ELSEVIER

Arthropod Structure & Development 30 (2001) 135–143

ARTHROPOD STRUCTURE & DEVELOPMENT

www.elsevier.com/locate/asd

Ultrastructure and function of nurse cells in phthirapterans. Possible function of ramified nurse cell nuclei in the cytoplasm transfer

Monika Żelazowska*, Szczepan M. Biliński

Department of Systematic Zoology, Institute of Zoology, Jagiellonian University, R. Ingardena 6, 30-060 Kraków, Poland

Received 11 June 2001; accepted 1 August 2001

Abstract

The structure of nurse cells as well as the distribution of cytoskeletal elements (actin filaments, microtubules) in three representatives of phthirapterans: the pig louse, *Haematopinus suis* (Anoplura) and bird lice, *Eomenacanthus stramineus*, *Columbicola columbae* (Mallophaga) were investigated. All three species have polytrophic–meroistic ovaries which means that each oocyte remains connected with a group of nurse cells via specialized cytoplasmic canals—intercellular bridges (ring canals). Throughout vitellogenesis, various macromolecules as well as organelles (mitochondria, endoplasmic reticulum vesicles, ribosomes) are transferred from the nurse cells to the oocyte. During this flow, the nurse cell nuclei do not enter the oocyte and are retained in the cell centers. In holometabolous insects (e.g. *Drosophila*, hymenopterans), the central position of nurse cell nuclei is maintained by cytoskeletal elements (actin filaments or microtubules). In the investigated species, the nurse cells are equipped with large, highly extended (irregularly lobed) nuclei. The inner nuclear membrane is lined with a relatively thick layer of nuclear lamina. Ultrastructural analysis and staining with rhodamine-labeled phalloidin revealed that the nurse cell cytoskeleton is poorly developed and represented only by: (1) single microtubules in the perinuclear cytoplasm; and (2) the F-actin layer in the cortical cytoplasm. In the light of this, we postulate that in phthirapterans the position of nurse cell nuclei during the cytoplasm transfer is maintained not by the cytoskeletal elements, but by a largely extended shape of the nuclei (i.e. their elongated extensions). © 2001 Published by Elsevier Science Ltd.

Keywords: Oogenesis; Cytoplasm transfer; Nurse cells; Cytoskeleton; Polytrophic ovary; Phthiraptera

1. Introduction

In insects, two principal types of ovaries are distinguished: panoistic and meroistic (for further characterization of insect ovaries see Biliński, 1998; Büning, 1994). In the panoistic ovaries, the oocyte itself is responsible for the production of various types of RNAs (e.g. rRNA, mRNA, snRNA) that are stored for future needs of the embryo. In the meroistic (polytrophic and telotrophic) ovaries, these macromolecules are synthesized in specialized germ-line cells termed nurse cells or trophocytes and later transferred to the growing oocytes (reviewed by Büning, 1994).

Attempts have been made to clarify the cellular regulation mechanisms underlying the unidirectional transport in meroistic ovaries (Cooley and Theurkauf, 1994; Gutzeit, 1986; Knowles and Cooley, 1994; Mahajan-Miklos and Cooley, 1994; Stebbings et al., 1987). This process is best characterized in the polytrophic ovary of *Drosophila melanogaster* (Bohrmann, 1997; Bohrmann and Biber, 1994; Bohrmann and Schill, 1997; Mahajan-Miklos and Cooley, 1994; Robinson and Cooley, 1997). In this type of ovary, each individual oocyte is accompanied by its own group of nurse cells. The nutritive role of the nurse cells is facilitated by the presence of stable cytoplasmic canals termed intercellular bridges or ring canals that link the nurse cells and the oocyte and serve as a transport route. Via these structures, synthetically active nurse cells supply the growing oocyte with different cytoplasmic components including mitochondria, endoplasmic reticulum vesicles, lipid droplets and ribosomes (Büning, 1994; Telfer, 1975). The other important function of the nurse cells is the synthesis of mRNAs (e.g. determinants necessary for embryonic pattern formation) that are subsequently transferred to the ooplasm (Stebbings et al., 1995; St Johnston and Nüsslein-Volhard, 1992; Wilsch-Bräuniger et al., 1997; Hurst et al., 1999; Stephen et al., 1999; Theurkauf and Hazelrigg, 1999).

It has been shown that in *Drosophila* the nurse-cell oocyte transport involves two easily distinguishable phases (Gutzeit, 1986; Cooley and Theurkauf, 1994; Knowles and Cooley, 1994; Mahajan-Miklos and Cooley, 1994).

* Corresponding author. Tel.: +48-12-633-63-77 ext. 2438; fax: +48-12-634-3716.

E-mail address: zawadz@zuk.iz.uj.edu.pl (M. Żelazowska).

1467-8039/01/$ - see front matter © 2001 Published by Elsevier Science Ltd.
PII: S1467-8039(01)00030-5

During the initial phase of oogenesis, the nurse cells grow in size and their cytoplasm containing maternal RNAs, organelles and proteins is slowly transferred into the oocyte through intercellular bridges. In the later stage, the cytoplasm accumulated by the nurse cells rapidly flows en masse into the oocyte. This process is termed the nurse cells dumping or 'terminal injection'. After the completion of this process, the nurse cells degenerate (Mahajan-Miklos and Cooley, 1994; Foley and Cooley, 1998). Cavaliere et al. (1998); McCall and Steller (1998); Matova et al. (1999); Nezis et al. (2000) presented evidence that in *Drosophila melanogaster* nurse cells are cleared from the egg chamber by apoptosis and suggested that rapid transport of nurse cells' cytoplasm to the oocyte is a developmentally regulated phenomenon associated with programmed cell death.

As a general rule, the terminal injection involves only the cytoplasm of nurse cells. The nuclei remain in cell centers and are kept from moving into intercellular bridges. In *Drosophila* ovarian follicles, the nuclei of nurse cells are anchored in place by F-actin cables extending inward from the plasma membrane toward the nucleus (Riparbelli and Callaini, 1995; Guild et al., 1997) (Fig. 16A).

The mechanism and cytoplasmic background of the rapid nurse cell cytoplasm transport described in *Drosophila* is not universal for all insects, not even for dipterans (Gutzeit and Huebner, 1986; Rübsam and Büning, 1996; Rübsam, personal communication; Mazurkiewicz and Kubrakiewicz, 2001). Recently, it has been shown that in hymenopterans, each nurse cell nucleus is surrounded by a three-dimensional cage of microtubules. This cage seems to be responsible for maintaining the position of the nurse cell nucleus during dumping (Biliński and Jaglarz, 1999) (Fig. 16B).

In this paper, we describe the structure of the nurse cells and the distribution of cytoskeletal elements in the polytrophic ovaries of two mallophagans: *Eomenacanthus stramineus*, *Columbicola columbae* and one anopluran species: the pig louse, *Haematopinus suis*. We also suggest a new mechanism responsible for maintaining the nuclear position in the nurse cells during the cytoplasm transfer in phthirapterans.

2. Material and methods

2.1. Animals

The adult females of *Haematopinus suis* were collected from domestic pigs, *Eomenacanthus stramineus* from hens and *Columbicola columbae* from pigeons.

