64 (2) 115–126

Ovariole Structure Supports Sistergroup Relationship of Neuropterida and Coleoptera

JÜRGEN BÜNING

Friedrich-Alexander-Universität Erlangen-Nürnberg, Institut für Biologie, Lehrstuhl für Entwicklungsbiologie, Staudtstr. 5, 91058 Erlangen, Germany [jbuening@biologie.uni-erlangen.de]

Received 1.iii.2006, accepted 25.x.2006.
Available online at www.arthropod-systematics.de

> Abstract

Insect ovaries consist of functional units, the ovarioles. Each ovariole is a polarized tube with a germarial region at its anterior end. Undifferentiated germarial germ cells may differentiate either into oocytes alone (so-called panoistic ovarioles) or in oocytes and nurse cells (so-called meroistic ovarioles). Whenever nurse cells accompany an oocyte through all growth periods in a separated physiological unit – the follicle – the subtype is called polytrophic meroistic. In telotrophic meroistic ovarioles all nurse cells remain in an anterior trophic chamber and contribute their products finally into all growing oocytes via nutritive cords. Differences in the mode of germ cell cluster formation and the specific interaction of germ cells with the somatic tissues were causing these different types. All three types are constant at the family to order level or even at a supra-order level. Therefore the characters which lead to these types are excellent candidates to unravel order and supra-order ranked taxa. Data from the analysis of germ cell cluster formation are presented which corroborate the sistergroup relationship of Neuropterida and Coleoptera.

> Key words

Ovariole, germ cell cluster analysis, insect, phylogeny.

1. Introduction

Insects are by far the largest animal taxon. Today we count 31 subtaxa of so-called "ordinal" rank. Palaeontological data show that the overwhelming majority of ordinal ranking taxa evolved during Devonian (perhaps Ordovician) through Permian periods (KRISTENSEN 1981; WHEELER et al. 2001; KLASS et al. 2002; BEUTEL 2005; GRIMALDI & ENGEL 2005). Until now, most morphological data used in phylogenetic work on insects came from parts of the exoskeleton, like segments, legs, wings, mouthparts and so on. In the past three decades a growing set of data have been derived from the analysis of internal organs such as muscles, hemolymph pulsatory organs, brain structures, gonads, etc. Here, I report some recent observations on the development of insect ovarioles that shed new light on the evolution of ovarioles, especially within females of the taxa Neuropterida and Coleoptera.

Insect ovaries are of dual origin. The somatic component derives from mesoderm to which pole cells (= germ cells) migrate during embryongenesis (LASKO & ASHBURNER 1990). These gonad anlagen are located on either side of the alimentary canal in the abdomen. In most species the gonad anlagen are globular or elongated and lie parallel to the alimentary canal. Germ cells are situated centrally inside the anlage while the somatic cells differentiate into two mesenchymal tissues: a posterior plug lying next to the alimentary canal and the anterior tissue in front of the germ cell population. The posterior tissue gives rise to the lateral oviducts, the pedicels and the prefollicular and follicular tissue of the ovary and the anterior tissue to the terminal filaments, cap, inner sheath and interstitial cells. In the well investigated acalyptrate fly *Drosophila melanogaster*, differentiation into functional units of insect ovary, the ovarioles, starts by cell to cell communications between germ cells and anterior cells from which the terminal filaments derive. A basement membrane separates the rising ovarioles laterally as they emerge, from front to back, from the ovariole anlage (KING 1970).

2. The *Drosophila* ovariole

First I will provide a short summary of this process in the ovary of *Drosophila*, the best known organ in terms of molecular, developmental and morphological data.

Figure 1A represents a typical polytrophic meroistic ovariole of this fly showing the earlier stages of oogenesis. A *germarium* produces growing entities, the *follicles*, which undergo previtellogenic and vitellogenic growth and chorionogenesis in the *vitellarium* (vitellogenic and choriogenic phases are omitted from the scheme). Each follicle consists of a clone (= cluster) of germ cells: one oocyte syncytially connected to a set of 15 sister cells, which differentiate to nurse cells and supply the oocyte with euplasmatic elements needed by the oocyte and by early stages of embryogenesis. The cluster of germ cells is surrounded by a monolayer of follicular cells that function in vitellogenesis and synthesize and deposit the egg envelopes at the end of oogenesis.

The cluster of germ cells develops in a stereotypic manner from stem cells (Fig. 1B, red), which reside in a *niche* at the apex of the ovariole. The niche consists of a few somatic cells, named cap cells (green) near the anterior tip of each ovariole, next to the terminal filament. Permanent cell to cell communication between cap cells and the germ line stem cells is necessary to maintain the function of stem cells and to keep them in the anterior most position (LIN et al. 1994; SPRADLING et al. 2001; ZHU & XIE 2003; DECOTTO & SPRADLING 2005). As a rule, stem cells divide regularly only in an anterior-posterior direction, giving birth, with each division, to a *cystoblast* (Fig. 1B; stem cell: red; cystoblast: pink), which undergoes a strict program of four synchronous mitotic cycles, each followed by incomplete cytokinesis. The result is a cluster of 16 *cystocytes* (blue; KING 1970; STORTO & KING 1989). Cystocyte divisions and differentiation into nurse cells and oocytes take place subsequently in four compartments of the germarium (Fig. 1A). Cystoblasts and clusters are guided by specialized somatic cells (DECOTTO & SPRADLING 2005; not shown here). Only one of the two innermost cells of each cluster becomes the oocyte (dark turquoise) while all other cystocytes develop into nurse cells (turquoise). This final differentiation is regulated by intrinsic factors (DE CUEVAS & SPRADLING 1998; GRIEDER et al. 2000; HUYNH & ST JOHNSTON 2004; LIN et al. 1994; MCGRAIL & HAYS 1997; ROPER & BROWN 2004; ZHU & XIE 2003). In *Drosophila*, these germarial processes continue throughout adult life. Production of eggs depends exclusively on differential mitotic activity of germ line stem cells (* in Fig. 1C). Now we know that sequential expression of many genes control these developmental processes and we can use this knowledge as a basis for understanding the development of other types of ovarioles occurring in insects.

Subsequent growth of oocytes proceeds in the vitellarium in so-called *follicles* (Figs. 1, 2). In polytrophic ovaries, each follicle consists of one oocyte and its sister cells, which develop as nurse cells surrounded by follicular cells. Follicular cells deposit the vitelline membrane and egg shell as a final act of oogenesis.

3. The insect ovarian types

As already mentioned, the *Drosophila* ovary belongs to the *polytrophic meroistic* type. This type, as well as the other two principal types discussed below combine special characteristics that have probably evolved from different ancestral entities in different ways. This report attempts to summarize this evolution. Apart from the polytrophic meroistic ovary (Fig. 2B), characterizing most Holometabola and Acercaria (BÜNING 1994), most of the more basal insect orders have *panoistic* ovaries in which all germ cells develop into oocytes (Fig. 2A). In these, as in polytrophic meroistic ovaries, each ovariole originates as a germarial region which usually persists into the adult. However, the cystoblasts either develop directly into oocytes or the clusters of cystocytes separate into single cells each of which develops into an oocyte.

The third type of ovarioles is the *telotrophic meroistic* ovary (Fig. 2C). Instead of an anterior germarial region in which the proliferation of germ cells takes place, each telotrophic ovariole has an anterior *tropharium* in which nurse cells synthesize all cytoplasmic products stored in the oocyte. Thus, in contrast to polytrophic and panoistic ovarioles, all germ cells maintain their anterior position, except for growing oocytes. In other words, the function of the cap cells (green) to maintain the anterior position of stem cells has expanded to all inner sheath cells (yellow, green) surrounding the germarium/tropharium. Thus the functional ovarioles of telotrophic ovaries lack a germarium. Consequently, the number of presumptive oocytes is fixed and the proliferation of germ cells is shifted into the larval stages. The transport of products from nurse cells to oocyte is maintained through *nutritive (trophic) cords*, which are anterior extensions of the oocytes (BÜNING 1994, 1998). A similar, but shorter and, in most cases, preliminary, extension of oocytes occurs also in clusters of many polytrophic females where it is called the *nutritive appendix* (BILIŃSKI & JAGLARZ 1987; Fig. 3). Therefore, the existence of nutritive cords is not unique to telotrophic ovarioles but the character belongs to all meroistic ovaries.

