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Ovariole Development in Telotrophic Ovaries of Snake Flies (Raphidioptera)

Izabela JĘDRZEJOWSKA and Janusz KUBRAKIEWICZ

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Snake flies (Raphidioptera), alder flies (Megaloptera: Sialidae) and also some myxophagan coleopterans share the same, peculiar telotrophic organization of their ovarioles usually referred to as ovarioles of the *Sialis*-type. Ovariole ontogenesis in *Raphidia* sp. is described and the basic events that lead to the formation of germ cell clusters and their subsequent transformations are reported. It was found that the major cellular events during ovariole formation in *Raphidia* and *Sialis* are essentially the same. Discrepancies concern details of germ cell cluster formation, differentiation of cystocytes within clusters and their location within the developing tropharium. Based on these results the hypothetical model of the *Sialis*-type ovariole formation, previously presented by KING and BÜNING (1985) is verified. A hypothesis on the mechanisms of oocyte determination in telotrophic ovaries is also presented.

Key words: Germ cell clusters, syncytium formation, oocyte determination, *Sialis*-type ovariole

Izabela JĘDRZEJOWSKA, Janusz KUBRAKIEWICZ, Zoological Institute, University of Wrocław, Sienkiewicza 21, 50-335 Wrocław, Poland.
E-mail: jkubrak@biol.uni.wroc.pl

Regardless of histological organization, insect meroistic ovaries are formed by germ cell clusters (clones, cysts) or their derivatives. Clones of germ cells (cystocytes) arise during gonial cells' mitotic divisions followed by incomplete cytokineses. In consequence sibling cystocytes usually remain interconnected. Cystocyte clusters in meroistic ovaries can be regarded as a peculiar form of syncytia. Nevertheless, sibling cystocytes within these syncytia do not follow the same developmental pathway, but instead differentiate into two directions and hence their ultimate fate is totally different. Some of the cystocytes become oocytes, while the remaining ones transform into trophocytes (nurse cells). The primary role of the trophocytes is to synthesize various macromolecules (mostly different classes of RNPs) that are subsequently transported and eventually stored in the cytoplasm of the growing oocytes. To fulfill this function, trophocytes usually exhibit high transcription activity that is often additionally reinforced by the elevated ploidy of their nuclei.

Insect meroistic ovaries fall into two basic categories (for a review on insect ovary structure and function see: BILIŃSKI 1998; BÜNING 1994, 1996). In polytrophic ovaries particular clusters represent distinct structural-functional units, each containing a single oocyte and a group of its sibling nurse cells. Throughout oogenesis, the oocyte and the nurse cells remain directly connected by stable cytoplasmic canals termed intercellular bridges that arise during the incomplete cytokinetic divisions. On the contrary, in telotrophic ovaries cytoplasmic continuity between cystocytes is established during complex cytoarchitectural alterations that involve membrane fusion and degradation. During these rearrangements the primary structure of the intercellular bridges may be entirely lost. Moreover, the trophocytes and oocytes occupy distinct compartments. Trophocytes (or trophocyte syncytia) form a common trophic chamber (tropharium), while the linear array of oocytes at progressively advanced phases of oogenesis constitutes the vitellarium. The trophic chamber is linked with particular oocytes in the vitellarium by elongated oocyte projections termed nutritive cords.

Comprehensive comparative analyses of insect ovary structure have shown that polytrophic ovaries are usually morphologically similar, whereas telotrophic ovaries may exhibit significant structural variety. To date the occurrence of four different types of telotrophic ovary organization have

been evidenced (BÜNING 1993, 1994, 1996). The distinction between these types was essentially based on fundamental differences in the organization of their tropharia. The specific architecture of the tropharium emerges during subsequent stages of ovariole development and is conditioned by the pattern of germ cell cluster formation, their possible fusion, and also by the mode of germ cell diversification. The most ancient and peculiar telotrophic condition was found in the ovaries of mayflies (Ephemeroptera) (GOTTANKA & BÜNING 1993; BÜNING 1994, 1996). The tropharia in mayfly ovarioles house linear clusters of cystocytes that remain connected by persistent intercellular bridges. In hemipteran ovaries each telotrophic ovariole consists of only one germ cell cluster with trophocytes that surround a centrally located, anucleate cytoplasmic region referred to as the trophic core. The trophocytes are connected to the trophic core by cytoplasmic processes. A comparative analysis of telotrophic ovary structure in Hemiptera revealed that in several significant details the overall organization of tropharia may show considerable diversity (for a review on hemipteran ovary organization see: SIMICZYJEW *et al*. 1998; SZKLARZEWICZ 1998 a, c). Telotrophic ovaries were also reported in polyphage beetles (BÜNING 1979a; KING & BÜNING 1985; BÜNING 1994, 1996). Their tropharia consist of numerous trophocytes but also contain somatic, interstitial cells that penetrate deeply into the tropharium and form a complex, three dimensional network among the trophocytes. Trophocyte membranes tend to disappear and so the syncytial areas may be formed in trophic chambers. Particular taxa may differ in the range and extent of syncytium formation in their ovarioles (BÜNING 1979 a, b; MATUSZEWSKI *et al*. 1985). Previous histological and ultrastructural studies unequivocally indicated that the telotrophic ovarioles of alder flies (Megaloptera: Sialidae) and snake flies (Raphidioptera), but also those of some Coleoptera: Myxophaga, are structurally alike and thus belong in the same category traditionally termed "ovariole of the *Sialis* type" (MATSUZAKI & ANDO 1977; BÜNING 1979 c, 1980, 1994, 1996; BÜNING & MADDISON 1998; KUBRAKIEWICZ *et al*. 1998). In this type of telotrophic ovary the major part of the tropharium is occupied by the central syncytial core that contains numerous germ cell nuclei, whereas the tropharium cortex is formed by a simple layer of trophocytes, usually referred to as tapetum cells. Each tapetum cell is linked directly to the central syncytial core by a delicate intercellular bridge, whereas cytoplasmic bridges were never found to occur between tapetum cells. Oocytes that differentiate in the basal part of tropharium in direct contact with the somatic prefollicular tissue are also connected to the central syncytium by stable intercellular bridges. During the initial stages of oogenesis (up to the early previtellogenic phase), tapetum cells and oocytes are of equal size and show the same structural organization.