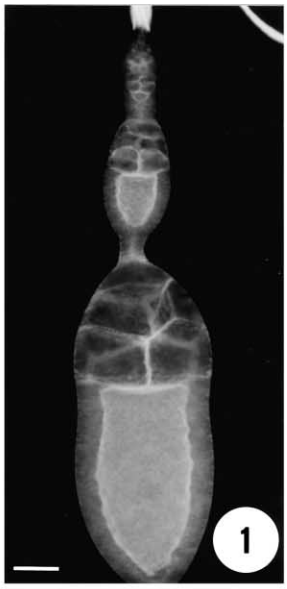
2.2. Light (LM) and transmission electron microscopy (TEM)

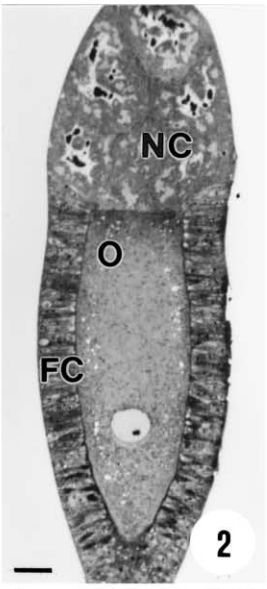
The ovaries were dissected and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 at room temperature for several weeks, rinsed and postfixed for 1.5 h in 1% osmium tetroxide in the same buffer. After dehydration in a series of ethanols and acetone, the material was embedded in Epon 812. Semithin sections (0.5 μm thick) were stained with 1% methylene blue in 1% borax or 1% toluidine blue, and examined in a Jenalumar (Zeiss, Jena, Germany) light microscope. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in a Philips 300 TEM at 60 kV.

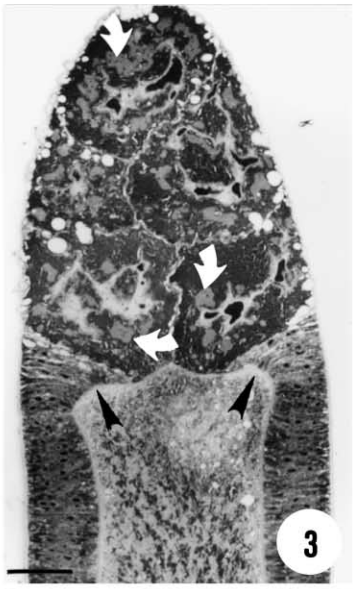
2.3. Triton X-100 extraction

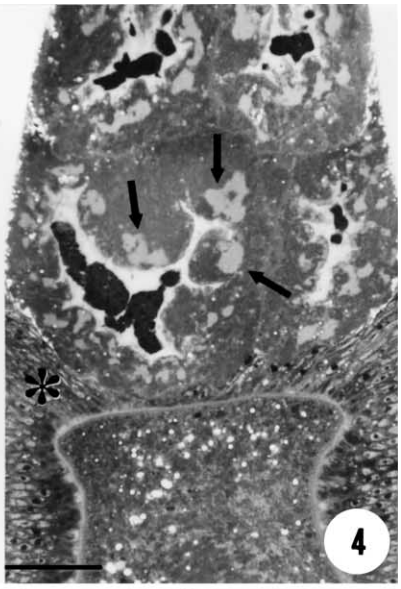
Dissected ovaries were Triton X-100 extracted (for a detailed description see Biliński et al., 1995). In brief, isolated ovarioles were incubated in modified Hanks' buffer (0.05 M phosphate buffer pH 7.3, 2 mM EGTA, 50 mM MES, 12 mM $MgCl_2$) containing 1% Triton X-100. After extraction, the material was fixed in 2.5% glutaraldehyde in the same buffer at room temperature, rinsed and postfixed in

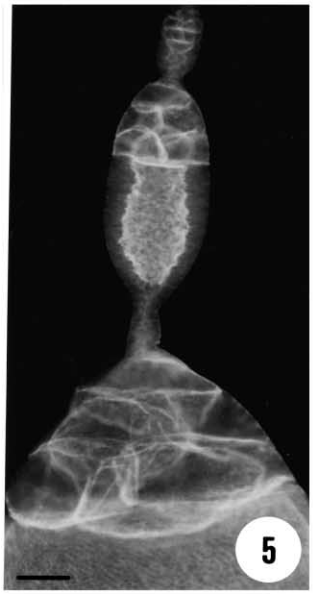
Figs. 1–12. Fig. 1: *Haematopinus suis*. Distribution of actin filaments in previtellogenic ovarian follicles. F-actin is associated with the cortices of nurse cells, and the oocyte. FLM, rhodamine-labeled phalloidin, scale bar = 50 μm. Fig. 2: *Haematopinus suis*. Early previtellogenic ovarian follicle, nurse cells (NC), oocyte (O), follicular cells (FC). LM, semithin section, methylene blue, scale bar = 25 μm. Fig. 3: *Haematopinus suis*. Anterior part of previtellogenic ovarian follicle. Note highly ramified nurse cell nuclei, 'translucent' nuage aggregations (white arrows), follicular cells of the fold (arrowheads). LM, semithin section, methylene blue, scale bar = 25 μm. Fig. 4: *Haematopinus suis*. Anterior part of previtellogenic ovarian follicle. Within nurse cell nuclei large, lobate nucleoli are present, nuage aggregations (arrows), follicular cells of the fold (asterisk). LM, semithin section, methylene blue scale bar = 25 μm. Fig. 5: *Haematopinus suis*. Distribution of actin filaments in early previtellogenic (anterior), late previtellogenic (medial) and vitellogenic (posterior) ovarian follicles. F-actin is associated with the cortices of nurse cells and the oocyte. FLM, rhodamine-labeled phalloidin, scale bar = 50 μm. Fig. 6: *Haematopinus suis*. Anterior part of an early vitellogenic ovarian follicle. Chromatin is localized only in peripheral parts of nurse cell nuclei (arrowheads), follicular cells of the fold (asterisk). FLM, semithin section, DAPI, scale bar = 25 μm. Fig. 7: *Haematopinus suis*. Anterior part of an early vitellogenic ovarian follicle. The extensions of nuclei radiate towards nurse cell membranes (arrows), within nuclei large, lobate nucleoli are present, follicular cells of the fold (asterisk). LM, semithin section, methylene blue scale bar = 25 μm. Fig. 8: *Haematopinus suis*. Anterior part of late vitellogenic ovarian follicle once the transfer of nurse cells cytoplasm is completed. The nurse cells acquire an 'empty' appearance, note that the nuclei remain in central position within the cells. The anterior follicular cells (FC) separate the ooplasm from the nurse cells. LM, semithin section, methylene blue, scale bar = 25 μm. Fig. 9: *Columbicola columbae*. Distribution of actin filaments in previtellogenic and vitellogenic (asterisk) ovarian follicles. F-actin is associated with the cortices of nurse cells and the oocyte. FLM, rhodamine-labeled phalloidin, scale bar = 15 μm. Fig. 10: *Eomenacanthus stramineus*. Anterior part of late previtellogenic ovarian follicle. Follicular cells of the fold (arrowheads), the nutritive appendix (asterisk). LM, toluidine blue, scale bar = 15 μm. Fig. 11: *Eomenacanthus stramineus*. Anterior part of vitellogenic ovarian follicle. Cytoplasmic continuity between nurse cells and oocyte is maintained by means of the nutritive appendix (asterisk), follicular cells of the fold (arrowheads). LM, semithin section, methylene blue, scale bar = 15 μm. Fig. 12: *Eomenacanthus stramineus*. Transition period between vitellogenesis and choriogenesis, anterior part of ovarian follicle. Degenerating nurse cells (DNC), anterior follicular cells (FC). LM, toluidine blue, scale bar = 15 μm.