4. Telotrophic meroistic ovarioles in Ephemeroptera and Hemiptera

Telotrophic ovarioles are common to Ephemeroptera, Hemiptera, Sialidae (Megaloptera), Raphidioptera, Hydroscaphidae (Coleoptera-Myxophaga) and all investigated Coleoptera-Polyphaga (Fig. 4). These insects have evolved four different types of telotrophic ovarioles with unique sets of characters which could only have arisen after long and independent evolution. In all mayflies (Ephemeroptera) so far observed (Fig. 4A), the tropharium houses linear clusters of germ cells. One eventually develops as the oocyte, while all others remain morphologically unaltered and serve as nurse cells. The decision as to which sibling cystocyte in a clone will develop as an oocyte depends on the contact of that germ cell with posterior somatic cells (Gottanka & Büning 1993).

In all Hemiptera so far examined (Fig. 4B), the telotrophic ovariole consists of one huge syncytial cluster from which the posterior germ cells will be determined and differentiated very early as oocytes. Nurse cells enhance their productivity by additional S-phases. Nutritive cords develop a unique and specialized set of longitudinally disposed microtubules by action of microtubule- associated motor proteins (Huebner & Anderson 1972; Büning 1985, 1994; Ksiazkiewicz-Kapralska 1991; Anastasi et al. 1990; Szklarzewicz 1997; Huebner & Diehl-Jones 1998; Kugler et al. 2006).

5. Telotrophic meroistic ovarioles in Neuropterida and Coleoptera

The only type which is found in more than one order-level taxon, called the *Sialis*-type (Fig. 4C,D), is known to occur in Sialidae (a sub-taxon of Megaloptera) and in Raphidioptera, a taxon closely related to Megaloptera (Matsuzaki & Ando 1977; Achtelig 1978; Büning 1979c, 1980). Surprisingly, this type occurs also in Hydroscaphidae, a sub-taxon of Myxophaga, which itself is one of the four equally ranked sub-taxa of Coleoptera (Büning 2005; Beutel 2005). Our investigations clearly show that the development of this ovariolar type is similar in all three taxa, indicating a common evolutionary origin (Büning 1994; Rübsam & Büning in preparation).

The fourth type of telotrophic ovarioles is unique to all Coleoptera-Polyphaga (Fig. 4E; Büning 1972, 1979a,b, 1994, 1998). The tropharium is filled with moderately polyploid nurse cells which are embedded in a three-dimensional network of somatic *interstitial cells*.

Before we knew about the ovariolar structure in *Hydroscapha*, there was no doubt about the independent origin of each of these four telotrophic types. The Ephemeroptera-type probably developed from that of a panoistic ancestor, while all others seem to have evolved from polytrophic meroistic ancestors (Büning 1994, 1998). However, the fundamental similarities between ovarioles of Sialidae/Raphidioptera (Figs. 4C, 5, 6) and *Hydroscapha* (Figs. 4D, 7) could not be explained by parallel evolution. To clarify this evolution, in the past years we reinvestigated ovariolar structure in representatives of all higher taxa belonging to the supraordinal taxon Neuropterida and the Coleoptera.

A reinvestigation of the *Sialis* and *Raphidia* ovarioles confirmed our previous findings and added some new data related to the proliferation of germ cells during the larval stages:

- Only few germ cells (1~5) invade the ovariole anlagen (Fig. 6).
- Germ cells undergo a few cycles of incomplete mitosis and form small rosette clusters with central polyfusomes (Figs. 5B–D,F, 6).
- The proliferation is maintained by cluster splitting (not shown). We did not find a niche for maintaining stem cells.
- Starting from the posterior end of each ovariole, cell membranes disappear in central regions and clusters begin to fuse (Figs. 5E, 6). Only those germ cells in contact with somatic inner sheath cells or posterior (prefollicular) tissue maintain their cellular character. These cells are called *tapetum cells*. Each tapetum cell remains connected to the central syncytium via its intercellular bridge (Fig. 6D).
- Posterior tapetum cells, contacting prefollicular cells, develop into the functional oocytes.

An ovariole of *Hydroscapha* develops in exactly the same way as the *Sialis* ovariole (Figs. 4D, 6, 7; Büning 2005). It has a central syncytium bordered by tapetum cells and posterior tapetum cells develop into oocytes (Fig. 7F; Büning 2005). In some ovarioles of freshly eclosed adults, separated anterior clusters of germ cells occur which are in varying stages of membrane reduction (Fig. 7D). Such stages are also known from *Sialis* ovarioles.

Recently, we reinvestigated the telotrophic ovary of *Tribolium castaneum* (Tenebrioninae, Polyphaga; Figs. 8–10; Trauner & Büning in press). We confirmed the well known morphology of adult and older pupa ovarioles (Kozhanova & Pasichnik 1979; Matuszewski et al. 1985; Büning 1972, 1978, 1979a,b, 1994, 1998; Ullmann 1973). In addition, we investigated larval and younger pupal stages and revealed the following sequence of ovariole development:

- The larval ovariole is polarized into an anterior and a posterior somatic tissue. The middle region is filled with small, irregularly branched, germ cell clusters, most of which form rosettes (Figs. 8A, 9A).
- Some cluster cells show closed intercellular bridges, indicating cluster splitting. The number of clusters is about 15–20 and therefore we assume that only a small number of germ cells invade the ovariole anlagen (Figs. 8A, 10).
- Anterior clusters undergo apoptosis (Figs. 8A, 10; not shown in detail)
- Posterior clusters in contact with posterior somatic cells survive (Figs. 9, 10). Germ cells with direct contact to somatic posterior cells are determined as future oocytes (*pro-oocytes*).
- A second period of incomplete germ cell mitoses starts in the pupal stage (Figs. 8B,C, 10) by which linear strings (sub-clusters) of future nurse cells (*pro-nurse cells*) arise. Here and there the strings become branched. Four to six synchronized waves of mitosis occur, and each wave starts from the pro-oocyte.
- In anterior regions the pro-nurse cell nuclei undergo additional S-phases and the polyploid nuclei finally divide amitotically (Fig. 10; not shown in detail).

Comparing both developmental paths leading to the *Sialis*-type and the Polyphaga-type of telotrophic ovariole, we note a great similarity during early stages (Figs. 6, 10):

- They both start with a few enclosed germ cells.
- They both enhance their content of germ cells by incomplete mitosis and cluster splitting.
- In neither type could we find a stem cell niche.

Thereafter, the developmental path diverges as shown above. What might have happened at the time of emergence of the Coleoptera-Polyphaga? We can imagine that the appearance of apoptosis was a key event that arose after a period of cluster splitting. Apoptosis might be evoked by a graded signal emanating from anterior somatic tissue but not influencing posterior clusters which survive. This single event could have generated a new but functional type of telotrophic ovariole having few or no nurse cells. Instead, all cells of posterior clusters that are in tight contact with posterior somatic cells develop into pro-oocytes. In a second step, pro-oocytes commenced a limited number of mitotic cycles to generate a telotrophic ovariole typical of Polyphaga.

