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REDUCTIONS AND NEW INVENTIONS DOMINATE OOGENESIS OF STREPSIPTERA (INSECTA)

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Abstract—The endoparasitic life of strepsipterans (Insecta), especially neotenic females, reduces to a great extent external and internal organs. Light and electron microscopic investigation of ovaries of *Elenchus tenuicornis* (Kirby) confirms the following: (1) somatic tissues of ovaries are totally reduced, with the exception of some cells surrounding germ cell clusters; (2) a previtellogenic growth phase of oocytes is reduced; (3) nurse cells remain diploid and their membranes degenerate at the onset of vitellogenesis; (4) vitellogenesis is reduced, vitellin and fat vacuoles contribute only 50% to the final egg volume; and (5) chorionogenesis is reduced to a vitellin membrane. However, some features of normal development remain, allowing classification of the ovary type as polytrophic meroistic: (1) germ cells undergo synchronized, incomplete divisions, following the 2^n rule, where all former intercellular bridges become localized in one cystocyte, while the other has none; and (2) only one cell is determined as the oocyte, all other cystocytes serve as nurse cells and the surrounding somatic cells transform into follicular cells. Novel events in oogenesis of strepsipterans include fission of clusters during the phase of cluster mitoses, and protection of oocyte nuclei, while nurse cell nuclei degenerate in the same cytoplasm. © 1998 Elsevier Science Ltd. All rights reserved

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INTRODUCTION

Strepsipterans are a group of holometabolic, endoparasitic insects in which larvae and female adults parasitize insects, such as bees, wasps, small homopterans or silverfish. One of the most common species in Europe is *Elenchus tenuicornis* (Kirby) which parasitizes small homopterans of the delphacid group, and was used in this study. The systematic position of Strepsiptera is still under debate: some specialists give them the rank of an order (Kinzelbach, 1971) near Coleoptera, while others prefer ranking as a family (Stylopidae) within polyphage Coleoptera (Crowson, 1981). Polyphage Coleoptera possess telotrophic meroistic ovarioles, while adephage Coleoptera and all related orders have polytrophic meroistic ovaries. Just recently, it has been suggested that the Strepsiptera are the sister group to the Diptera and the argument is supported by morphological and molecular data (Chalwatzis *et al.*, 1995; Whiting, 1996). So, the elucidation of the ovary structure of Strepsiptera may shed some light on the systematic question. Furthermore, it is of general interest to see how reductions can occur in insect ovaries without the loss of function, and how new inventions can rescue special reductions.

MATERIALS AND METHODS

Larvae and adult females of *Elenchus tenuicornis* were dissected from their host, mainly *Stiroma* sp. (Hemiptera: Delphacidae), which live on grasses in wet meadows. The species is bivoltine in a habitat near Münster. Ten per cent of *Stiroma* sp. was stylopized in the late summer season, and up to 2% the following spring season. The development is more or less synchronized, i.e., Delphacidae were stylopized by strepsipteran larvae of similar developmental stages. Within each larva, the development of rosette stages is also synchronized; i.e., young and old rosette stages are never found together, and vitellogenic stages are also never found between old rosettes. This necessitates preparation of larvae for electron microscopy every two weeks in each season in order to get the whole series of developmental stages. Baumert (1958) reported that, *E. tenuicornis* has five larval stages before pupation. Determination of larval stages for this study was according to Baumert's definitions, i.e., larval stage 2 is defined by a total length of <250 μm, and rosette clusters are found only on the ventral side and are less than 100 in total number. Larval stage 3 is defined by a body length of >250 μm, <600 μm and distribution of rosette clusters (Telfer, 1975; Büning, 1994; definition of cystoblast, cystocyte is that of King, 1970) throughout the body of female larvae. Stage 4 is up to 1500 μm in length and is characterized by transformation of the rosette stage to the mid-vitellogenic stage. In the young adult, oogenesis is complete and fertilization can occur. Larvae and female adults were prepared by standard methods for electron microscopy and semi-thin and ultra-thin serial sections were prepared as described in Büning and Sohst (1988).