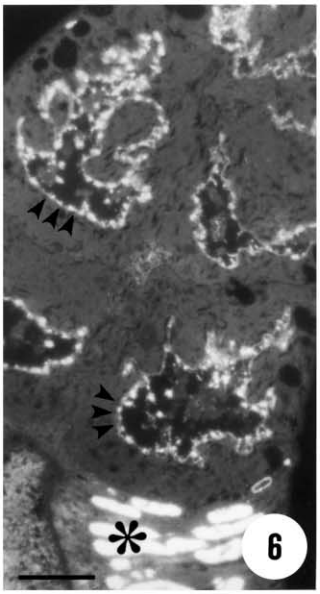

1

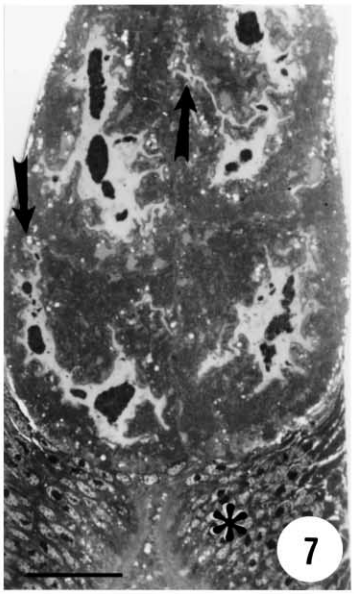

NC
O
FC
2

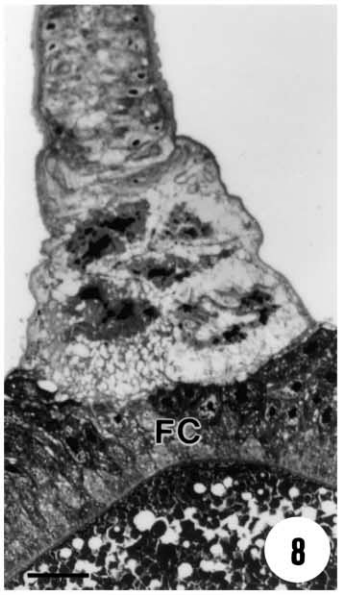

3

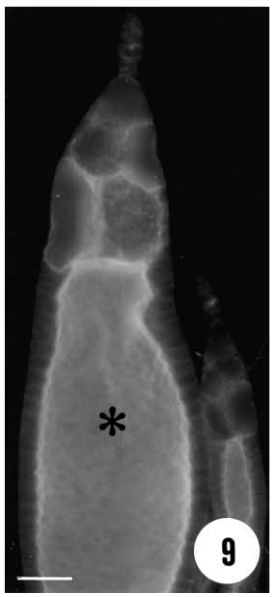

4

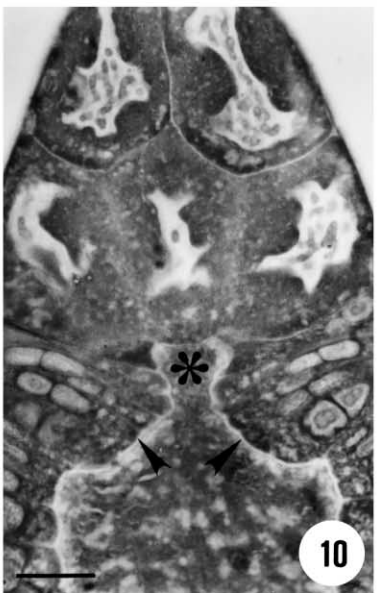

5

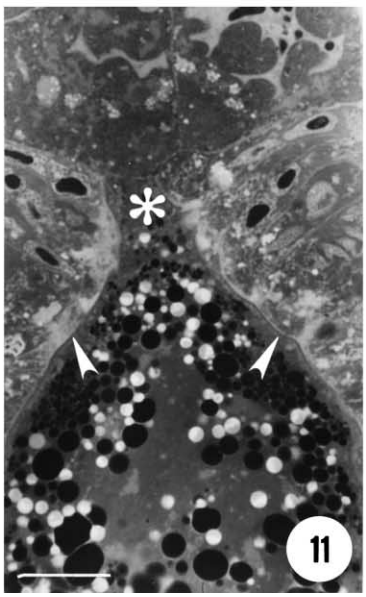

6

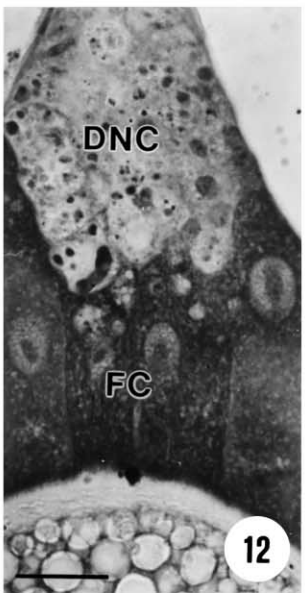

7


FC
8


9


10


11


DNC
FC
12

2% osmium tetroxide and 0.8% potassium ferricyanide in 0.1M phosphate buffer pH 7.4 for 1 h. The ovarioles were rinsed in water and additionally fixed in 0.15% tannic acid and contrasted with uranyl acetate. After dehydration in a series of ethanols and acetone, the material was embedded in Epon 812. Ultrathin sections were contrasted with lead citrate and examined in a Philips 300 TEM at 60 kV.

2.4. Fluorescence microscopy (FLM)

The ovaries were dissected and fixed in 4% formaldehyde (freshly prepared from paraformaldehyde) in phosphate-buffered saline (PBS) for 40 min at room temperature. The ovaries were then rinsed with PBS and after dehydration in a graded ethanol series the material was infiltrated and embedded in acrylic resin (Histocryl, Agar, Stansted, UK). For detection of DNA, semithin sections (0.5 μm thick) were stained with 1 μg/ml of diamidino-2-phenylidole dihydrochloride (DAPI, Sigma Chemical Co., St Louis, MO, USA) in PBS for 20 min in darkness. The sections were then rinsed three times with PBS, mounted in a medium containing 80% glycerol, 20% Tris buffer, pH 9 and 50% *n*-propylgalate, and examined in a Jenalumar (Zeiss) epifluorescence microscope equipped with appropriate filters.

For detection of filamentous actin, the ovaries were dissected and fixed as described above. The specimens were then rinsed in PBS and stained with rhodamine-labeled phalloidin (Sigma Chemical Co.) in PBS (1 μg/ml) for 30 min at room temperature in the dark. Following several washes in PBS, the ovaries were mounted on microscopic slides in a medium containing 80% glycerol, 20% Tris buffer, pH 9 and 50% *n*-propylgalate to reduce photobleaching. The preparations were examined in a Jenalumar (Zeiss) epifluorescence microscope equipped with appropriate filters.