6. Arguments for the *Sialis*-type in the ground plan of Neuropterida + Coleoptera

If we compare the ovariolar types within taxa of the clade Neuropterida + Coleoptera, we can identify three taxa with polytrophic meroistic ovaries, four with telotrophic meroistic ovaries and additional four with panoistic ovaries (Fig. 11). A panoistic ovariole can easily evolve by reduction from a meroistic ovariole. One way might be the introduction of a female-specific stop signal on mitotic cycles in the cystoblasts; the other would be a total splitting of each cystocyte cluster into single cells with each developing as oocyte (Fig. 2). We have examples from outside the clade Neuropterida + Coleoptera for both ways (BÜNING 1994, 1998). Therefore, I assume a meroistic ovary to be characteristic of the stem species generating the clades Neuropterida + Coleoptera. This assumption is supported by the discovery of polytrophic meroistic ovarioles in many taxa of Acercaria and Holometabola so that one can assume that the rise of this ovariole type is at its stem species or earlier (BÜNING 1994). However, based on our present knowledge, I prefer a telotrophic meroistic ovariole of the *Sialis*-type to be at the base of the clades Neuropterida + Coleoptera for the following reasons:

- This type occurs nearly unchanged in Neuropterida and Coleoptera. If a parallel evolution of this type had occurred, one would expect a greater diversity in ovariolar type (MATSUZAKI & ANDO 1977; ACHTELIG 1978; BÜNING 1979, 1980, 2005).
- Such diversity exists in the development of polytrophic meroistic ovarioles in neuropterans compared to those of Coleoptera-Adephaga (GIARDINA 1901; KUBRAKIEWICZ 1997, 1998; Rübsam & Büning unpublished):
  - In Neuroptera, the anterior germarial region has few isolated germ cells which may function as stem cells. Cluster splitting was never found in these ovarioles. In Coleoptera-Adephaga stem cells are not yet described. Instead, there are numerous small clusters consisting of two sister germ cells each, resting and growing in perennial cycles. They multiply by synchronized mitosis and additional cluster splitting (GIARDINA 1901; unpublished results from our group).
  - Neuroptera develop more or less linear clusters by synchronized mitosis with some irregular bifurcations (KUBRAKIEWICZ 1997, 1998; Rübsam unpublished). In Coleoptera-Adephaga the germ cell clusters follow the strict rules known from *Drosophila* development (Fig. 1) and most Dytiscidae develop 16-cell clusters or 8-cell clusters, while Carabidae may have more mitotic cystocyte cycles (JAGLARZ 1992).

These different developmental pathways among polytrophic ovaries of Neuroptera and Coleoptera-Adephaga can be explained best by action of independent restoration processes of polytrophic ovarioles from a telotrophic meroistic background. This telotrophic background has been conserved, so far as we know, almost entirely in Sialidae, Raphidioptera and Hydroscaphidae (Fig. 11).

As shown above, morphological data on the development of insect ovarioles support the *Sialis*-type hypothesis. However, the underlying net of genetic activities must be studied comparatively and in great detail. Such knowledge will provide a more powerful instrument for answering these phylogenetic questions much more precisely.

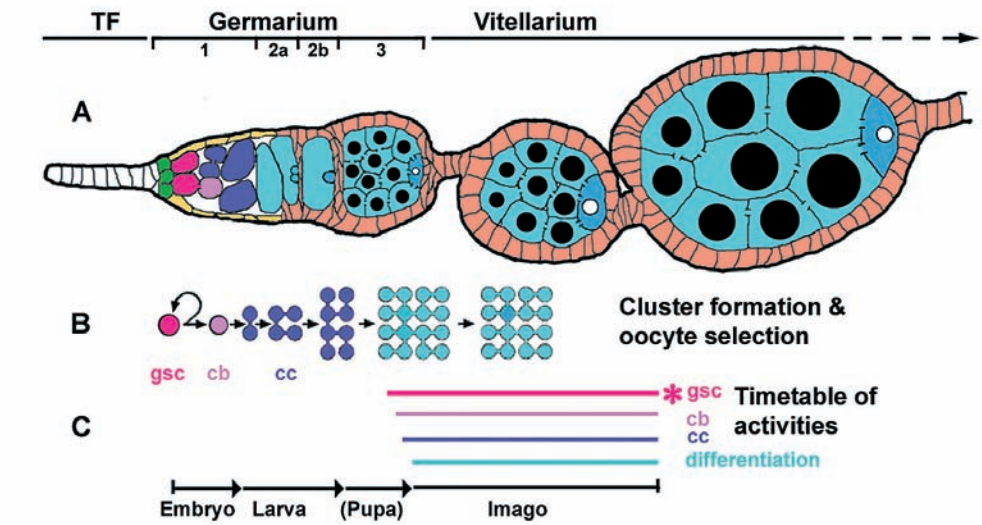


Fig. 1. *Drosophila* oogenesis. **A**: Drawing of the anterior part of an ovariole, showing the terminal filament (TF), germarial zones and the first two follicles of the vitellarium. Zone 1 houses the germarial stem cells (gsc; red), cystoblasts (cb; pink), and cystocyte clusters (cc; blue). In zone 2a the 16-cell clusters (turquoise) change their form from a rosette stage to a double layered pancake stage in which oocyte differentiation becomes visible by meiotic prophase in the two pro-oocytes (dark turquoise). In zone 2b the prefollicular cells surround the cluster while only the definitive oocyte remains in meiotic prophase. In zone 3 the follicular cells surround as an epithelium the cluster and nurse cells (dark nuclei) begin their additional S-phases. **B**: Germ cells proliferate from front to back by differential mitosis. The stereotype patterning of cystoblasts follows, giving birth to the 16-cell cluster (after KING 1970). **C**: During pupal stages ovarioles have finished the final architecture and germ stem cells proliferate (*) throughout imaginal life.

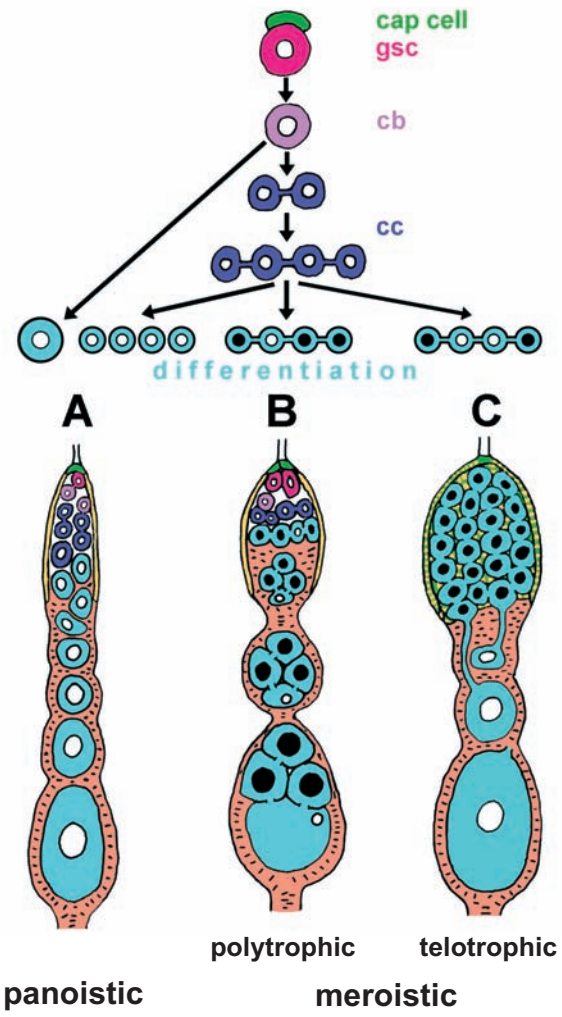


Fig. 2. The three types of ovarioles (panoistic **A**, polytrophic meroistic **B**, and telotrophic meroistic **C**) are shown, together with cluster genesis, adopted from *Drosophila*. Panoistic ovarioles emerge by stop signals to mitosis in cystoblasts or by cluster splitting into single cells, which all develop into oocytes. Polytrophic ovarioles develop as shown in Fig. 1; note that only one cell of a cluster can develop as an oocyte, while all other cystocytes differentiate as nurse cells. Telotrophic ovarioles retain all germ cells in the anterior germarium, which transforms into the tropharium. Several cystocytes of one cluster can develop into oocytes and only the oocytes will leave the tropharium, but still connected to the tropharium by their anterior elongations, the nutritive cords.