RESULTS

Primary larvae (L1) develop in synchrony in the body cavity of the mother. After hatching the L1 larva passes into a brood canal of the mother and emerges to the open. As the only free-living stage, the L1 larvae possess six legs and two long cerci, with which they are able to jump. The head bears two eyes with five ocelli each, and the main internal organ is a huge synganglion, which fills most of the head, thorax and the anterior ventral half of the abdomen. In the thorax, the synganglion is penetrated by the tube-like foregut. The middlegut is composed of a thin epithelium; the gut lumen, however, is blown up like a balloon and fills the rest of the abdomen. The hindgut has not developed at this stage. In the posterior half of

the abdomen, on both sides of the alimentary canal, is a small ellipsoid organ, in which some round, big cells, the germ cells, are enclosed by a few somatic cells. This is the gonad anlage (Fig. 1B).

The L1 larva jumps and attaches to the host, then penetrates the cuticle. Inside the host, the larva molts to an L2 stage. Early L2 and late L1 stages were not investigated in this study. However, during these periods, the ovary anlage does not develop ovarioles, but loses the outer envelope of the ovary very early, while the germ cells begin to divide in synchrony. These developmental processes are deduced from the morphology of later L2 stages. The L2 larva has no outer appendages on the body, and the gut epithelium cells are polyploid. Some sparse muscles are found between the gut and body wall. On the ventral side of the abdomen, next to the alimentary canal, about 50 small rosette balls float in the body cavity (Fig. 1D). Higher magnification reveals a central area in each ball, at which the tips of all cells assemble (Fig. 2A). Thus, each cell of the rosette has an elongated pyramidal shape, with a centrally ordered narrow tip and a broad basement area. The nuclei settle near the basement area. Each of these rosette balls is ensheathed by a layer of flat, somatic cells (Fig. 2 and Fig. 3A). Ultrastructural analysis clearly showed that the centre of each rosette contains a polyfusomal area to