3. Results

3.1. Structure of the ovary

The ovaries of *Haematopinus suis*, *Eomenacanthus stramineus* and *Columbicola columbae* as those of other anoplurans and mallophagans (Ries, 1932) are of the polytrophic–meroistic type (for description and classification of insect ovaries see Štys and Biliński, 1990; Büning, 1994; Biliński, 1998). They are composed of five loosely arranged ovarioles. Each ovariole is divided into a terminal filament, a germarium, a vitellarium and a short pedicel. The vitellarium comprises 3–5 linearly arranged ovarian follicles in subsequent stages of oogenesis. The process of oogenesis in phthirapterans has been previously divided into five consecutive stages: previtellogenesis (stages 1–2), vitellogenesis (stages 3–4) and choriogenesis (stage 5) (Biliński and Jankowska, 1987). The individual follicle is surrounded by somatic follicular cells and consists of seven polyploid nurse cells and a single oocyte (Figs. 1, 2, 5 and 9).

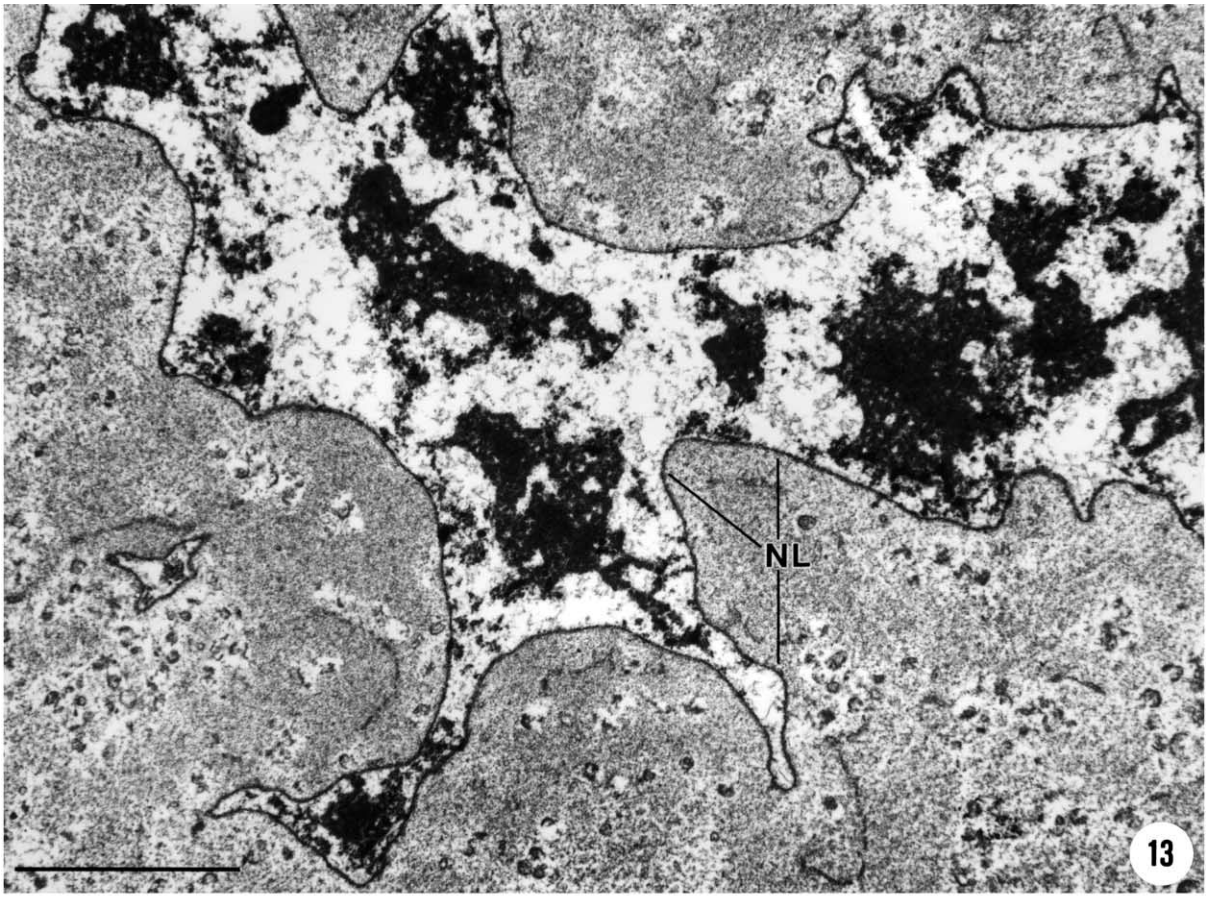
During oogenesis, the follicular cells (FC) gradually diversify into four subpopulations: anterior FC; main body FC; posterior FC; and cells covering nurse cells that also occupy spaces between them (for detailed description see Biliński and Jankowska, 1987; Zawadzka et al., 1997; Żelazowska and Biliński, 1999). At the late previtellogenesis, anterior FC become strongly elongated and form a fold that separates the ooplasm from the nurse cells (Figs. 3, arrowheads and 4, asterisk). The rim of this fold limits the cytoplasmic, funnel-shaped canal (referred to as the nutritive appendix) which ensures contact of the oocyte with the nurse cells (Figs. 10 and 11, asterisks) (stages 3–4). It is on the appendix that intercellular bridges connecting nurse cells and the oocyte are localized. In the transition period between vitellogenesis and choriogenesis (stages 4–5) the anterior FC grow and migrate centripetally, finally constricting the nutritive appendix and separating the ooplasm from the nurse cells (Figs. 8 and 12).

