates and particularly wide in Acari. Acarian oocyte-ovary wall contact can be realized in two main ways. In ticks (Ixodida), the oocytes are located on the stalks formed by one type microtubule-rich funicle cells (Diehl, 1970; Diehl et al., 1982). In Trombidiformes (Actinida), the stalks are also present but they contain centrally located nutritive protrusions connecting oocytes with nurse cells lying in the ovarian wall (Witte, 1975). The observations presented in this paper show the structure of oocyte-ovary wall contacts and their alterations during oogenesis in the representatives of three taxonomically distant suborders of mite and must be treated as preliminary ones.

MATERIAL AND METHODS

The following three species of mite were used in the study: Euryparasitus emarginatus (C. L. Koch) (Gamasida, Ologamasidae), Erythraeus phalangoides (de Geer) (Actinida, Erythraeidae), and Hafenrefria gilvipes (C. L. Koch) (Oribatida, Liacaridae). Females were collected in spring and summer, and after dissection in a fixative containing 3% glutaraldehyde in 0.09 M sodium cacodylate buffer, pH 7.4, the ovaries were fixed for 1.5 h at room temperature. Postfixation was carried out in 1.4 % OsO4 for 0.5 h. After dehydration in ethanol and propylene oxide the material was embedded in Epon 812. Semithin (0.3 μm) sections were stained with a mixture of methylene blue and azur II (Richardson et al., 1960). Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in a Tesla BS 500 electron microscope at 60 kV.

RESULTS

The ovaries in E. emarginatus and E. phalangoides are of grape type, i.e., the oocytes are located on the stalks protruding from the ovarian wall. The stalks may be more (E. phalangoides) or less (E. emarginatus) clearly visible. In both species at the site of oocyte-stalk cell contact the vitelline envelope is absent, forming a micropylar orifice of a variable diameter. The ovary of H. gilvipes is compact and the oocytes are tightly enveloped by follicular cells. The stalks and micropylar orifice in the vitelline envelope are absent.

Euryparasitus emarginatus

The first morphological evidence of stalk formation can be observed at the stage of early previtellogenic oocyte, to which progenitors of so called “central cells” become attached (Fig. 1). Those cells will later occupy the central position in the stalk. Their nuclei are large, roundish or flattened, with patches of condensed chromatin. The cytoplasm contains abundant microtubules, free ribosomes, tubular and vesicular ER, fine spheroidal vesicles with slightly thickened walls (Fig. 1, inset), and numerous electron-dense granules of irregular shape. Small Golgi complexes are frequently visible. When the oocyte grows the stalk becomes clearly visible and elongated (Fig. 2) simultaneously with the appearance of parietal cells which are located at the stalk periphery.

The parietal cells (Figs. 2, 4 and 5) have nuclei similar to those of central cells. A rather dense cytoplasm contains clusters of mitochondria and meandering agranular cisternae of ER with their dilatations filled with small electron-dense vesicles (Fig. 4) or granules which later fuse to form greater bodies showing a concentric internal structure (Fig. 5). As the stalk elongates, the cytoplasm of central cells becomes less condensed (Figs. 3 and 6) and the number of microtubules drastically decreases so that they are only occasionally visible. The basement lamina covers both the stalk and the oocyte surface.

Oolemma of the previtellogenic oocytes forms
FIG. 1: *E. emarginatus*: Site of attachment of the previtellogenic oocyte (Oc) to the ovarian wall (OW). In the progenitor of central stalk cell the nucleus (N) and numerous granules (arrows) can be seen. L, lipid droplet. × 7700.
Inset: A detail of cytoplasm of the central cell progenitor with numerous cross-sectioned microtubules. A part of nucleus (asterisk) and elongated vesicles (arrows) are visible. × 30700.

FIG. 2: *E. emarginatus*: Semithin section through the micropylar region of the vitellogenic oocyte. Vitelline envelope is absent along the line of attachment of the oocyte (Oc) to central (asterisk) and parietal (PC) cells of the stalk. Light microscope. × 580.