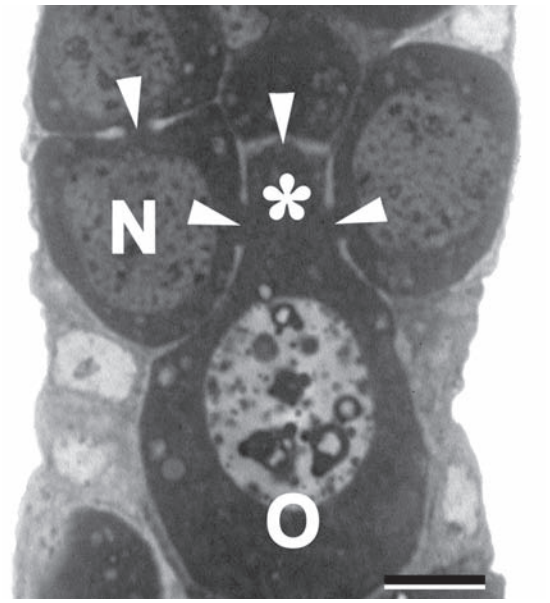


Fig. 3. Early previtellogenic growing follicle of *Agabus bipustulatus* (Coleoptera: Adephaga: Dytiscidae). Nurse cells (N) open via their intercellular bridges (arrowheads) into the anterior elongation of the oocyte (O), the nutritive appendix (*); scale bar = 10 μm.

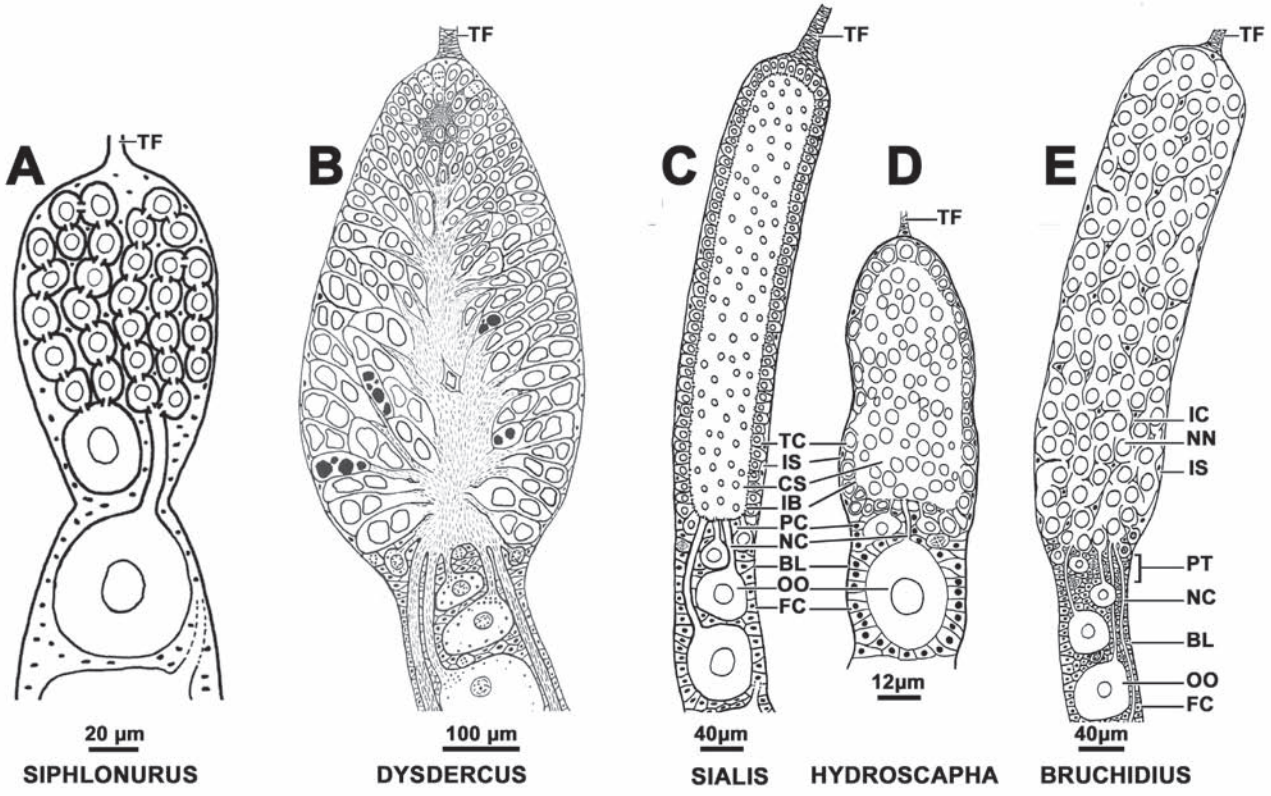


Fig. 4. The four different types of telotrophic meroistic ovarioles found among insects. **A**: The Ephemeroptera-type consists of linear clusters, one cell of which differentiates into an oocyte (GOTTANKA & BÜNING 1993). **B**: The Hemiptera-type consists of one cluster, in which posterior cystocytes develop as oocytes, all others as nurse cells (BÜNING 1994; DIEHL-JONES & HUEBNER 1998; KUGLER et al. 2006). **C**,**D**: The *Sialis*-type emerges by cluster splitting during the multiplication phase of germ cells and of cluster fusion of all germ cells, except tapetum cells (BÜNING 1994, 2005). **E**: The polyphagan-type emerges by apoptosis of all anterior germ cell clusters and by additional phases of mitosis of posterior clusters, by which pro-oocytes develop linear clusters of nurse cells with rare bifurcations (MATUSZEWSKI et al. 1985; BÜNING 1994; TRAUNER & BÜNING 2006).