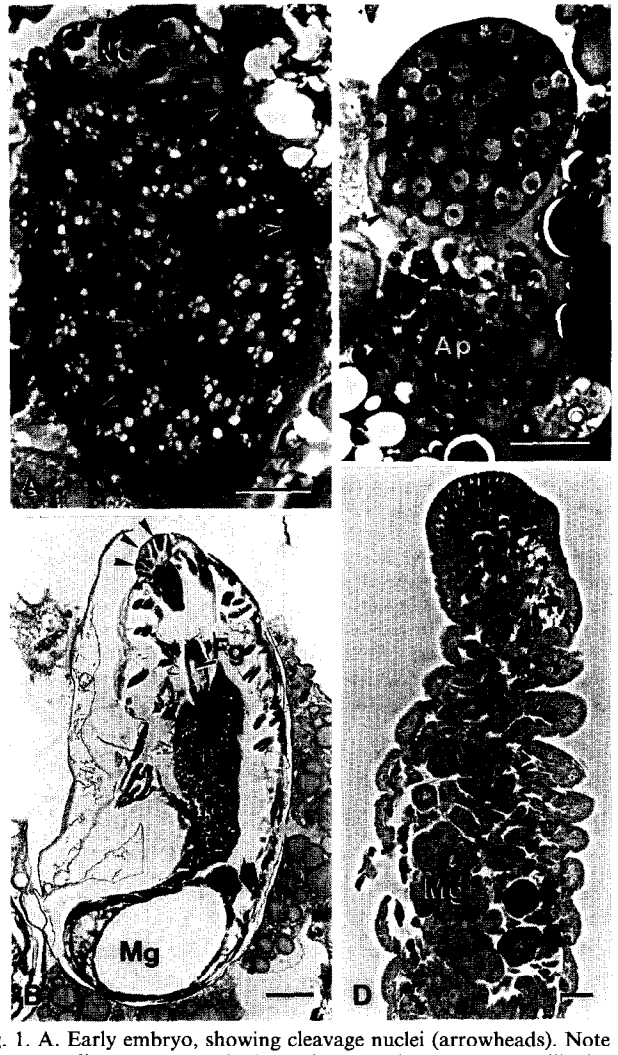

Fig. 1. A. Early embryo, showing cleavage nuclei (arrowheads). Note the nurse cell compartment (Nc), not integrated into oocyte. Follicular cells still exist. B. L1 larva, just before hatching. Note ommatidia (arrowheads), foregut (Fg), midgut (Mg) and synganglion (Sn). Gonad (big arrow). C. Apoptotic follicle (Ap). Follicle above is in a transition stage, showing first signs of oocyte differentiation, as can be deduced by enlargement of follicular cells (arrowheads). D. L2 larva. Pigmented cells (arrowheads) of reduced ommatidia; midgut (Mg); follicles of rosette stage (arrows). Bars = 20 μm.

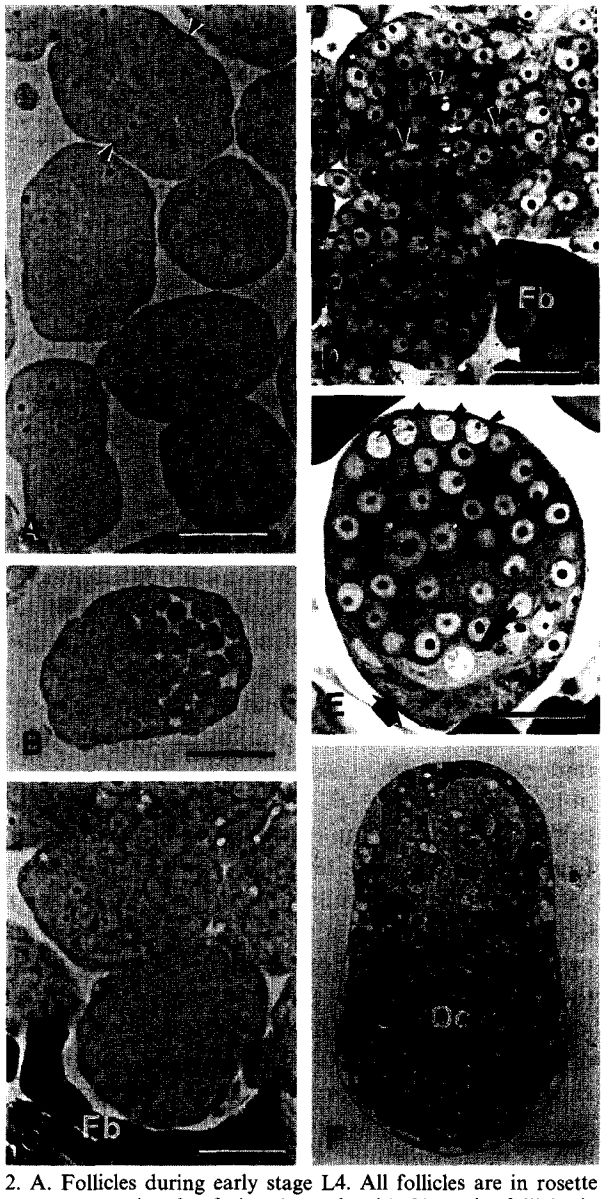

Fig. 2. A. Follicles during early stage L4. All follicles are in rosette stage, some preparing for fission (arrowheads). Note the follicle, in which one half is in metaphase, the other half in interphase. B. Half of the follicle is in cytokinesis, the other half in interphase. C. Last synchronous division leading to transition stage. Note amounts of fatbody (Fb). D. Transition stage. Remnants of polyfusome (arrowheads); fatbody (Fb). E. Follicle showing nurse cell-oocyte differentiation. Oocyte (arrow) is bordered by enlarged follicular cells (short arrow). Oocyte and some nurse cells (arrowheads) are in prophase, all other nurse cells in interphase. Star indicates the exceptional nurse cell, which might show low degree of polyploidy. F. Follicle in mid-vitellogenesis. Areas of syncytial nurse cell compartments (stars); oocyte (Oc). Bars = 20 μm.