FIG. 3: *E. emarginatus*: Fragment of central cell cytoplasm. Electron-dense granules are indicated by arrows. × 12300.
Figs. 4-5: *E. emarginatus*: Two fragments of parietal cell. In the cytoplasm clusters of mitochondria and dilated ER cisternae containing numerous vesicles (Fig. 4; asterisks) or concentric electron-dense granules (Fig. 5) are present. × 26,000.

Fig. 6: *E. emarginatus*: Site of attachment of oocyte (Oc) to central cell (CC). Spherical evaginations of the oocyte are placed on short pedicles (asterisks). L, lipid droplet. × 17,000.

Inset: Light micrograph of the same region. Asterisk marks the oocyte. × 1,300.
numerous microvilli, except for the place of oocyte-stalk attachment, i.e., the micropylar region. In that site small vesicular protrusions of oolemma interdigitate with central cells of the stalk. At the beginning of vitellogenesis, the oolemmal protrusions enlarge, assuming a spherical shape (Fig. 6). They communicate with the oocyte via petiolar connections showing subplasmalemmal densities at their bases. The protrusions are filled with a fine fibrous material containing scarce free ribosomes but are otherwise devoid of other cytoplasmic organelles.

In contrast to *E. phalangoides* and *H. gilvipes*, the microtubule-rich protrusions or germ cells were not observed.
**Erythraeus phalangoides**

In *E. phalangoides* oocytes are connected with the ovary by well developed stalks (Fig. 7) composed of four elements: (1) main stalk cells, (2) border cells, (3) nutritive protrusion, and (4) muscle cells. The main stalk cells form stalk body. In the stalks of previtellogenic oocytes these cells are roundish. During the development of oocyte and elongation of the stalk they become pear-shaped with their narrow poles directed towards the centre of the micropylar region of the oolemma (Fig. 8). The cytoplasm contains large amount of microtubules, Golgi-derived electron-dense granules with a diameter ranging from 0.2 to 0.5 μm, as well as larger electron-lucent vacuoles 0.7 μm in diameter, similar in appearance to lipid droplets of oocyte. The dilated part of the main stalk cell is occupied by large nucleus surrounded by mitochondria, free ribosomes, and occasional, well defined RER cisternae.

The main cells are separated from the other cells of ovary wall by several layers of strongly flattened cells with occasional mitochondria and elliptic nuclei containing large concentrations of condensed chromatin. According to their localization, these cells have been called border cells.

Along the centre of stalk runs a nutritive protrusion (1.8-2.0 μm in diameter) which connects each oocyte with its nutritive cell located in the ovary wall (Fig. 7). Protrusion is filled with closely packed microtubules interspersed with numerous fine vesicles of a diameter ca. 0.1 μm and a medium electron density, as well as free ribosomes. The ovoidal, extremely electron-dense granules, 1.3 × 1.5 μm, can also be occasionally encountered. Near the vitelline envelope, the protrusion slightly dilates, and the microtubules penetrate the oocyte through the micropyle.

The giant nutritive cells (Fig. 9) contain several nuclei of an irregular shape, devoid of larger concentrations of condensed chromatin. The nuclear envelope shows the presence of numerous pores. The cytoplasm possesses many free ribosomes, abundant microtubules with multidirectional orientation and large areas occupied by a dense, granular material of a “nuage” type with closely adjacent mitochondria containing large dense bodies in the matrix. Sometimes the ovoidal, electron-dense granules similar to those present in the nutritive protrusion can be seen.

The outer surface of the stalk is covered by one layer of basement lamina with adjacent visceral muscle cells occasionally twined around the stalk.