The development of the *Sialis*-type telotrophic ovariole was described by BÜNING (1980), who thoroughly traced the major cellular events that assist ovary formation in *Sialis flavilatera*, from the early larval stages till maturity. Assuming that all *Sialis*-type ovarioles should develop in a similar way, KING and BÜNING (1985) presented the general model describing the mechanisms that govern the ontogenesis of this type of ovariole. It should be emphasized, however, that other insects with *Sialis*-type ovaries were never studied with respect to their ovariole development. In this paper the results of observations on the crucial events that lead to the formation of the ovary in Raphidioptera are presented. This study was aimed to elucidate the most unclear and hypothetic items of the model postulated by KING and BÜNING (1985), and also to confirm which of the mechanisms of cluster formation and fusion are actually shared by Megaloptera and Raphidioptera.

Material and Methods

Insects

Larvae and pupae of *Raphidia* spp. were collected from under the bark of pine trees from March till May in SW Poland.

Histological and ultrastructural analysis

The ovaries were dissected and fixed at room temperature in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH=7.4) for long periods (usually for a few days). After several rinses in the same buffer, the material was postfixed for approximately one hour in 2% osmium tetroxide. After dehydration in a graded acetone series, single ovarioles were embedded in Epon 812. Semithin sections (0.6 m thick) were stained with 1% methylene blue in 1% borax and examined in an Olympus BHS light microscope. Ultrathin sections were cut on a Reichert UltraCut E ultramicrotome and analyzed with a Tesla BS 500 or Zeiss EM 900 electron microscopes at 60 kV and 80 kV, respectively.

Detection of microfilaments (F-actin)

The ovaries were fixed for 30 min in 4% formaldehyde, freshly prepared from paraformaldehyde, in modified MF buffer (solution B after BOND and

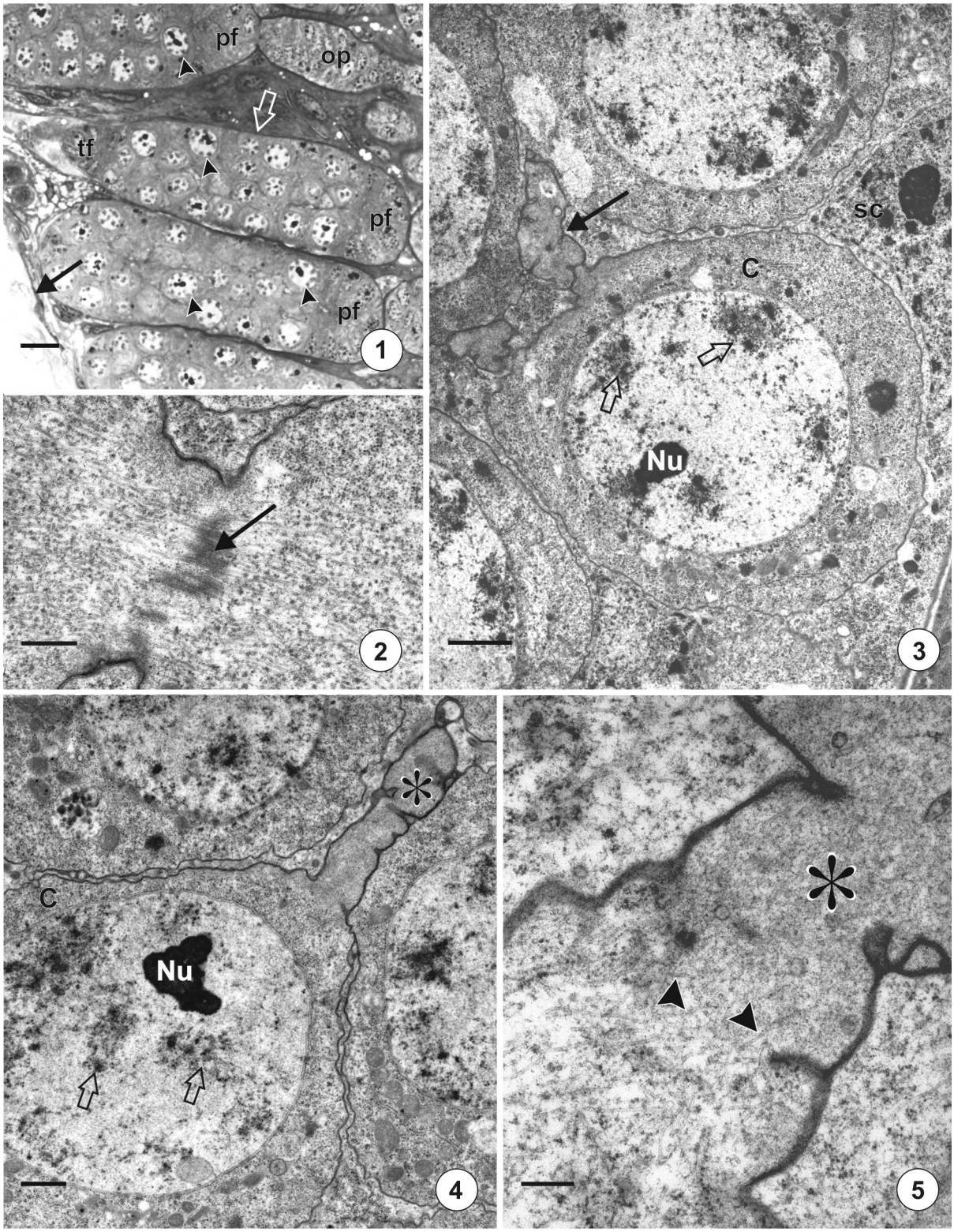
SOMLYO 1982) containing 80 mM KCl, 5.6 mM glucose, 1.5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 10 mM EGTA, 20 mM Pipes (pH 7.2) and 0.1% Triton X-100. The ovaries were then rinsed with the buffer, first with and then without Triton X-100. Single ovarioles, devoid of an outer muscle tissue layer, were stained with 2 μg/ml rhodamine-labeled phalloidin (Sigma) for 20 min in darkness. After rinsing with the buffer, the ovarioles were whole-mounted onto microscope slides and examined with an Olympus BHS epifluorescence microscope equipped with appropriate filters.