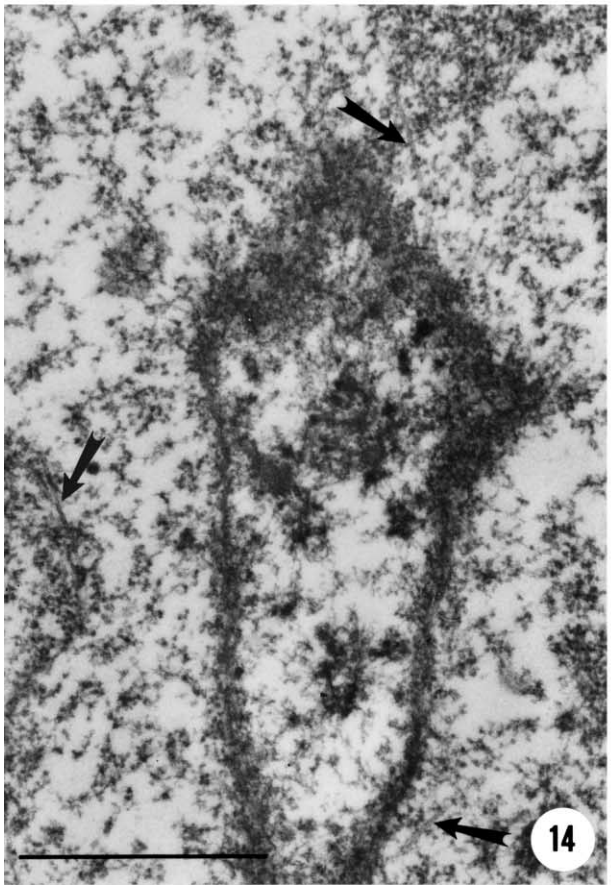
3.2. Structure of the nurse cells

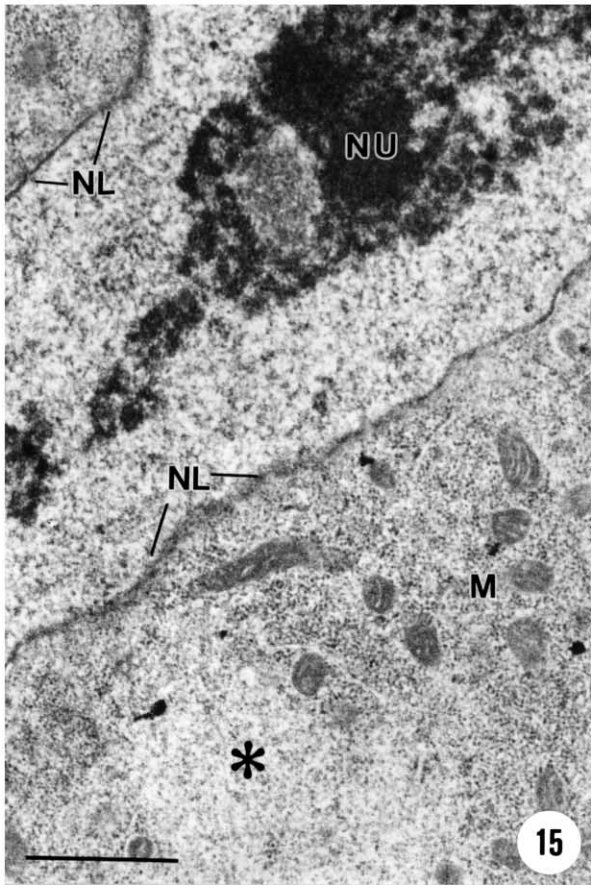
During early previtellogenesis (stage 1) the nurse cells are relatively small and contain large, lobate, centrally located nuclei (Fig. 2). In the karyoplasm, irregular nucleoli and patches of chromatin are present. The cytoplasm comprises numerous free ribosomes and mitochondria.

As previtellogenesis progresses (stage 2), the volume of the nurse cells grows considerably. Simultaneously, large, irregular, 'translucent' areas arise in the perinuclear cytoplasm (Figs. 3 and 4, arrows). Analysis in TEM revealed that these 'translucent' areas represent aggregates of nuage material that, apart from previously reported ribosomes and mitochondria, are present in the nurse cell cytoplasm. Nuage consists of fine-granular material of medium electron density (Fig. 15, asterisk). The nurse cell nuclei become highly ramified (irregularly lobed) (Figs. 3, 4, 6, 7, 10, 11 and 13). Their extensions radiate towards nurse cell membranes (Fig. 7, arrows). The nuclei contain prominent, dense, lobate nucleoli (Figs. 4, 7 and 15). Staining with DAPI revealed that chromatin is restricted only to the peripheral parts of nurse cell nuclei, often in contact with the nuclear envelope (Fig. 6, arrowheads). The inner membrane of this envelope is lined with relatively thick layer of nuclear lamina (Fig. 15). Measurements of the thickness of the nuclear lamina layer in the nuclei of various ovarian cells gave the following results: the nurse cells from

Figs. 13–15. Fig. 13: *Haematopinus suis*. Vitellogenesis, fragment of nurse cell. Highly ramified nucleus. Nuclear lamina (NL). TEM, Triton X-100 extraction, scale bar = 5 μm. Fig. 14: *Haematopinus suis*. Vitellogenesis, fragment of an extension of nurse cell nucleus. Single microtubules are present in the perinuclear cytoplasm (arrows). TEM, Triton X-100 extraction, scale bar = 1 μm. Fig. 15: *Haematopinus suis*. Late previtellogenesis, fragment of nurse cell. Nucleolus (NU), nuage aggregation (asterisk), nuclear lamina (NL), mitochondria (M). TEM, scale bar = 1 μm.


NL
13


14


NU
NL
NL
M
*
15

25 to 45 nm; the oocyte, about 15 nm; and in the follicular cells nuclear lamina was not recognizable.

During vitellogenesis (stage 3), the yolk spheres and lipid droplets are accumulated in the ooplasm (Fig. 11). In late vitellogenic ovarian follicles (stage 4), the nurse cell cytoplasmic content flows into the oocyte and enters the oocyte through the nutritive appendix. After this transfer, the nurse cells acquire an 'empty' appearance and are almost devoid of cytoplasm (Fig. 8). Nevertheless, their nuclei remain in the cell centers (Fig. 8).

Following completion of the nurse cell cytoplasm transfer (the transition period between vitellogenesis and choriogenesis, stages 4–5), the anterior FC migrate centripetally and cover the anterior pole of the oocyte (compare Figs. 7, 8 and 11, 12) (see also Section 3.1). The subsequent growth of these cells is accompanied by gradual degeneration of nurse cells (Figs. 8 and 12).

3.3. Cytoskeletal architecture of the nurse cells

Analysis of ovarioles stained with rhodamine-conjugated phalloidin revealed that throughout stages 1–5 microfilaments are restricted to nurse cell cortices and line their plasma membranes (Figs. 1, 5 and 9). On the other hand, the Triton X-100 extraction visualized single, randomly distributed microtubules in the perinuclear cytoplasm (Fig. 14, arrows). The latter technique also confirmed the existence of a thick nuclear lamina within nurse cell nuclei (Figs. 13 and 14).