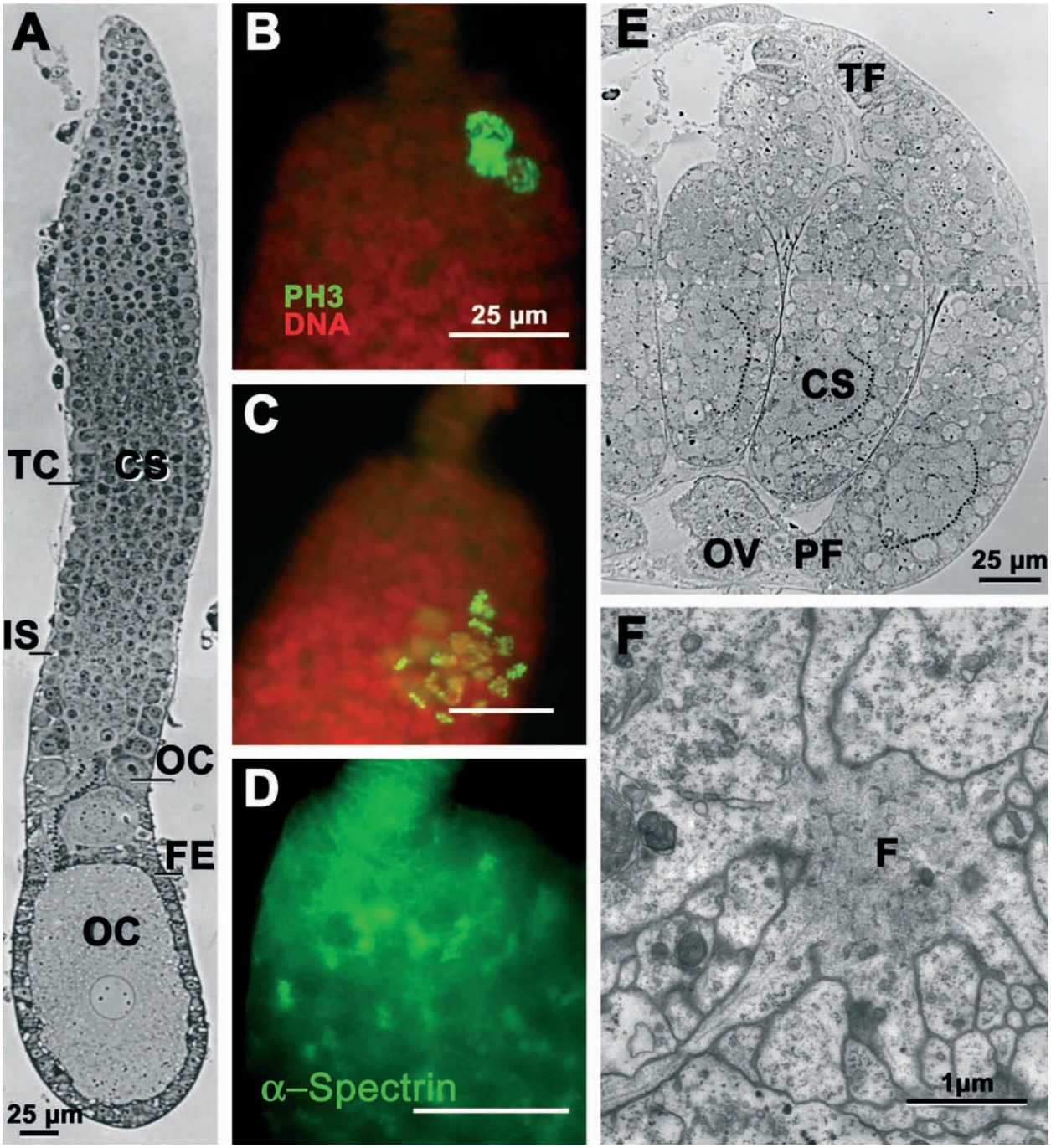


Fig. 5. Morphology and development of the *Sialis*-type telotrophic ovary. **A**: A functional ovariole of *Raphidia flavipes* (Raphidioptera) (Büning 1980). **B–F**: Larval ovarioles of *Sialis* sp. (Megaloptera). **B**,**C**: Fluorescence microscopy of anterior regions of larval ovarioles. Clusters arise in anterior regions; however, stem cell niches were not found. **D**: Clusters have polyfusomes as indicated by α-spectrin labelling. **E**: Later in larval development, posterior clusters fuse and all inner membranes dissolve, giving birth to the central syncytium, bordered by single germ cells, the tapetum cells. **F**: The electron micrograph shows the centre of a cluster of cystocytes in which fusomal material accumulates (Büning 1979c, 1994; Rübsam & Büning in preparation). Abbreviations: TF (terminal filament), IS (inner sheath), TC (tapetum cell), CS (central syncytium), OC (oocyte), FE (follicular epithelium), PH3 (antibody against phosphohistone 3, a mitosis marker), F (fusome).

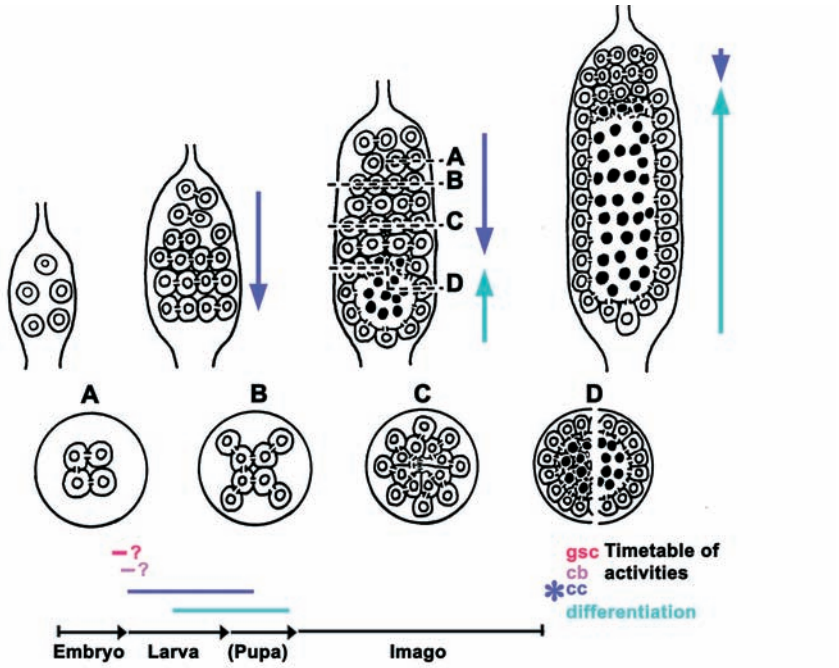
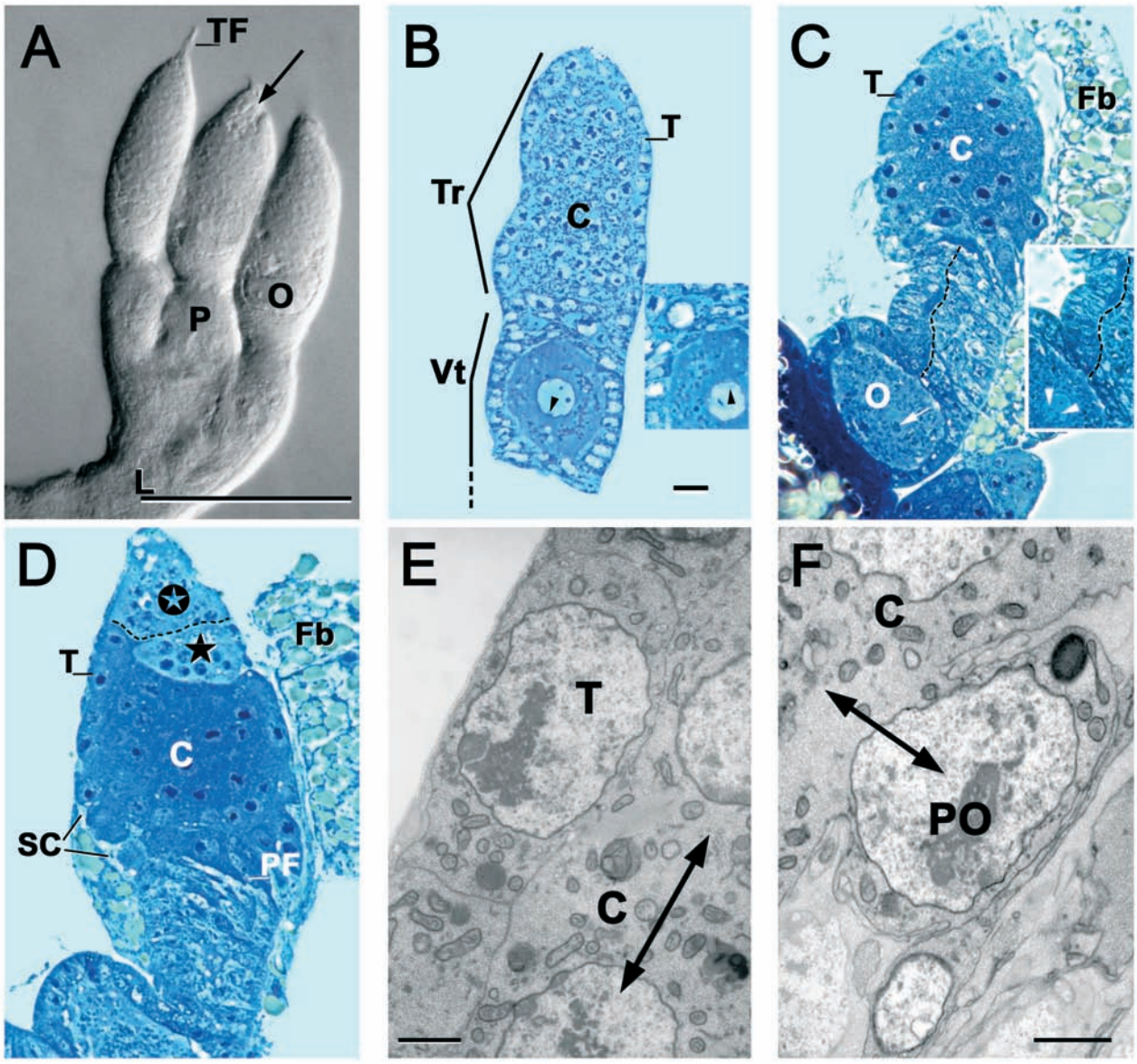


Fig. 6. Several stages of ovariole development during larval life of *Sialis* sp. (Megaloptera). A stem cell niche is not found in the anterior region. The proliferation of germ cells proceeds by cluster mitoses (A–C; *) and cluster spitting (not shown in detail), followed by cluster fusion in posterior-anterior direction (D).