Results

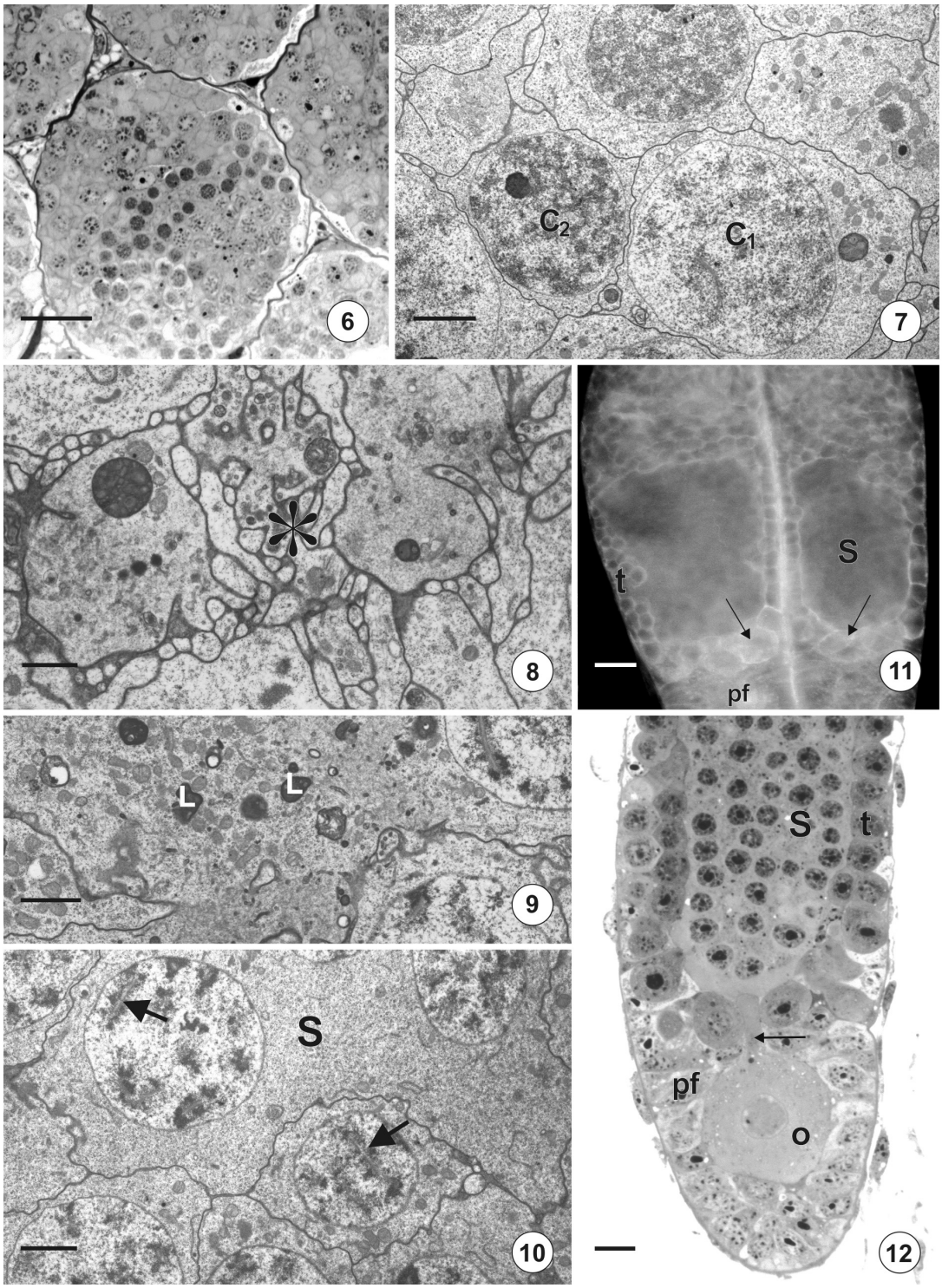
The ovaries of *Raphidia* sp. are paired and located in the abdomen on both sides of the alimentary canal. In the larval stages, each ovary is roughly spherical with a slightly pointed anterior pole. Its opposite, posterior part is concave and attached to the primordium of the lateral oviduct. The ovary is composed of several dozens, tightly packed, elongated ovarian tubes (ovarioles). The whole gonad is covered by a prominent extended layer of the somatic epithelium. Somatic cells also enter inside the ovary, penetrate among the ovarioles and separate them from each other (Fig. 1). From the early stages of ovary differentiation throughout the subsequent phases of oogenesis, all ovarioles within the ovary develop in synchrony. In the larval ovary the ovarioles are cylindrical and enclosed by a continuous basal lamina. At this developmental stage each ovariole can be subdivided into three morphologically distinguishable regions (Fig. 1). The anteriormost terminal filament is made up of flattened and stacked somatic cells which are not separated from the rest of the ovariole by any sort of transverse septum (not shown). The midpart of the ovariole is the largest region and houses mostly undifferentiated germ cells (gonial cells). A compact group of somatic prefollicular cells can be found in the posteriorly directed region. Posteriorly, the ovariole midpart passes into a short and solid ovariole pedicle. The pedicle consists of lens- shaped somatic cells and connects each ovariole directly to the lateral oviduct.