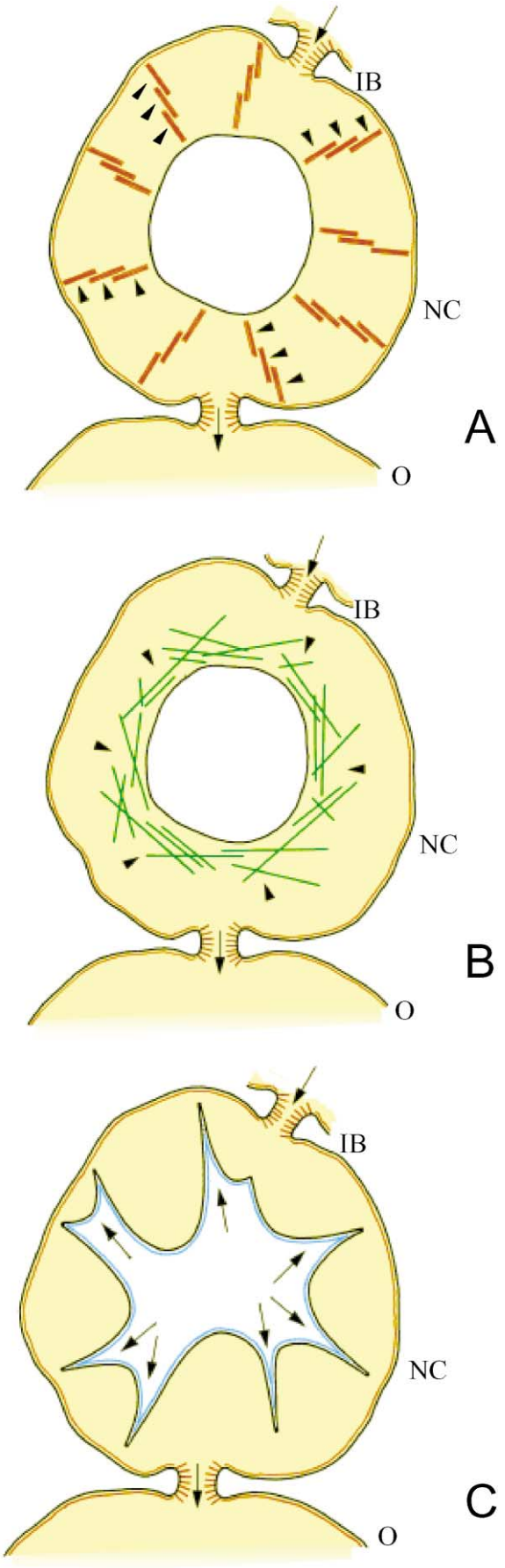
4. Discussion

In polytrophic–meroistic ovaries, an individual ovarian follicle consists of a single oocyte and accompanying nurse cells. The number of these cells depends on the number of successive divisions of the single germ cell termed the cystoblast and its progeny (cystocytes) (Giardina, 1901). Each oocyte of *Haematopinus suis*, *Eomenacanthus stramineus* and *Columbicola columbae* is associated with seven nurse cells. This indicates that in the germaria of the investigated species the oogonial cells undergo three synchronous and incomplete mitotic divisions.

Generally, in polytrophic ovarioles, the intercellular bridges connecting nurse cells with oocyte are situated at the more or less flat anterior pole of the oocyte (Büning, 1994). In the investigated phthirapterans, these bridges are located on the cytoplasmic extension of the anterior oocyte pole, termed the nutritive appendix. Similar modified connections have been described in hymenopterans (Cassidy and King, 1972; Biliński, 1991) and adephagous beetles (Biliński and Jaglarz, 1987; Jaglarz, 1992). In all investigated species, the nutritive appendices persist in ovarian follicles until the advanced stages of vitellogenesis, when the transfer of nurse cell cytoplasm into the oocyte takes place. Once this process is completed, they become constricted by growing and centripetally migrating follicular cells and eventually degenerate (Biliński and Jankowska, 1987; Biliński and Jaglarz, 1999; this paper).

It is generally accepted that the basic function of nurse cells is to synthesize different types of RNAs that are subsequently transported to the oocyte (Telfer, 1975; Büning, 1994). To intensify their transcriptional activity, nurse cells usually multiply their genomes by endomitotic polyploidization (reviewed by Büning, 1994). The nurse cell nuclear envelopes are perforated by numerous pores by which ribonucleoproteids (RNPs) are transported to the cytoplasm. Here, RNPs form characteristic aggregates of fine-granular material termed 'nuage aggregations' (Büning, 1994). In the investigated phthirapterans, these aggregations are exceptionally large and electron-transparent. Similar translucent nuage aggregations have been described in the nurse cells of psocopterans—*Peripsocus phaeopterus* and *Stenopsocus stigmaticus* (Büning and Sohst, 1990).

In the polytrophic–meroistic ovaries of *Drosophila* two phases of intercellular transport from nurse cells into the oocyte have been distinguished (see the Introduction). During the initial (= slow) transport phase, different types of mRNAs and proteins (e.g. *BicD* mRNA, *egalitarian* mRNA, *bicoid* mRNA, Exuperantia—reviewed by Cooley and Theurkauf, 1994) move selectively from nurse cells into the oocyte. The proper execution of this process depends on the organization of microtubules and motor proteins (Theurkauf et al., 1993; Cooley and Theurkauf, 1994; Pokrywka and Stephenson, 1991). The late (or massive) transport phase in which the cytoplasm stored in nurse cells is pushed into the oocyte starts in advanced vitellogenesis (Gutzeit and Koppa, 1982). Within the past few years it has been established that in *Drosophila* this massive transfer depends on the reorganization of F-actin cytoskeleton (Cooley et al., 1992; Mahajan-Miklos and Cooley, 1994; Wheatley et al., 1995; Robinson and Cooley, 1997). Before dumping, nurse cells comprise two subpopulations of microfilaments: the submembrane network; and the basket-like structures associated with the intercellular bridges. It was suggested that these subpopulations participate in the slow transport phase (Riparbelli and Callaini, 1995). During late vitellogenesis the cytoskeleton of the nurse cells becomes modified by the rapid assembly of thick actin cables. The latter project from the cell periphery towards the nuclear envelope (Riparbelli and Callaini, 1995). Each cable is composed of ~25 cross-linked actin filaments (modules). Adjacent modules of the cable overlap like the units of an extension ladder (Guild et al., 1997). During dumping, the subcortical layer contracts, the nurse cells shrink and the cytoplasm flows into the oocyte through intercellular bridges (Wheatley et al., 1995). Concomitantly, the actin modules slide past one another and anchor the nurse cell nucleus in the center of the cell, away from the bridges (Mahajan-Miklos and Cooley, 1994; Guild et al., 1997). Three genes required for the nurse cell actin cables formation have been identified so far: *chickadee*, *quail* and *signed*. The *chickadee* gene



encodes profilin that most likely allows fast actin polymerization (Cooley et al., 1992). *Drosophila signed* encodes a homologue of fascin, a filament bundler that produces hexagonally packaged actin filament bundles with a 12 nm periodicity caused by the fascin cross-links (Cant et al., 1994). The *quail* gene encodes a protein with similarity to villin that efficiently assembles actin filaments into cables in nurse cells (Matova et al., 1999). The assembly of nurse cell actin cables is accompanied by an extensive perforation of the nurse cell nuclear envelopes and both these phenomena are regarded as manifestations of nurse cell apoptosis (Matova et al., 1999; Nezis et al., 2000).

The mechanisms that prevent nurse cell nuclei from physically blocking the intercellular bridges described in *Drosophila* are characteristic for higher dipterans only (Gutzeit and Huebner, 1986; Nezis et al., 2001). Further comparative studies have shown that in other insects (including lower dipterans) other ways of ensuring transport of macromolecules and organelles from nurse cells to the oocyte have evolved (Biliński and Jabłońska, 1996; Adamska and Biliński, 1997; Biliński and Jaglarz, 1999; Mazurkiewicz and Kubrakiewicz, 2001). In hymenopterans, each nurse cell nucleus is surrounded by a three-dimensional cage of microtubules (Biliński and Jaglarz, 1999). These cages are gradually assembled during oogenesis and at the onset of the rapid cytoplasm flow they anchor the nuclei in the cell centers. Furthermore, another subset of microtubules is involved in transferring nuage aggregates from the vicinity of the nucleus towards the nurse cell periphery and the nearest intercellular bridge (Biliński and Jaglarz, 1999).