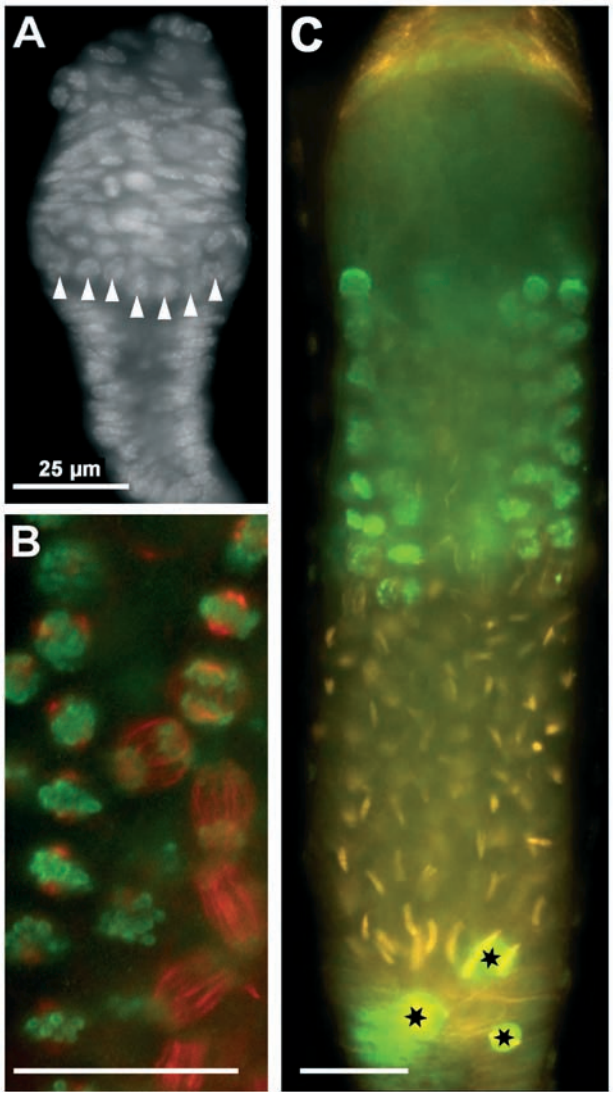


Fig. 8. Late larval and pupal ovarioles of *Tribolium castaneum* (Coleoptera: Polyphaga: Tenebrionidae). In late larval ovarioles a posterior cluster of pro-oocytes (triangles) nests on the floor of posterior somatic tissue (**A**). In a second phase of synchronized mitoses, linear clusters of nurse cells arise (**B**). In late pupae, nurse cell divisions are still going on in posterior and middle regions, while in the anterior region nurse cell nuclei undergo additional S-Phases, followed by amitotic nucleus divisions (not shown in detail) (**C**; mitotic spindles in yellow [acetylated tubulin antibody]; mitotic chromosomes in green [Phosphohistone 3 antibody]; black stars sign somatic mitoses of the prefollicular tissue).

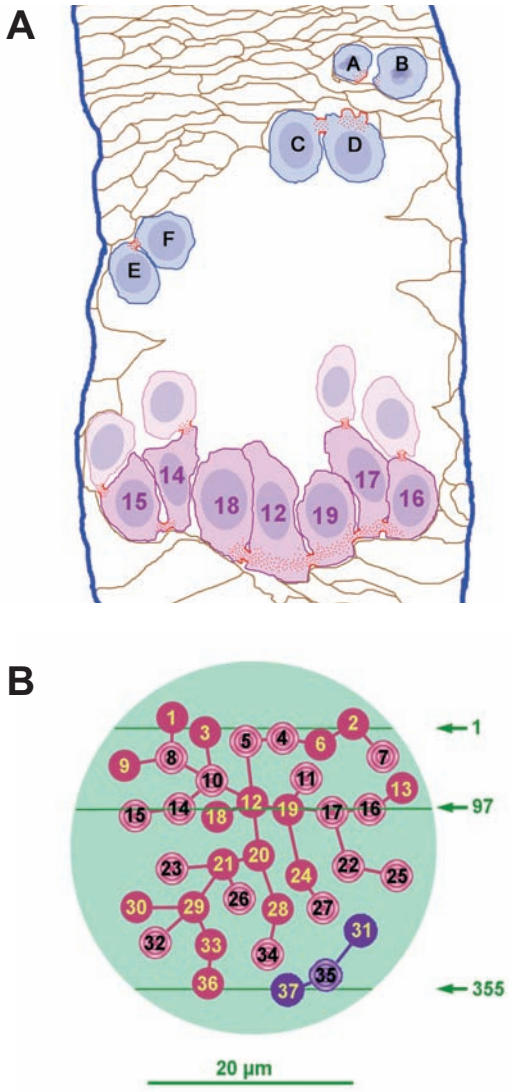


Fig. 9. Early cluster formation in larval *Tribolium* ovarioles. In late larval ovarioles 10–20 germ cell clusters (blue, only three are figured: AB, CD, EF) are spaced in middle regions of ovarioles, enclosed by anterior and posterior somatic tissues (brown) (**A**). Some cluster cells show closed bridges, indicating cluster splitting. Some other clusters are in different stages of apoptosis. Posterior clusters survive. Their cells grow and they become pro-oocytes (purple). Some pro-oocytes have started the second period of mitotic activity, giving birth to nurse cells (pale purple). Intercellular bridge rims in red; red stippling indicates fusomal material. In a serial section analysis of 355 ultrathin sections the two clusters reaching the posterior somatic tissue are shown (**B**). The small cluster has 3 pro-oocytes, the large one has 34 pro-oocytes. Those pro-oocytes which have additional pro-nurse cells are indicated by black numbers. The section 97 was the pattern for figure **A**.

Fig. 7 (left). Morphological details of ovarioles of *Hydroscapha natans* (Coleoptera: Myxophaga: Hydroscaphidae). Three ovarioles are found in each ovary (**A**). The tropharium (Tr) consists of a central syncytium, surrounding tapetum cells (T) and somatic inner sheath cells (SC) (**B**–**D**). Oocytes (O) are connected to the central syncytium (C) by nutritive cords (**C**, stippled lines). Sometimes anterior clusters do not fuse (arrow in **A**; stars in **D**). The central syncytium is devoid of cell membranes and each tapetum cell opens via an intercellular bridge to the central syncytium (**E**, **F**). Posterior tapetum cells develop as pro-oocytes (PO); arrows indicate the longitudinal axis of the ovariole. Additional abbreviations: FB (fat body), P (pedicel), L (lateral oviduct), Vt (vitellarium); scale bars A, B = 10 µm; E, F = 1µm.