The gonial cells make up the midpart of the ovariole and are separated from the basal lamina by a layer of stretched somatic cells that form a continuous inner sheath within the ovariole (Fig. 3). The gonial cells are round. They can be easily recognized by the characteristic appearance of their nuclei, which contain numerous dense clumps of chromatin and single irregular nucleoli (Fig. 1). Gonial cells divide mitotically and so their number gradually increases. Ultrastructural analysis has shown that starting from the advanced larval stages, at least some of these divisions are followed by incomplete cytokineses. In consequence the arising cells preserve cytoplasmic continuity and become connected by intercellular bridges. Initially the bridges are filled with bundles of parallel microtubules that represent the remnants of the mitotic spindle (mid-body) (Fig. 2). Somewhat later the bridges become elongated while the microtubules are replaced by a fine fibrillar material – fusome (Figs 3-5). The microtubules of the mitotic spindle do not disappear completely. Many of them can be still found in the cytoplasm adjacent to the fusomal material in both connected cells, while other microtubules remain closely associated with the fusome or can be embedded in it (Fig. 5). The membrane forming the wall of the bridge is usually slightly folded and thickened (Figs 4, 5). Mitotic divisions followed by incomplete cytokineses become more intense during the subsequent stages of ovariole development. As a result several multicellular clones (clusters) of sibling germ cells appear within the ovariole. At the advanced larval and early pupal stages, single gonial cells can hardly be found and so the ovariole contains almost exclusively germ cell clusters. Descendants of gonial cells (cystocytes) are relatively small while their connecting intercellular bridges are minute. Therefore, even a closer inspection of ovariole whole mounts stained with rhodamine-conjugated phalloidin or observations of serial sections did not reveal either the ultimate number of cystocytes per cluster or their spatial arrangement within clusters. The presence of cystocytes connected by more than two intercellular bridges with their siblings (not shown) unequivocally indicates that clusters are branched. Cystocytes forming an individual clone develop synchronously but particular clusters within the ovariole are found at different stages of their formation and differentiation (Fig. 6). In some of them gonial cells still divide mitotically, whereas others are formed by the cystocytes that have already entered the prophase of the first meiotic division. The developmental stage reached by particular clusters could be easily distinguished by the appearance of their cystocyte nuclei, e.g. the prophase of the first meiotic division can be recognized by the presence of synaptonemal complexes in the germ cell nuclei (Fig. 7). Histological or ultrastructural analyses did not show any correlation between developmental stage of cystocytes within the clone and the location of the latter in the ovariole. The profiles of the growing and differentiating germ cell clusters are mostly irregular. None of them, however, were found to spread over the whole diameter of the ovarian tube.

During cluster development the cystocytes undergo a specific spatial rearrangement. Intercellu-



Figs 1-5. Larval stages of ovariole formation. Fig. 1. Elongated midparts of the ovarioles are filled mainly with gonial cells (arrowheads). Posteriorly, gonial cells border the somatic prefollicular tissue (pf), while the latter is in direct contact with the cells of the ovariole pedicle (op). Terminal filament (tf) forms the anteriormost part of the ovariole. White hollow arrow indicates the ovariole sheath, while black arrow denotes the somatic ovarian epithelium. Semithin section stained with methylene blue. Bar = 0.1 μm. Fig. 2. Gonial cell division. The lumen of the forming intercellular bridge is initially filled with the remnants of the mitotic spindle (midbody) (arrow). TEM. Bar = 0,6 μm. Figs 3-5. Cystocytes (C) of the newly formed clusters are connected by elongated intercellular bridges (denoted by arrows in Fig. 3). Fine fibrillar material (fusome) appears in their lumen (asterisks in Figs 4, 5). Microtubules that are stuck in or neighbour the fusomal material are denoted by arrowheads (Fig. 5). Note dense patches of chromatin (hollow arrows) and single, irregular nucleoli (Nu) within cystocyte nuclei (Figs 3, 4). Somatic cell of the ovariole inner sheath denoted by sc in Fig. 3. TEM. Bar in Fig. 3 = 1.7 μm; 1.1 μm in Fig. 4 and 0.4 μm in Fig 5.



Figs 6-12. Pupal stages of ovariole formation. Figs 6-7. The developmental stage of a cluster within the ovariole can best be identified based on the appearance of the nuclei of its cystocytes. Fig. 6. Cross section through the ovariole midparts. Note that the cystocyte clusters at different stages of their differentiation assume irregular shapes but none of them extends over the whole ovariole width. Semithin section stained with methylene blue. Bar = 0.2 μm. Fig.7. Cystocytes of neighbouring clusters. Those at meiotic prophase (C_1) have conspicuously larger nuclei containing synaptonemal complexes; nuclei of those belonging to the adjacent cluster (C_2) are smaller and contain patches of more condensed chromatin. TEM. Bar =1,7 μm. Figs 8-10. Subsequent stages of membrane disintegration and cystocyte fusion. Fig. 8. Cystocyte membrane disintegration is preceded by their extensive folding (asterisk). Fig. 9. Membranes within clusters gradually disappear resulting in the progressive cystocyte fusion and formation of syncytial areas. L – dense lamellar bodies in the area of membrane disintegration. Fig. 10. Cystocyte nuclei at the prophase of the first meiotic division can either be found in individual cystocytes prior to their fusion or within the common cytoplasm of the newly formed syncytium (S). Synaptonemal complexes are indicated by arrows; TEM. Bars in Figs 8-10 = 1,7 μm. Fig. 11. Syncytium formation is initiated in the posterior zone of the tropharium and gradually progresses anteriorly. The arising central syncytial core of the tropharium (S) is externally covered by individual cells that form the tapetum layer (t). Early previtellogenic oocytes rest at base of the tropharium in contact with prefollicular tissue (pf). Whole mount preparation stained with rodamin conjugated phalloidin. Bar = 0.2 μm. Fig. 12. In the late pupal ovary, the tropharium consists of a well developed central syncytium (S) encompassed by tapetum cells (t). Posteriorly located previtellogenic oocytes (o) are embedded in the prefollicular tissue (pf); note the nutritive cords (arrows) connecting the oocytes with the core of tropharium. Semithin section stained with methylene blue. Bar = 0.1μm.