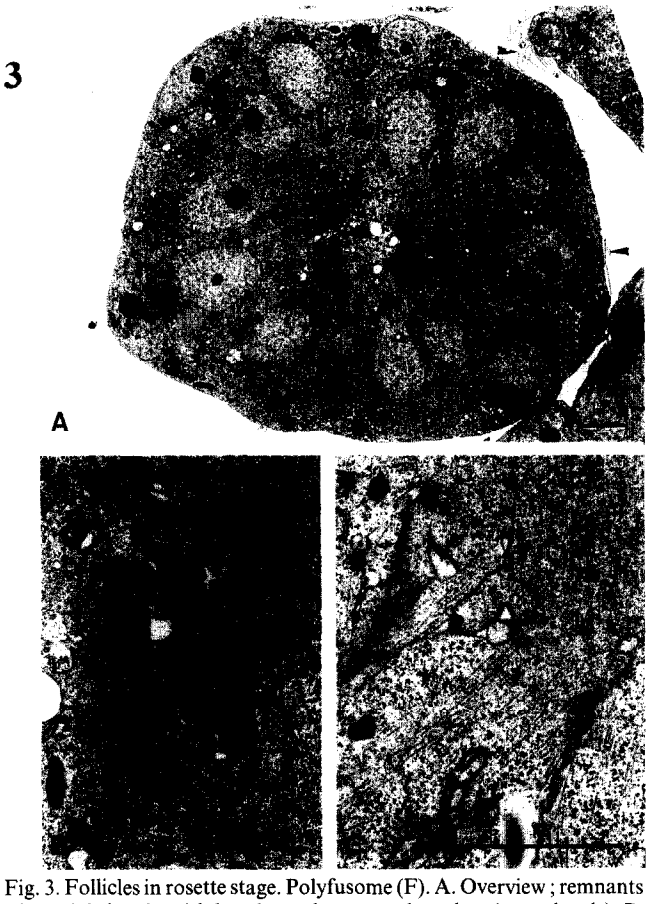

Fig. 3. Follicles in rosette stage. Polyfusome (F). A. Overview; remnants of ovarial sheath, with basal membranes and trachea (arrowheads). B. Center of the rosette showing polyfusome and intercellular bridges (double-headed arrows). C. Part of central region with polyfusome and associated bundles of microtubules; rims of intercellular bridges (arrowheads). Bars = 2 μm.

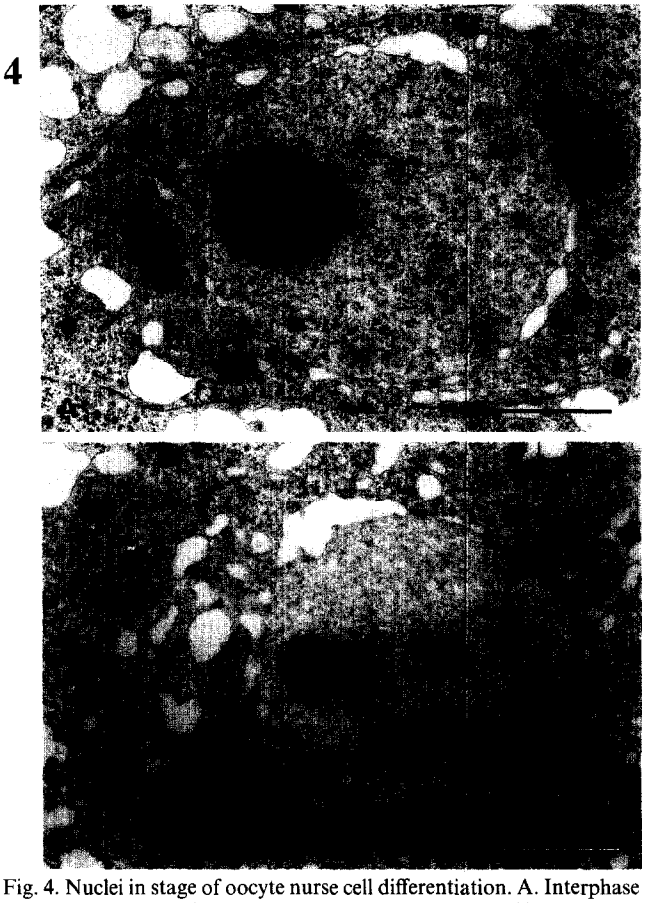

Fig. 4. Nuclei in stage of oocyte nurse cell differentiation. A. Interphase nurse cell nucleus with nucleolus. Note the nuage material in cytoplasm (arrows). B. Tangential sectioned oocyte nucleus with nucleolus and synaptonemal complexes (arrowheads). Bars = 2 μm.