**Hafenrefferia gilvipes**

In the ovary of cross-sectioned *H. gilvipes* four germaria, i.e., groups of oogonia in a rosette arrangement can be distinguished (Figs. 10 and 11). The previtellogenic oocytes occupy the periphery of the rosettes, whereas the vitellogenic oocytes are distributed on each side of the ovary near the ventral cuticle. Oogonia and previtellogenic oocytes possess thin protrusions which join each other in the centre of germarium to form anucleate, microtubule-rich mass (central structure) (Figs. 11 and 12). Protrusions, ca. 1.1 μm in diameter, are filled — like the nutritive protrusions in *E. phalangoides* — by densely packed microtubules. The microtubules, especially in the vicinity of the central structure, are interconnected by bands of a denser material showing on cross sections a regular, rectangular arrangement (Fig. 12, inset). As the oocytes grow, they move towards the periphery of the germarium and loose the protrusions. The vitellogenic oocytes occur as single cells showing no connections with other germ cells but enveloped by follicular cells only.

Hence, the protrusions which connect oogonia and previtellogenic oocytes in the ovary of *H. gilvipes* disappear rather early. The vitelline envelope, formed later, is continuous and does not show the presence of the micropylar orifice.

Schematic representation of stalk structure in *E. emarginatus* and *E. phalangoides* is shown in Fig. 13.

**DISCUSSION**

During description of oocyte-ovarian wall contacts, special attention has been paid to the micropylar region. In that site no vitelline envelope is formed,
**Fig. 8**: *E. phalangoides*: Main stalk cells with nuclei (N) located in dilated regions, and numerous electron-dense granules and electron-lucent vacuoles (asterisks) present in parts directed towards oocyte. BC, border cells; OW, cells of ovarian wall; VE, vitelline envelope. × 4 300.

**Fig. 9**: *E. phalangoides*: Fragment of the nutritive cell with two nuclei (N) and concentrations of electron-dense material (black asterisks). White asterisk marks an electron-dense granule. × 6 300.

Inset: High magnification of the marginal part of the nutritive cell filled with microtubules. × 22 600.
and the oocyte can communicate with other cells of the ovary, in particular, with the cells of the stalk. In Gamasida *E. emarginatus*, the oolemma forms large corrugations embedded in one of the two adhering cell types, i.e., with the central cells. A similar albeit more simple attachment type formed exclusively by interdigitated microvilli of oocyte and ovarian cells was found in ticks (BRINTON and OLIVER, 1971; DIEHL *et al.*, 1982), and in other Chelicerata: in horseshoe crab (DUMONT and ANDERSON, 1967), and harvestmen (WITALIŃSKI and ŻUWAŁA, 1981). In *E. phalangoides*, the oocyte does not contact directly somatic cells because the micropylar orifice is very small and only the nutritive protrusion passes through it. Such a protrusion is also present in other mites belonging to this family (WITTE, 1975). The way of formation of such a long protrusion which, perhaps, is a remnant of intercellular bridge, as well as formation of multinuclear nutritive cells should be the subject of further investigations.

The possible functions of oocyte-ovary wall contact zones and micropyle in arthropods may be versatile.

1) They can serve as mechanical attachment of the oocyte. Moreover, in ticks and other taxa possessing the grape-like ovary the ovulation, i.e., entrance of oocyte into the ovarian lumen, is believed to occur due to some action of stalk cells (DIEHL *et al.*, 1982). The details of such process, however, have never been observed.
2) In species, in which the spermatozoa penetrate the egg cells after the vitelline envelope starts to form, the micropyle constitutes the way of entrance for the spermatozoon. Brinton and co-workers (1974) in ticks and Witaliński (unpublished data) in *E. emarginatus* and other gamasid mites observed the presence of spermatozoa in a close vicinity of the micropyle. On the other hand, in the parthenogenetic tick *Haemaphysalis longicornis* the micropyle is not being formed (Khalil, 1972), what supports their function as an entrance for the spermatozoon.