lar bridges that connect the sibling cells tend to group in the central area of the cluster and thus the latter obtains a rosette shape. Neighbouring fusomes merge and form a polyfusome, a common, fine fibrillar structure that seems to penetrate all the bridges within the cluster (not shown). At the initial stages of rosette formation membranes of adjacent cystocytes get extensively folded and tangled thus forming a spatially complex labirynth (Fig. 8). These membranes gradually disintegrate while the cystocytes fuse with each other (Fig. 9). Disintegration of cystocyte membranes is accompanied by the appearance of electron dense bodies in the arising syncytial areas and in the cytoplasm of those cystocytes that are currently fused (Fig 9). Polyfusomes persist within the clusters till the initial stages of cystocyte fusion and disappear completely soon after. As a rule cystocyte fusion is initiated in the central part of each cluster and expands externally. Because of these transformations the cystocyte nuclei are not separated by plasma membranes but inhabit a common cytoplasm, thus forming a syncytium (Fig. 10). Fusion of cystocytes is not only limited to particular clusters but is followed by fusion of adjacent clusters. As a result the area occupied by a common, multinuclear syncytium conspicuously grows within the ovariole. Analysis of whole mount ovariole preparations stained with rhodamine-conjugated phalloidin showed that the formation of a syncytium is regularly initiated in the posterior, basal region of the ovariole midpart (Fig. 11). This common syncytial region progressively enlarges anteriorly comprising a growing number of cystocyte clusters. Eventually almost all cystocytes within the ovariole undergo fusion. The only exceptions are those that contact the inner ovariole sheath or the prefollicular tissue. Each of these cells retains its cellular identity and is directly connected with the central multinuclear syncytium by means of a single, well preserved intercellular bridge (not shown)[1].

The cystocytes that do not contribute to the formation of the syncytium behave differently during their further differentiation. Differences were found to depend on the position of these cystocytes within the ovariole. The cells that adhere to the inner ovariole sheath form an external, one cell thick layer of germ cells referred to as tapetum cells (Figs 11,12), whereas more posteriorly located cystocytes that contact the prefollicular tissue are potential oocytes. Initially the tapetum cells and oocytes are morphologically almost identical and of equal size (Fig. 11). In the advanced pupal stages the early previtellogenic oocytes become conspicuously larger than the tapetum cells. Growing oocytes sink into the prefollicular tissue and become invested by a simple follicular epithelium (Fig. 12). Individual oocytes together with their follicular epithelium form ovarian follicles. Eventually four distinct regions can be easily recognized along the ovariole. The anteriormost terminal filament is followed by an elongated, cylindrical tropharium (trophic chamber). The core of tropharium is filled with a multinuclear syncytium, while a one cell thick layer of tapetum cells forms the cortex (Fig. 12). A linear row of a few ovarian follicles at progressively advanced stages of previtellogenesis and vitellogenesis constitutes the vitellarium. The intercellular bridges connecting particular oocytes with the central trophic syncytium persist unchanged till the final stages of vitellogenesis. The anterior cytoplasm of each oocyte in the vitellarium is significantly extended and forms a cytoplasmic projection termed a nutritive cord (Fig.12). The vitellarium passes posteriorly into a short ovariole pedicle (not shown) (the gross morphology of the snake fly ovariole has been described previously: KUBRAKIEWICZ *et al*. 1998; for details on nutritive cord formation and structure in the snake fly ovariole see: JĘDRZEJOWSKA & KUBRAKIEWICZ (2002).

Discussion

Based solely on light microscopy investigations ACHTELIG (1978) was the first to show that the ovaries in Raphidioptera are telotrophic. Soon after, these results were confirmed but also significantly detailed by TEM analysis of the ovary structure in *Raphidia flavipes* (BÜNING 1980). Data obtained by BÜNING clearly indicated that snake flies share the same architecture of the telotrophic ovariole with alder flies. Morphology, ultrastructure and ontogenesis of telotrophic ovarioles in *Sialis* were described first, and subsequently this type of telotrophic ovariole organization was termed the "*Sialis* type" after this genus. Since the ovarioles in alder flies and snake flies are structurally alike, it was reasonable to postulate that their ontogenetic development should follow a similar pathway. Indeed, results presented in this paper indicate that in general the scenario of the major cellular events that lead to the formation of the mature ovary in *Sialis* and *Raphidia* is essentially the same. The formation of the *Sialis* type ovariole is a process that comprises at least 3 major steps. After the gonial cells have invaded the ovariole primordia they multiply by mitotic divisions with com-

[1] The presence and ultrastructure of these intercellular bridges have been presented previously (KUBRAKIEWICZ *et al*. 1998; JĘDRZEJOWSKA & KUBRAKIEWICZ 2002).

plete cytokineses. Somewhat later gonial cells' divisions followed by incomplete abscission of daughter cells are initiated. As a result the dividing germ cells remain interconnected and form clusters of cystocytes. The progressive fusion of cystocyte membranes within the clusters and also between them finally results in the emergence of an extensive internal syncytium. Fusion of clusters and hence the extension of the syncytial areas within the ovariole is initiated in the posterior region of the forming tropharium and progresses anteriorly. Only the most external cells preserve cellular identity and remain connected with the central syncytium by means of delicate intercellular bridges. Among these are the cells that rest in the basal part of the ovariole in direct contact with the somatic prefollicular tissue. These cells eventually become oocytes, while those located externally in the anterior section of the ovariole tube (tropharium) transform into the so called tapetum cells.

Data presented in this paper indicate that although basically similar, the course of tropharium formation in Sialidae and Raphidioptera exhibits noticeable discrepancies. These discrepancies concern some details of germ cell cluster formation, differentiation of cystocytes within clusters and also their location within the developing tropharium.