which all cells open with an intercellular bridge (Fig. 3). The whole central area is filled with a polyfusome. The polyfusome includes some vacuoles as known from Golgi complexes or smooth ER. Furthermore, microtubules stretch from fusomes into the cytoplasm, while ribosomes are excluded from fusomal areas (Fig. 3C). The diameters of the intracellular bridges are 1.0–1.5 μm (Fig. 3B). During vitellogenesis, the bridges reduce their diameter to below 1 μm (Fig. 5A, C).

Several synchronous mitotic events in rosette clusters were analysed on semi-thin and ultra-thin sections. In each rosette, the cystocytes divide in synchrony. One spindle pole is thereby oriented next to the pre-existing polyfusome, while the other pole is situated just opposite. The new intercellular bridge moves centripetally, and the midbody of the spindle fuses and transforms to fusomal material. The synchrony of mitotic events embraces all cystocytes. However, when the rosette cluster divides into two equal halves, the synchrony is maintained in each of the future halves, but not necessarily between the two halves (Fig. 2). Such fission events can happen during larval stages 2–4, and these events are responsible for the multiplication from about 50 rosettes to about 1500 rosettes in late stage 4 (Table 1). This number is deduced from the volume of the mother and the middle distribution of embryos in sections. The fission is completed by immigrating somatic cells that surround the new, symmetrical halves of the daughter rosettes (Fig. 2A). The first sign of later fission is the occurrence of large vacuoles in the centre, which finally split the polyfusome and close the syncytium near to the oldest intercellular bridge (Fig. 2A and Fig. 3). During the larval stage 2–4, this multiplication leads to loosely connected, sometimes ramified, strings of rosettes. Each of the rosettes in the last larval stage houses about 130 cells, from which a minimum of seven mitotic cycles can be deduced. Loosely connected to the follicles, some pieces of the intercellular matrix are found, which contain trachea (Fig. 3A).

During the final multiplication of rosettes in larval stage 4, the amount of fat body tissue also increases. Immediately thereafter, the rosette configuration is lost, and simultaneously the polyfusome vanishes (Fig. 2C, D). The former pyramidal cells change their shape and become isodiametric, connected to their siblings via still persisting intercellular bridges. The cytoplasm in intercellular bridges is not different from that of the surrounding areas, and mitochondria, ribosomes and vacuoles can shift easily from one cell to the other. However, microtubular aggregates stretch through the