3) In cases when intercellular bridge (nutritive protrusion) exists between the oocyte and the other ovarian cells it may function as a way for nutritive cell-oocyte transfer of ribosomes which are possibly involved in the yolk or vitelline envelope formation. Such protrusions (trophic cords) connecting oocytes and nutritive cells were described, for example, in meroistic-telotrophic ovaries in hemipterans (Lutz and Huebner, 1981).

4) The oocyte-ovary wall contacts may be, at least in some cases, the communication sites for hormonal signals. The secretion of hormones (antagonotropins) inhibiting the maturation of young oocytes, directly by the older oocytes or indirectly by the adjacent ovarian cells under the influence of the older oocytes, was found in insects characterized by a discontinuous egg production (Huebner and Davey, 1973; Thomas, 1979).

The function of stalks and micropylar orifices in mites should be interpreted with a considerable caution. Although some morphological features could suggest the occurrence of all four functions mentioned above, only the first three seem to be evident. In the studied *E. emarginatus*, a mechanical contact of the oocyte with the stalk cells has a form of interdigitating invaginations of the membranes of oocyte and the central cells. In *E. phalangoides*, the stalk cells are separated from the oocyte by the vitelline envelope, a process of the latter cell containing numerous microtubules passes, however, through the micropyle, as also observed in other representatives of this family (Witte, 1975) and can play an auxiliary role in anchoring the oocyte to the apex of the stalk. A morphological similarity of such oocyte processes to the nutritive processes present in the telotrophic insect ovaries suggests that in mites they can also serve as routes of supply oocytes with ribosomes. This supposition requires, however, a further proof.

The function of micropyle in some mites as an entrance for the spermatozooza is supported by the observed occurrence of spermatozoa in the invaginations of stalk cells in *E. emarginatus* and other gamasid mites (unpublished data).

The presence in the stalks of several cell types containing various secretory granules is unclear. Moreover, in case of *E. emarginatus*, the central stalk cells contain abundant vesicular ER, while the parietal cells are characterized by large amount of meandering agranular ER cisternae. Such structures strongly suggest some secretory activity of the stalk cells in the investigated mites, especially in *E.*

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![Fig. 13](image-url)  
FIG. 13: Schematic drawing of oocyte-ovarian wall contacts in *E. emarginatus* (a) and *E. phalangoides* (b). BC, border cell; BL, basement lamina; CC, central stalk cell; MC, muscle cell; MSC, main stalk cell; NP, nutritive protrusion; OC, oocyte; OW, ovarian wall; PC, parietal stalk cell; VE, vitelline envelope.
The existence and chemical character of a secretory substance(s) produced by the stalks in mites remains, however, an open question.

In the ovary of oribatid mite *H. gilvipes* oogonia and previtellogenic oocytes are provided with microtubule-rich protrusions by aid of which they are interconnected in the centre of each rosette-like group of cells, but real stalks are not present. These connections, due to the absence of nutritive cells, play a supportive and/or synchronizing role rather than a nutritive one. In the course of growth the oocytes move centrifugally from the rosette, leading to a very early rupture of protrusions. During formation of the vitelline envelope, the oocytes have no protrusions and neither the micropyle nor specialized sites of contact with other ovarian elements are formed (WITALINSKI, 1986). In conclusion, it should be emphasized that the stalks and micropylar orifices are present in a gamasid mite *E. emarginatus* and in an actinedid mite *E. phalangoides*. Their supposed functions in both species include a mechanical support of the oocyte and route for oocyte ovulation, as well as the entrance for spermatozoa on their way to oocyte. In *E. phalangoides*, the stalk penetrated by the oocyte protrusion can play a mediating role between the oocyte and the nutritive cells. The presence of large amounts of secretory granules accompanied by a well developed ER in stalk cells of *E. emarginatus* suggest some secretory function of these cells. In an oribatid mite, *H. gilvipes*, there are only temporary connections between the germ cells and their function seems to be limited to mechanical support and/or coordination during early oogenesis.

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