The divisions of cystocytes within particular clusters seem to be synchronous and so the number of germ cells per cluster presumably obeys the rule $N=2^n$ (where "N" is the number of cystocytes, while "n" defines the number of cystocyte divisions). However, due to the small size of cells and their tangled spatial arrangement, the ultimate number of cystocytes within a clone (and so the number of cystocyte divisions) was never exactly evaluated. BÜNING (1980) suggested that in *Sialis* 32-cell clusters result from 5 synchronous rounds of cystocyte divisions. Clusters in *Raphidia* probably consist of the same number of cystocytes, but it should be emphasized here that these estimations in both cases are rough.

In the majority of insect meroistic ovaries studied so far, the spatial organization of their cystocytes within clusters was described as branched or ramified (TELFER 1975; KING *et al*. 1982; BÜNING 1993, 1994; KING & BÜNING 1985; MCKEARIN 1997). This means that the clusters were usually found to contain cystocytes interconnected with their sibling neighbours by more than only two intercellular bridges. Although the branched condition of spatial organization of cystocyte clusters was reported repeatedly in different insects with meroistic ovaries, the cellular and molecular mechanisms of their formation was most extensively studied only in the polytrophic ovary of *Drosophila* (STORTO & KING 1989; LIN *et al*. 1994; LIN & SPRADLING 1995; reviewed in: SPRADLING *et al*. 1997; PEPLING *et al*. 1999; DENG & LIN 2001). In this model organism the rise of branched arrangements of cystocytes was found to depend on a peculiar polarization of mitotic divisions during cluster formation (DENG & LIN 1997; MCKEARIN 1997). When cystocytes divide, the poles of their mitotic spindles are attracted by fusomal material which appears within the intercellular bridges during the previous round of cystocyte divisions. Fusomes in *Drosophila* contain several specific proteins, like α-and β-spectrin, ankyrin and an adducin-like protein encoded by the *hu-li tai shao* (*hts*) gene (LIN *et al*. 1994; DE CUEVAS *et al*. 1996; DE CUEVAS & SPRADLING, 1998), but also cytoplasmic dynein (MCGRAIL & HAYS 1997). For its affinity to microtubules, cytoplasmic dynein seems to be the best candidate responsible for the attraction of mitotic spindles and thus for the generation of branching within clusters (DENG & LIN 1997; THEURKAUF 1997). Fusomes were regularly found in arising clusters of germ cells and it is now widely accepted that their appearance is a prerequisite for the generation of the branched spatial arrangement of clusters. Although serial ultrathin sections of the ovarioles were never analysed either in *Sialis* or *Raphidia*, available electron microscopy data (BÜNING 1979c; present paper) suggest that in both cases cystocyte clusters are branched. Groups of sibling cystocytes form rosettes containing germ cells with more than two intercellular bridges. Moreover, the observations presented in this paper clearly indicate that intercellular bridges in *Raphidia* are filled with fusomal material. On the other hand, however, according to data obtained by BÜNING (1979c), clones of cystocytes in *Sialis* lack recognizable fusomes or polyfusomes. Since the formation of branches within clusters in the absence of fusomes can hardly be explained, fusomal material probably appears within intercellular bridges in the *Sialis* ovary, but contrary to the situation found in the *Raphidia* ovary where they persist for a longer time, in *Sialis* they presumably represent only transient structures and that is why their presence can be easily overlooked during ultrastructural analysis.

In the hypothetical model of ontogenesis of the *Sialis*-type ovariole, KING and BÜNING (1985) postulated that single-layered, 32-cell clusters are flat, disc-shaped and oriented perpendicularly to the long axis of the ovariole, while each occupies the whole diameter of the ovariole. In this model the outermost cystocytes (i.e. terminal cystocytes within a single cluster) retain their cellular identity, while those located internally undergo fusion and thus contribute to the formation of the central syncytium. The present data do not seem to fully

support these postulations. First, particular germ cell clusters in snake flies were never observed to extend over the whole width of the ovariole, neither were they disc-shaped, but rather more irregular instead. Second, according to the model presented by KING and BÜNING (1985), at least some of the oocytes that are specified at the base of the forming tropharium should be connected with the central syncytium by more than a single intercellular bridge. Obviously such a situation has never been observed. The present study show that clusters of germ cells are irregular in shape and located at random within the premature ovariole. All cystocytes undergo fusion and lose their cellular identity, except for those that remain in contact with the somatic tissue of the ovariole. No matter where the cystocyte is positioned within the cluster, whether it is located in the branched midzone of a rosette or is an external, terminal cell with only one intercellular bridge connecting it with the remaining part of a clone, the lack of direct contact with the somatic tissue will always result in fusion. This may indicate that germ cells are somehow prevented from fusion when they remain in direct contact with the somatic tissue. Since the tapetum cells and oocytes are always terminal cells within clusters, they remain connected with the syncytial core of the tropharium only by single intercellular bridges, and are never connected with each other. Tapetum cells and early previtellogenic oocytes share the same developmental history and are morphologically alike. The only difference is that oocytes start previtellogenic growth, while tapetum cells seem to be arrested in the pre-previtellogenic phase. Why do some terminal cystocytes become tapetum cells while others are specified as oocytes? Probably the character of the somatic-germ cells' interactions may influence the ultimate fate of germ cells. In contrast to the tapetum cells, future oocytes are located at the base of the tropharium and so are able to develop broad and elaborate connections with the somatic prefollicular tissue. While the significance of germ-somatic cells' interactions for oocyte specification in telotrophic ovaries was postulated previously (BÜNING 1979 c; GOTTANKA & BÜNING 1993; BÜNING 1994), the mechanisms of germ cell determination remain elusive. It seems possible that the intimate connections between future oocytes and somatic tissue could be reinforced by (over)expression of the adhesion molecules, which in turn, could play an important role in oocyte specification. This postulation suggests that the mechanisms underlying oocyte selection in telotrophic ovaries would be based almost entirely on the activity of some extrinsic cues, and so would be significantly different from those postulated for polytrophic ovaries. The mechanisms that govern oocyte determination in polytrophic ovaries were extensively studied in *Drosophila*. Several lines of evidence indicate that in the fruit fly the specification of the oocyte is a complex, multistep process which is fundamentally based on the activity of intrinsic factors and events. These comprise the asymetric distribution of fusomal material during cystocyte divisions, polarization of the cytoskeleton, directed transport of specific markers and their concentration in the future oocyte and also events that enable oocyte fate maintenance (for a recent review on oocyte specification in *Drosophila* see: DENG & LIN 2001). The mechanisms of oocyte determination in insect telotrophic ovaries were never studied at the molecular level, however, based on morphological observations, fusomes and polyfusomes were recognized in the forming germ cell clusters in different types of telotrophic ovarioles, e.g. in Ephemeroptera (GOTTANKA & BÜNING 1993), in Hemiptera (BÜNING 1994; SZKLARZEWICZ 1997, 1998 a,b,c), Coleoptera: Polyphaga (MATUSZEWSKI *et al*. 1985; BÜNING 1994; ŚWIĄTEK 2002) and Raphidioptera (this report). The relative location of germ cell clusters (and their fusomes/polyfusomes) and the places where oocytes originate, strongly implicates that fusomes do not play a role in oocyte specification. More probably their occurrence conditions the generation of branching within clusters (this function has also been evidenced in *Drosophila* – STORTO & KING 1989; LIN & SPRADLING 1995; DENG & LIN 1997). Fusomes may also be engaged in the synchronization of certain events during germ cell cluster formation, e.g. mitotic divisions, entry into meiosis etc.