Previous investigations have shown that in phthirapterans the nurse cell–oocyte cytoplasmic transport at final stages of oogenesis proceeds relatively slowly and does not have as massive character (Biliński and Jankowska, 1987) as in *Drosophila* and hymenopterans. In phthirapteran nurse cells, all actin filaments occur peripherally, in contact with the cell membranes. Neither 'radial' F-actin cables nor microtubular cages were observed in these cells. In the light of this observation, we postulate that the mechanism responsible for maintaining the position of nurse cell nuclei during the cytoplasm transfer does not rely on the cytoskeleton.

From advanced previtellogenesis, phthirapteran nurse cell nuclei are ramified, irregularly lobed. The extensions

Fig. 16. Schematic representation of three known mechanisms responsible for maintaining nuclear position during the flow of nurse cell cytoplasm into the oocyte: A, higher dipterans—cytoplasmic actin cables (red, arrowheads) (according to Guild et al., 1997); B, hymenopterans—cytoplasmic, perinuclear microtubule cages (green, arrowheads) (according to Biliński and Jaglarz, 1999); C, phthirapterans—highly ramified (arrows) shape of nurse cell nuclei, stabilized by a thick layer of nuclear lamina (blue) (this study). Subcortical actin filaments of nurse cells (red), F-actin associated with rims of intercellular bridges (IB) connecting neighboring nurse cells (NC) and nurse cells–oocyte (O) is represented by red dashed lines. Black arrows point the direction of the cytoplasmic flow. For further description see text.

of these nuclei radiate towards plasma membranes. We suggest that in phthirapterans the ramified shape of nurse cell nuclei is stabilized by a thick layer of the nuclear lamina. We further postulate that the position of nurse cell nuclei during the cytoplasm transfer is maintained due to the extended shape of the nuclei. More precisely, the rigid extensions keep the nuclei in the cell centers while the cytoplasm flows (in between these extensions) towards the intercellular bridges leading to the oocyte (Fig. 16C).

Among three mechanisms allowing the transport of the nurse cell cytoplasm to the oocyte (Fig. 16A–C), the one described in this paper seems to be the most ancestral (plesiomorphic). This hypothesis is substantiated by the comparison of the nurse cell structure in hemi- and holometabolous insect orders. In more 'primitive' hemimetabolous insects (Dermaptera, Psocoptera, Phthiraptera), the nurse cells are equipped with ramified nuclei that are not associated with prominent cytoskeletal structures (Yamauchi and Yoshitake, 1982; Büning and Sohst, 1990; this paper). In contrast, nurse cells of more 'advanced' holometabolous insects (e.g. Diptera, Hymenoptera) are characterized by peculiar cytoskeletal 'innovations' (F-actin cables, microtubular cages) that surround nurse cell nuclei during the cytoplasm transfer (Riparbelli and Callaini, 1995; Guild et al., 1997; Biliński and Jaglarz, 1999; Nezis et al., 2001).

Acknowledgements

The authors wish to thank Dr J. Kubrakiewicz (Zoological Institute, University of Wrocław, Poland) and Dr M. Jaglarz (Institute of Zoology, Jagiellonian University, Kraków, Poland) for critical reading of the manuscript. We are also indebted to MSc N. Pośpiech (Department of Zoology and Ecology, Academy of Agriculture, Kraków, Poland) and Dr J. Pośpiech who kindly collected the specimens of the pig louse. Skillful technical assistance of MSc W. Jankowska and MSc E. Kisiel is acknowledged. This work was supported by funds from the research grant BW/IZ/2000 to M.Ż.

References

Adamska, M., Biliński, S.M., 1997. Microtubules are responsible for the asymmetrical distribution of organelles in the oocytes of the hymenopteran, *Chrysis ignita*. Folia Histochemica et Cytobiologica 35, 221–225.

Biliński, S.M., 1991. Morphological markers of anteroposterior and dorsoventral polarity in developing oocytes of the hymenopteran *Cosmoconus meridionator* (Ichneumonidae). Roux's Archives of Developmental Biology 200, 330–335.

Biliński, S.M., 1998. Ovaries, oogenesis and insect phylogeny. Introductory remarks. Folia Histochemica et Cytobiologica 36, 143–145.

Biliński, S.M., Jabłońska, A. (1996) Formation and differentiation of germ cell clusters in hymenopterans. In: Proceedings of XXth International Congress of Entomology, Firenze, p. 111.

Biliński, S.M., Jaglarz, M., 1987. Oogenesis in the common tiger beetle, *Cicindela campestris* (Coleoptera: Adephaga). II. Unusual structure ensuring the contact between the oocyte and accompanying nurse cells. Zoologische Jahrbücher. Abteilung für Anatomie 116, 353–359.

Biliński, S.M., Jaglarz, M.K., 1999. Organization and possible functions of microtubule cytoskeleton in hymenopteran nurse cells. Cell Motility and the Cytoskeleton 43, 213–220.

Biliński, S.M., Jankowska, W., 1987. Oogenesis in the bird louse, *Eomenacanthus stramineus* (Insecta, Mallophaga) I: general description and structure of egg capsule. Zoologische Jahrbücher. Abteilung für Anatomie 116, 1–12.

Biliński, S.M., Klag, J., Kubrakiewicz, J., 1995. Subcortical microtubule network separates the periplasm from the endoplasm and is responsible for maintaining the position of accessory nuclei in hymenopteran oocytes. Roux's Archives of Developmental Biology 205, 54–61.

Bohrmann, J., 1997. *Drosophila* unconventional myosin VI is involved in intra- and intercellular transport during oogenesis. Cellular and Molecular Life Sciences 53, 652–662.

Bohrmann, J., Biber, K., 1994. Cytoskeleton-dependent transport of cytoplasmic particles in previtellogenic to midvitellogenic ovarian follicles of *Drosophila*. Time-lapse analysis using video-enhanced contrast microscopy. Journal of Cell Science 107, 849–858.

Bohrmann, J., Schill, S., 1997. Cytoplasmic transport in *Drosophila* ovarian follicles: the migration of microinjected fluorescent probes through intercellular bridges depends neither on electrical charge nor on external osmolarity. International Journal of Developmental Biology 41, 499–507.

Büning, J. (Ed.), 1994. The Insect Ovary: Ultrastructure, Previtellogenic Growth and Evolution Chapman and Hall, London.

Büning, J., Sohst, S., 1990. Ultrastructure and cluster formation in ovaries of bark lice, *Peripsocus phaeopterus* (Stephens) and *Stenopsocus stigmaticus* (Imhof and Labram) (Insecta: Psocoptera). International Journal of Insect Morphology and Embryology 19, 227–241.

Cant, K., Knowles, B.A., Mooseker, M.S., Cooley, L., 1994. *Drosophila singed*, a fascin homolog, is required for actin bundle formation during oogenesis and bristle extension. Journal of Cell Biology 125, 369–380.

Cassidy, J.D., King, R.C., 1972. Ovarian development in *Habrobracon juglandis* (Ashmead) (Hymenoptera: Braconidae). I. The origin and differentiation of the oocyte–nurse cell complex. Biological Bulletin 143, 483–505.

Cavaliere, V., Taddei, C., Gargiulo, G., 1998. Apoptosis of nurse cells at the late stages of oogenesis of *Drosophila melanogaster*. Development, Genes and Evolution 208, 106–112.