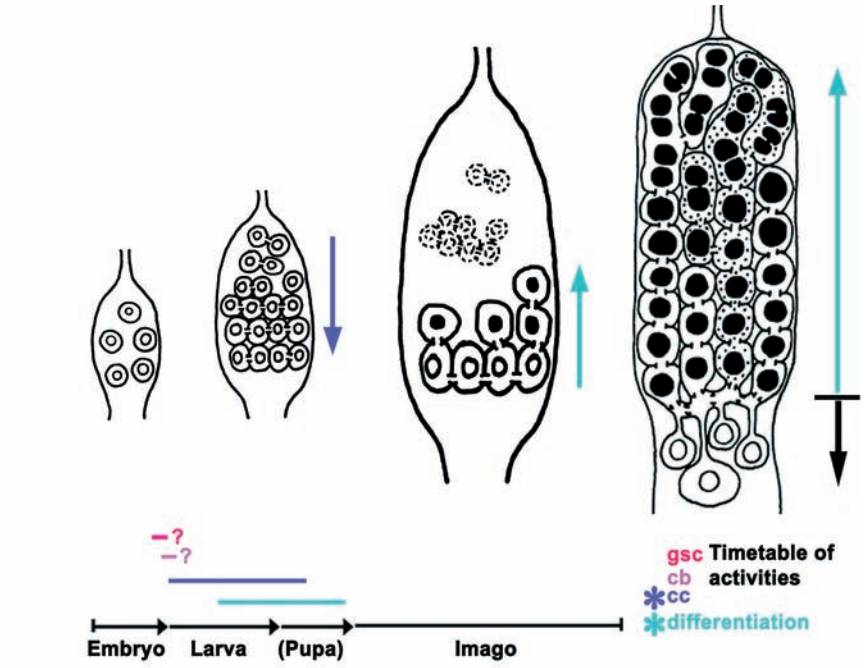


Fig. 10. The development of ovarioles of *Tribolium castaneum* during larval and pupal stages. Few germ cells invade an ovariole anlage. A germ cell niche was not found. Germ cells proliferate in the beginning by cystocyte mitoses and cluster spitting. Except for posterior clusters, the clusters undergo apoptosis (stippled cells). Posterior clusters transform into pro-oocytes and in a second period of mitosis linear strings of pro-nurse cells arise, transforming later to nurse cells by additional S-phases and amitotic divisions of nuclei. Germ cell multiplication occurs by cystocyte mitoses and during the differentiation period (*). Vertical arrows indicate the polar developmental gradients.

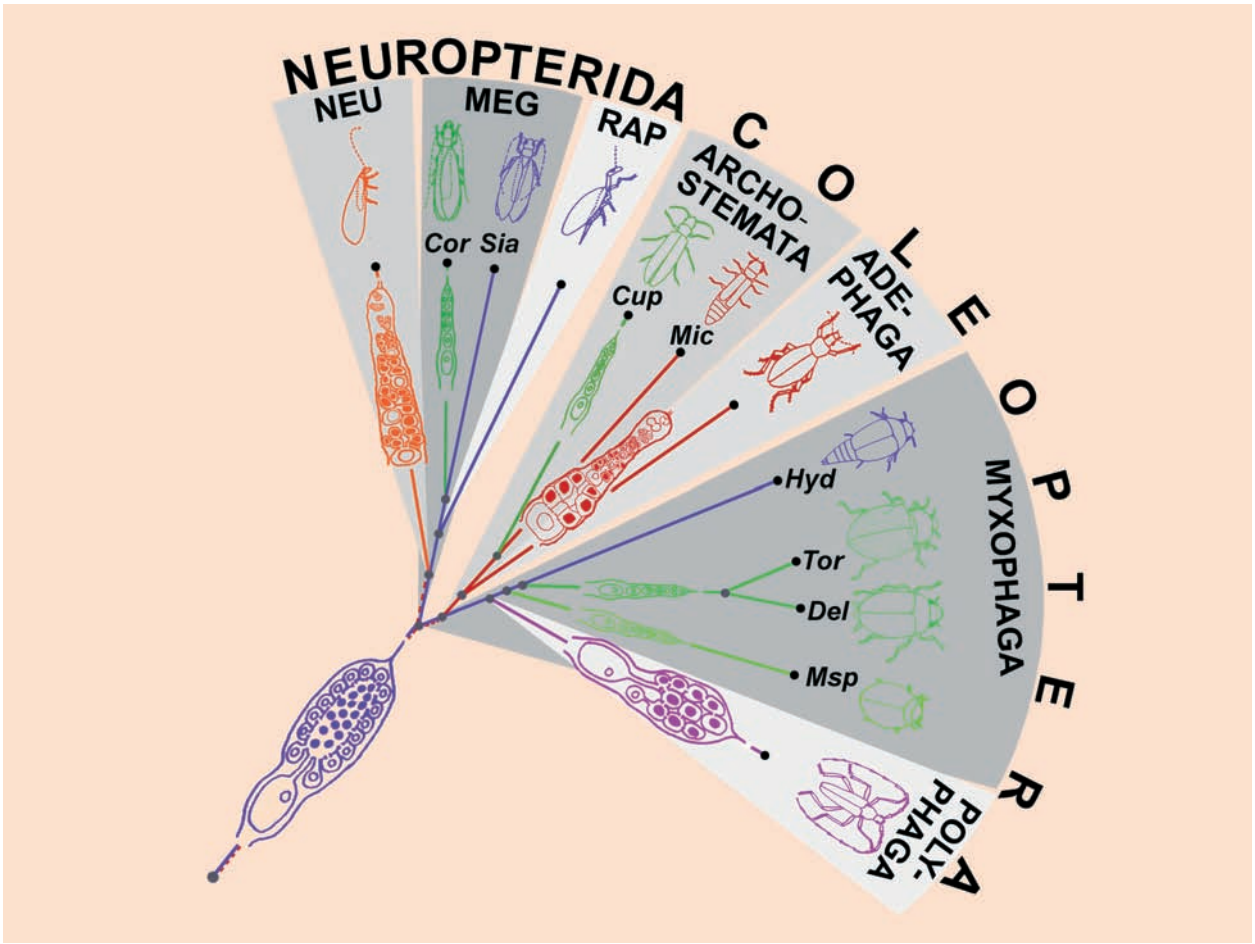


Fig. 11. Proposed phylogeny of ovarioles among lineages of the clade Neuropterida + Coleoptera. The direct ancestors had telotrophic meroistic ovarioles of the *Sialis*-type (blue). This type evolved from polytrophic meroistic ancestors (BÜNING 1994, 1998), indicated by the stippled red line, which parallels the blue line of the *Sialis*-type. Several reversals to panoistic ovarioles occurred in some sub-taxa (diverse green figures, not shown in detail). On at least two occasions a reversion to the polytrophic ovary occurred (Neuroptera, orange; Coleoptera: Archostemata + Adephaga, red). The telotrophic ovary type of Polyphaga (violet) developed directly out of the *Sialis*-type. Abbreviations: Neu (Neuroptera), Meg (Megaloptera), Rap (Raphidioptera), Cor (Corydalidae), Sia (Sialidae), Cup (Cupedidae), Mic (Micromalthidae), Hyd (Hydroscaphidae), Tor (Torridinculidae), Del (*Delevea*), Msp (Microsporidae).

7. References

Achtelig, M. 1978. Entwicklung und Morphologie der inneren und äußeren weiblichen Genitalorgane der Kamelhalsfliegen (Neuropteroidea: Rhapidioptera). – Entomologica Germaniae **4**: 140–163.

Anastasi, A., C. Hunt & H. Stebbings 1990. Isolation of microtubule motors from an insect ovarian system: characterization using a novel motility substratum. – Journal of Cell Science **96**: 63–69.

Beutel, R.G. 2005. 1. Systematic position and early evolution. Pp. 1–9 *in*: Handbook of Zoology, vol. IV Arthropoda: Insecta. Part 38. Coleoptera, vol. 1: Morphology and Systematics (Archostemata, Adephaga, Myxophaga, Polyphaga (partim)). – Walter De Gruyter, Berlin, New York.