How the oocytes are specified in telotrophic ovaries is still unclear. The available data seem to point to some putative external factors e.g. adhesion molecules, however, the problem remains unresolved since molecular data are missing completely. The function of adhesion molecules (e.g. DE-cadherin) during germ cell cluster formation has been quite recently defined in *Drosophila* based on molecular studies (GODT & TEPASS 1998, GONZÁLEZ-REYES & St JOHNSTON 1998). Rather than determing the oocyte fate itself, adhesion was found to play a central role in oocyte positioning within the cluster after the oocyte has already been selected. Whether germ cell determination in telotrophic ovaries relies on inductive signalling, what is the nature of the signalling molecules, and to what extent oocyte determination depends on adhesion interactions can only be elucidated on molecular grounds.

Although the external factors seem to be essential for oocyte specification in telotrophic ovaries, it should be emphasized herewith that the influence of some intrinsic regulatory mechanisms cannot be entirely ruled out. Indications of such

internal factors come from the observations of the architecture of some hemipteran ovaries, and may also be found in the course of ovariole development in polyphage beetles. In hemipteran ovarioles, arrested oocytes were found in different locations within tropharia (SZKLARZEWICZ & BILIŃSKI 1995; SZKLARZEWICZ 1997, 1998 a,b; SZKLARZEWICZ *et al*. 2000). This peculiar position of oocytes indicates that their specification could not be solely conditioned by somatic-germ cells' interactions. In a polyphage beetle, *Creophilus maxillosus*, fusome precursors (spectrosomes) are asymmetrically distributed only to potential oocytes (MATUSZEWSKI *et al*. 1999) implying that this material may play some role in oocyte determination.

References

ACHTELIG M. 1978. Entwicklung und Morphologie der inneren und usseren weiblichen Genitalorgane der Kamelhalsfliegen (Neuropteroidea, Raphidioptera). Ent. Germ. **4**: 140-163.

BILIŃSKI S. M. 1998. Introductory remarks. Folia Histochem. Cytobiol. **36**: 143-145.

BÜNING J. 1979a. The trophic tissue of telotrophic ovarioles in Polyphage Coleoptera. Zoomorphologie **93**: 33-50.

BÜNING J. 1979b. The telotrophic nature of ovarioles of Polyphage Coleoptera. Zoomorphologie **93**: 51-57.

BÜNING J. 1979c. The telotrophic-meroistic ovary of Megaloptera I. The ontogenetic development. J. Morph. **162**: 37-66.

BÜNING J. 1980. The ovary of *Raphidia flavipes* is telotrophic and of the *Sialis* type (Insecta, Raphidioptera). Zoomorphologie **95**: 127-131.

BÜNING J. 1993. Germ cell cluster formation in insect ovaries. Int. J. Insect Morphol. Embryol. **22**: 237-253.

BÜNING J. 1994. The Insect Ovary. Ultrastructure, previtellogenic growth and evolution. Chapman & Hall, London.

BÜNING J. 1996. Germ cell cluster variety creates diversity of ovary types in insects. Verh. Dtsch. Zool. Ges. **89**: 123-137.

BÜNING J., MADDISON D. R. 1998. Surprising ovary structures at the base of Coleoptera. VI[th] European Congress of Entomology. Ceske Budejovice, August 23-29.

CHOI W. C., NAGL W. 1977b. Patterns of DNA and RNA synthesis during the development of ovarian nurse cells in *Gerris najas* (Heteroptera). Dev. Biol. **61**: 262-272.

DE CUEVAS M., LEE J. K., SPRADLING A. C. 1996. á-spectrin is required for germline cell division and differentiation in the *Drosophila* ovary. Development **122**: 3959-3968.

DE CUEVAS M., SPRADLING A.C. 1998. Morphogenesis of the *Drosophila* fusome and its implications for oocyte specification. Development **125**: 2781-2789.

DENG W., LIN H. 1997. Spectrosomes and fusomes anchor mitotic spidles during asymmetric germ cell divisions and facilitate the formation of a polarized microtubule array for oocyte specification in *Drosophila*. Dev. Biol. **189**: 79-94.

DENG W., LIN H. 2001. Asymmetric germ cell division and oocyte determination during *Drosophila* oogenesis. Int. Rev. Cytol. **203**: 94-138.