Cooley, L., Theurkauf, W.E., 1994. Cytoskeletal functions during *Drosophila* oogenesis. Science 266, 590–596.

Cooley, L., Verheyen, E., Ayers, K., 1992. *Chickadee* encodes a profilin required for intercellular cytoplasm transport during *Drosophila* oogenesis. Cell 69, 173–184.

Foley, K., Cooley, L., 1998. Apoptosis in late stage *Drosophila* nurse cells does not require genes within the H99 deficiency. Development 125, 1075–1082.

Giardina, A., 1901. Origine dell' oocyte e delle cellule nutrici nei *Dytiscus*. International Monatsschreibe für Anatomie und Physiologie 18, 417–419.

Guild, G.M., Connelly, P.S., Shaw, M.K., Tilney, L.G., 1997. Actin filament cables in *Drosophila* nurse cells are composed of modules that slide passively past one another during dumping. Journal of Cell Biology 138, 783–797.

Gutzeit, H.O., 1986. Transport of molecules and organelles in meroistic ovarioles of insects. Differentiation 31, 155–165.

Gutzeit, H.O., Huebner, E., 1986. Comparison of filament patterns in nurse cells of different insects with polytrophic and telotrophic ovarioles. Journal of Embryology and Experimental Morphology 93, 291–301.

Gutzeit, H.O., Koppa, R., 1982. Time-lapse film analysis of cytoplasmic streaming during late oogenesis of *Drosophila*. Journal of Embryology and Experimental Morphology 67, 101–111.

Hurst, S., Talbot, N.J., Stebbings, H., 1999. A staufen-like RNA-binding protein in translocation channels linking nurse cells to oocytes in

Notonecta shows nucleotide-dependent attachment to microtubules. Journal of Cell Science 112, 2947–2955.

Jaglarz, M., 1992. Peculiarities of organization of egg chambers in carabid ground beetles and their phylogenetic implications. Tissue and Cell 24, 397–409.

Knowles, B.A., Cooley, L., 1994. The specialized cytoskeleton of the *Drosophila* egg chamber. Trends in Genetics 10, 235–241.

Mahajan-Miklos, S., Cooley, L., 1994. Intercellular transport during *Drosophila* oogenesis. Developmental Biology 165, 336–351.

Matova, N., Mahajan-Miklos, S., Mooseker, M.S., Cooley, L., 1999. *Drosophila* Quail, a villin-related protein, bundles actin filaments in apoptotic nurse cells. Development 126, 5645–5657.

Mazurkiewicz, M., Kubrakiewicz, J., 2001. Intercellular cytoplasm transport during oogenesis of a moth midge, *Tinearia alternata*, (Diptera: Psychodidae). Folia Biologica (Kraków) 49 in press.

McCall, K., Steller, H., 1998. Requirement for DCP-1 caspase during *Drosophila* oogenesis. Science 279, 230–234.

Nezis, I.P., Stravopodis, D.J., Papassideri, I., Margaritis, L.H., 2001. Actin cytoskeleton reorganization of the apoptotic nurse cells during the late developmental stages of oogenesis in *Dacus oleae*. Cell Motility and the Cytoskeleton 48, 224–233.

Nezis, I.P., Stravopodis, D.J., Papassideri, I., Robert-Nicoud, M., Margaritis, L.H., 2000. Stage specific apoptotic patterns during *Drosophila* oogenesis. European Journal of Cell Biology 79, 610–620.

Pokrywka, N.J., Stephenson, E.C., 1991. Microtubules mediate the localization of *bicoid* RNA during *Drosophila* oogenesis. Development 113, 55–66.

Ries, E., 1932. Die Prozesse der Eibildung und das Eiwachstum bei Pediculiden und Mallophagen. Zeitschrift für Zellforschung und mikroskopische Anatomie 16, 314–388.

Riparbelli, M.G., Callaini, G., 1995. Cytoskeleton of the *Drosophila* egg chamber: new observations on microfilament distribution during oocyte growth. Cell Motility and the Cytoskeleton 31, 298–306.

Robinson, D.N., Cooley, L., 1997. Genetic analysis of the actin cytoskeleton in the *Drosophila* ovary. Annual Review of Cell and Developmental Biology 13, 147–170.

Rübsam, R., Büning, J., 1996. The cytoskeleton in ovarian clusters of polytrophic meroistic insects. In Proceedings of XX International Congress of Entomology, Firenze, p. 132.

Stebbings, H., Anastasi, A., Indi, S., Hunt, C., 1987. Binding of mammalian brain microtubule-associated proteins (MAPs) to insect ovarian microtubules. Cell Motility and the Cytoskeleton 8, 174–181.

Stebbings, H., Lane, J.D., Talbot, N.J., 1995. mRNA translocation and microtubules: insect ovary models. Trends in Cell Biology 5, 361–365.

Stephen, S., Talbot, N.J., Stebbings, H., 1999. Poly(A) mRNA is attached to insect ovarian microtubules in vivo in a nucleotide-sensitive manner. Cell Motility and the Cytoskeleton 43, 159–166.

St Johnston, D., Nüsslein-Volhard, C., 1992. The origin of pattern and polarity in the *Drosophila* embryo. Cell 68, 201–219.

Štys, P., Biliński, S.M., 1990. Ovariole types and the phylogeny of hexapods. Biological Reviews 65, 401–429.

Telfer, W.H., 1975. Development and physiology of the oocyte–nurse cell syncytium. Advances in Insect Physiology 11, 223–319.

Theurkauf, W.E., Alberts, B.M., Jan, Y.N., Jongens, T.A., 1993. A central role for microtubules in the differentiation of *Drosophila* oocytes. Development 118, 1169–1180.

Theurkauf, W.E., Hazelrigg, T.I., 1999. In vivo analyses of cytoplasmic transport and cytoskeletal organization during *Drosophila* oogenesis: characterization of a multi-step anterior localization pathway. Development 125, 3655–3666.

Wheatley, S., Kulkarni, S., Karess, R., 1995. *Drosophila* nonmuscle myosin II is required for rapid cytoplasmic transport during oogenesis and for axial nuclear migration in early embryos. Development 121, 1937–1946.

Wilsch-Bräuniger, M., Schwarz, H., Nüsslein-Volhard, C., 1997. A sponge-like structure involved in the association and transport of maternal products during *Drosophila* oogenesis. Journal of Cell Biology 139, 817–829.

Yamauchi, H., Yoshitake, N., 1982. Origin and differentiation of the oocyte–nurse cell complex in the germarium of the earwig, *Anisolabis maritima* Borelli (Dermaptera: Labiduridae). International Journal of Insect Morphology and Embryology 11, 293–305.

Zawadzka, M., Jankowska, W., Biliński, S.M., 1997. Egg shells of mallophagans and anoplurans (Insecta: Phthiraptera): morphogenesis of specialized regions and the relation to F-actin cytoskeleton of follicular cells. Tissue and Cell 29, 665–673.

Żelazowska, M., Biliński, S.M., 1999. Distribution and transmission of endosymbiotic microorganisms in the oocytes of the pig louse, *Haematopinus suis* (L.) (Insecta: Phthiraptera). Protoplasma 209, 207–213.