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

Büning, J. 1972. Untersuchungen am Ovar von *Bruchidius obtectus* Say. (Coleoptera-Polyphaga) zur Klärung des Oocytenwachstums in der Prävitellogenese. – Zeitschrift für Zellforschung **128**: 241–282.

Büning, J. 1978. Development of telotrophic-meroistic ovarioles of polyphage beetles with special reference to the formation of nutritive cords. – Journal of Morphology **156**: 237–256.

Büning, J. 1979a. The trophic tissue of telotrophic ovarioles in polyphage Coleoptera. – Zoomorphology **93**: 33–50.

Büning, J. 1979b. The telotrophic nature of ovarioles of polyphage Coleoptera. – Zoomorphology **93**: 51–57.

Büning, J. 1979c. The telotrophic-meroistic ovary of Megaloptera I. The ontogenetic development. – Journal of Morphology **162**: 37–66.

Büning, J. 1980. The ovary of *Rhaphidia flavipes* is telotrophic and of the *Sialis*-type. – Zoomorphology **95**: 127–131.

Büning, J. 1985. Morphology, ultrastructure, and germ cell cluster formation in ovarioles of aphids. – Journal of Morpholology **186**: 209–221.

Büning, J. 1994. The insect ovary: Ultrastructure, previtellogenic growth and evolution. – Chapman and Hall, London.

Büning, J.1998. The ovariole: structure, type, and phylogeny. Pp. 957–993 *in*: F.W. Harrison (ed.), Microscopic Anatomy of Invertebrates, vol. 11C Insecta: F.W. Harrison & M. Locke (eds.) – Wiley-Liss, New York.

Büning, J. 2005. The telotrophic ovary known from Neuropterida exists also in the myxophagan beetle *Hydroscapha natans*. – Development Genes & Evolution **215**: 597–607.

Decotto, E. & A.C. Spradling 2005. The *Drosophila* ovarian and testis stem cell niches: similar somatic stem cells and signals. – Devopmental Cell **9**(4): 501–510.

De Cuevas, M. & A.C. Spradling 1998. The morphogenesis of the *Drosophila* fusome and its implications for oocyte specification. – Development **125**: 2781–2789.

Giardina, A. 1901. Origine dell'oocite e delle cellule nutrici nel *Dytiscus*. – Internationale Monatsschrift für Anatomie und Physiologie **18**: 417–477.

Gottanka, J. & J. Büning 1993. Mayflies (Ephemeroptera), the most "primitive" winged insects, have telotrophic meroistic ovaries. – Roux's Archives of Developmental Biology **203**: 18–27.

Grieder, N.C., M. De Cuevas & A.C. Spradling 2000. The fusome organizes the microtubule network during oocyte differentiation in *Drosophila*. – Development **127**: 4253–4264.

Grimaldi, D. & M.S. Engel 2005. Evolution of the Insects. – Cambridge University Press, NY.

Huebner, E. & E. Anderson 1972. A cytological study of the ovary of *Rhodnius prolixus*. III. Cytoarchitecture and development of the trophic chamber. – Journal of Morphology **138**: 1–40.

Huebner, E. & W. Diehl-Jones 1998. Developmental biology of insect ovaries: germ cells and nurse cell oocyte polarity. Pp. 957–993 *in*: F.W. Harrison (ed.), Microscopic Anatomy of Invertebrates, vol. 11C Insecta: F.W. Harrison & M. Locke (eds.). – Wiley-Liss, New York.

Huynh, J.R. & D. St Johnston 2004. The origin of asymmetry: early polarisation of the *Drosophila* germline cyst and oocyte. – Current Biology **14**: 438–449.

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

King, R.C. 1970. Ovarian development in *Drosophila melanogaster*. – Academic Press, New York.

Klass, K.-D., O. Zompro, N.P. Kristensen & J. Adis 2002. Mantophasmatodea: a new insect order with extant members in the Afrotropics. – Sience **296**: 1456–1459.

Kozhanova, N.I. & M. Pasichnik 1979. Differentiation of oocytes and nurse cells in telotrophic ovarioles of the beetle *Coccinella septempunctata*. – Citologija **18**: 824–833.

Kristensen, N.P. 1981. Phylogeny of insect orders. – Annual Review of Entomology **26**: 125–157.

Ksiazkiewicz-Kapralska, M. 1991. Organization of the trophic chamber of homopteran insects Membracidae: Cicadomorpha. – Cytobios **66**: 113–119.

Kubrakiewicz, J. 1997. Germ cell cluster organization in polytrophic ovaries of Neuroptera. – Tissue & Cell **29**: 221–228.

Kubrakiewicz, J., I. Jedrzejowska & S.M. Biliński 1998. Neuropteroidea – different ovary structure in related groups. – Folia Histochemica et Cytobiologica **36**: 179–187.

Kugler, J., R. Rübsam, J. Trauner & J. Büning 2006. The larval development of the telotrophic meroistic ovary in the bug *Dysdercus intermedius* (Heteroptera, Pyrrhocoridae). – Arthropod Structure & Development **35**: 99–110.

Lasko, P.F. & M. Ashburner 1990. Posterior localization of vasa protein correlates with, but is not sufficient for, pole cell development. – Genes & Development **4**: 905–921.

Lin, H., L. Yue & A.C. Spradling 1994. The *Drosophila* fusome, a germline-specific organelle, contains membrane skeletal proteins and functions in cyst formation. – Development **120**: 947–956.

MATSUZAKI, M. & H. ANDO 1977. Ovarian structures of the adult alderfly, *Sialis mitsuhashi* Okamoto (Megaloptera: Sialidae). – International Journal of Insect Morphology & Embryology **8**: 257–263.

MATUSZEWSKI, B., K. CIECHOMSKI, J. NURKOWSKA & M. KLOC 1985. The linear clusters of oogonial cells in the development of telotrophic ovarioles in polyphage Coleoptera. – Roux's Archives of Developmental Biology **194**: 462–469.

MCGRAIL, M. & T. HAYS 1997. The microtubule motor cytoplasmic dynein is required for spindle orientation during germ line stem cell divisions and oocyte differentiation in *Drosophila*. – Development **124**: 2409–2419.

ROPER, K. & N.H. BROWN 2004. A spectraplakin is enriched on the fusome and organizes microtubules during oocyte specification in *Drosophila*. – Current Biology **14**: 99–110.

SPRADLING, A.C., D. DRUMMOND-BARBOSA & T. KAI 2001. Stem cells find their niche. – Nature **414**: 14–18.

STORTO, P. & R.C. KING 1989. The role of polyfusomes in generating branched chains of cystocytes during *Drosophila* oogenesis. – Developmental Genetics **10**: 70–86.

SZKLARZEWICZ, T. 1997. Structure and development of the telotrophic ovariole in ensign scale insects (Hemiptera, Coccomorpha: Ortheziidae). – Tissue & Cell **29**: 31–38.

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

TRAUNER, J. & J. BÜNING 2006. Larval and pupal development of the telotrophic meroistic ovary of *Tribolium castaneum* (Tenebrionidae, Coleoptera Polyphaga). – Development Genes & Evolution, in press.

ULLMANN, S.L. 1973. Oogenesis in *Tenebrio molitor*: Histological and autoradiographical observations on pupal and adult ovaries. – Journal of Embryology & Experimental Morphology **30**: 179–217.

WHEELER, W.C., M. WHITING, Q.D. WHEELER & J.M. CARPENTER 2001. The phylogeny of the extant hexapod orders. – Cladistics **17**(2): 113–169.

ZHU, C.-H. & T. XIE 2003. Clonal expansion of ovarian germline stem cells during niche formation in *Drosophila*. – Development **130**: 2579–2588.