GODT D., TEPASS U. 1998. *Drosophila* oocytes localization is mediated by differential cadherin-based adhesion. Nature **395**: 387-391.

GONZÁLEZ-REYES A., St JOHNSTON D. 1998. The *Drosophila* AP axis is polarised by the cadherin-mediated positioning of the oocyte. Development **125**: 3635-3644.

GOTTANKA J., BÜNING J. 1993. Mayflies (Ephemeroptera), the most "primitive" winged insects, have telotrophic meroistic ovaries. Roux's Arch. Dev. Biol. **203**: 18-27.

JĘDRZEJOWSKA I., KUBRAKIEWICZ J. 2002. Formation and structure of nutritive cords in telotrophic ovarioles of snake flies (Insecta: Raphidioptera). Folia Histochem. Cytobiol. **40**: 77-83.

KING R. C., BÜNING J. 1985. The origin and functioning of insects oocytes and nurse cells. (In: Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol. 1 Embriogenesis and Reproduction. G. A. Kerkut & L. I. Gilbert eds. Pergamon Press, Oxford): 37-82.

KING R. C., CASSIDY J. D., ROUSSET A. 1982. The formation of clones of interconnected cells during gametogenesis in insects. (In: Insect Ultrastructure, vol. 1. R. C. King, H. Akai eds. Plenum Press, New York): 3-31.

KUBRAKIEWICZ J., JĘDRZEJOWSKA I., BILIŃSKI S. M. 1998. Neuropteroidea – different ovary structure in related groups. Folia Histochem. Cytobiol. **36(4)**: 179-187.

LIN H., YUE L., SPRADLING A. C. 1994. The *Drosophila* fusome, a germ-line organelle, contains membrane skeletal proteins and functions in cyst formation. Development **120**: 947-956.

LIN H., SPRADLING A.C. 1995. Fusome asymmetry and oocyte determination in Drosophila. Dev. Genet. **16**: 6-12.

MATSUZAKI M., ANDO H. 1977. Ovarian structure of the adult alderfly, *Sialis mitsuhashii* Okamoto (Megaloptera, Sialidae). Int. J. Morphol. Embryol. **6**: 17-29.

MATUSZEWSKI B., CIECHOMSKI K., NURKOWSKA J., KLOC M. 1985. The linear clusters of oogonial cells in the development of telotrophic ovarioles in Polyphage Coleoptera. Reux's Arch. Dev. Biol. **194**: 462-469.

MATUSZEWSKI B., CIECHOMSKI K., KLOC M. 1999. Extrachromosomal rDNA and polarity of pro-oocytes during ovary development in *Creophilus maxillosus* (Coleoptera, Staphylinidae). Folia Histochem. Cytobiol. **37(3)**: 179-190.

MCGRAIL M., HAYS T. S. 1997. The microtubule motor cytoplasmic dynein is required for spindle orientation during germ line division and oocytes differentiation in *Drosophila*. Development **124**: 2409-2419.

MCKEARIN D. 1997. The *Drosophila* fusome, organelle biogenesis and germ cell differentiation: if you build it. BioEssays **19**: 147-152.

PEPLING M., DE CUEVAS M., SPRADLING A. C. 1999. Germline cyst: a conserved phase of germ cell development? Trends Cell Biol. **9**: 257-262.

SIMICZYJEW B., OGORZAŁEK A., ŠTYS P. 1998. Heteropteran ovaries: variation on the theme. Folia Histochem. Cytobiol. **36**: 147-156.

SPRADLING A. C., DE CUEVAS M., DRUMMOND-BARBOSA D., KEYES L., LILLY M., PEPLING M., XIE T. 1997. The *Drosophila* germarium: stem cells, germ line cyst, and oocytes. Cold Spring Harbor Symposia on Quantitive Biology **62**: 25-33.

STORTO P. D., KING R. C. 1989. The role of polyfusomes in generating branched chains of cystocytes during *Drosophila* oogenesis. Dev. Genet. **10**: 70-86.

SZKLARZEWICZ T. 1997. Structure and development of the telotrophic ovariole in ensign scale insect (Hemiptera, Coccomorpha: Ortheziidae). Tiss. Cell **29/1**: 31-38.

SZKLARZEWICZ T. 1998a. Structure of ovaries in scale insects. I. Pseudococcidae, Kermesidae, Eriococcidae, and Cryptococcidae (Insecta, Hemiptera, Coccinea). Int. J. Morphol. Embryol. **27**: 167-172.

SZKLARZEWICZ T. 1998b. Structure of ovaries in scale insects: II. Margarodidae (Insecta, Hemiptera, Coccinea). Int. J. Morphol. Embryol. **27**: 319-324.

SZKLARZEWICZ T. 1998c. The ovaries of scale insects (Hemiptera, Coccinea). Morphology and phylogenetic conclusions. Folia Histochem. Cytobiol. **34**: 157-165.

SZKLARZEWICZ T., BILIŃSKI S.M. 1995. Structure of ovaries in ensign scale insects, the most primitive representatives of Coccomorpha (Insecta, Hemiptera). J. Morphol. **224**: 23-29.

SZKLARZEWICZ T., WNĘK A., BILIŃSKI S.M. 2000. Structure of ovarioles in *Adelges laricis*, a representative of the primitive aphid family Adelgidae. Acta Zool. **81**: 307-313.

ŚWIĄTEK P. 2002. Structure and development of ovaries in the weevil, *Anthonomus pomorum* (Coleoptera, Polyphaga). II. Germ cells of the trophic chamber. Folia biol. (Kraków) **50**: 153-163.

TELFER W.H. 1975. Development and physiology of the oocyte-nurse cell syncytium. (In: Advances in Insect Physiology, vol. 11. J.E. Treherne, M.J. Berrige & V.B. Wigglesworth eds. Academic Press, London): 223-319.

THEURKAUF W. E. 1997. Oocyte differentiation: A motor makes a difference. Curr. Biol. **7**: 548-551.