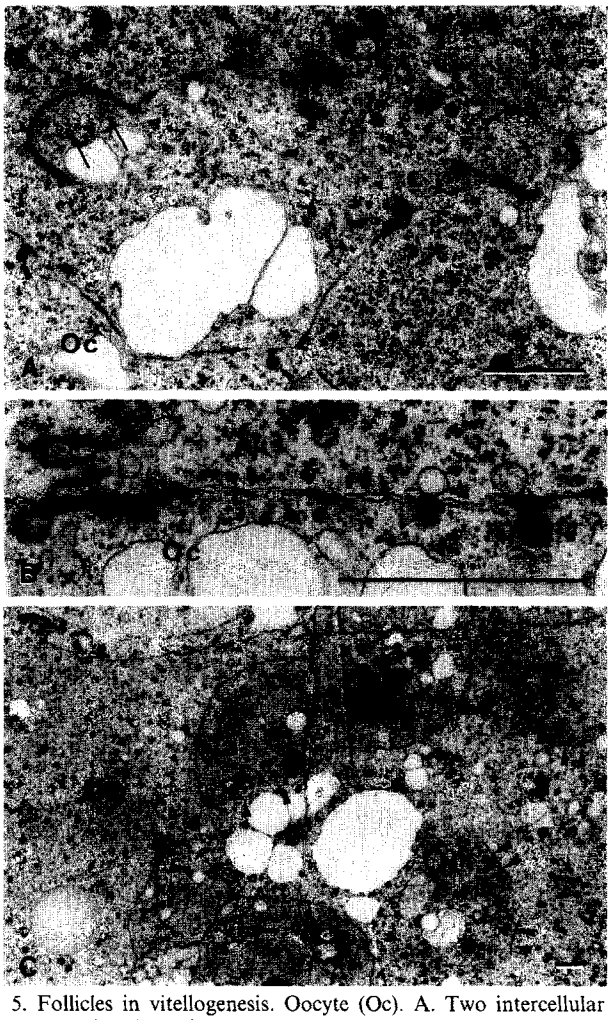

Fig. 5. Follicles in vitellogenesis. Oocyte (Oc). A. Two intercellular bridges with bundles of microtubules (arrows). Note dark-staining material in clefts between oocyte and nurse cells and coated vesicles (arrowheads). B. Intercellular cleft between follicle cells (arrowheads) and coated vesicles. C. Degraded nurse cell membranes (arrowheads) result in syncytial nurse cell areas, which are integrated into the oocyte. Persisting nurse cell nuclei (N); persisting intercellular bridge (arrows). Bars = 1 μm.

bridges, supporting the idea of active transport mechanisms (Fig. 5A). A strong ring of actin filaments, like those known to exist in intercellular bridges in *Drosophila* could not be identified ultrastructurally. During the transformation of rosettes, which is defined as the transition stage, the volume increases roughly fourfold compared with the preceding stage (Table 1). The amount of nuclei doubles from about 130 to 260. Some of the cell nuclei reach a prophase status, which is meiotic, as seen by synaptonemal complexes (Fig. 2E and Fig. 4B). These pachynema cells contain a single nucleolus, which is much smaller than that found in interphase germ cells that serve as nurse cells (Fig. 4). The distribution of the few cells remaining in prophase within the cluster seems to be random: some of them are in the central area and others at the periphery, bordering somatic cells. One of the latter, which borders the somatic cells, develop as the definitive oocyte. As a first morphological sign, the bordering somatic cells become cuboid in shape (Fig. 1C and Fig. 2E). The oocyte itself immediately begins to accumulate yolk spheres by endocytosis (Fig. 2F and Fig. 5A, B). The majority of interconnected cystocytes remain in interphase and do not change the volume of cytoplasm, nuclei or nucleoli, indicating their constant 4C status. The only nurse cell, which might have undergone endomitosis, is shown in Fig. 4E. In nurse cell cytoplasm, nuage material is often found near the nucleus membrane (Fig. 4A), which is typical for germ cells. Thus, the loosely connected follicles have now reached the developmental stage as occurs in polytrophic meroistic ovaries during growth phases. Such characteristics include flat follicle cells around the nurse cell compartment, while follicle cells surrounding the growing oocyte become cuboid. Ultrastructural analysis of oocyte-follicle cell boundaries clearly showed a small intercellular cleft, filled with highly contrasting material (Fig. 5). In most species, the oocyte membrane develops microvilli during vitellogenesis. This is not the case in *E. tenuicornis* oocytes; nevertheless, coated pits are found, which is the first morphological sign of yolk protein uptake. Some coated pits also occur at oocyte membranes next to nurse cells, in which intercellular spaces are also enlarged and filled with electron-dense material (Fig. 5A). However, coated pits are never found in nurse cell membranes, but develop only in oocytes, which indicates the presence of special yolk protein receptors on the oocyte membrane. Simultaneous with yolk protein uptake, fat vacuoles occur in the oocyte

Table 1.

Stage of females	Amount of germ balls/ female	Stage of germ balls	Volume of germ ball, egg, embryo [μm^3]	Volume of oocyte [μm^3]	Volume of nurse cells [μm^3]
L2	≈50	Rosette	4990	—	—
L3	≈150	Rosette	5650	—	—
L4 early	≈300	Rosette	11,000	—	—
L4 late	≈1500	Transition	18,000	—	—
Pupal	≈1300	Oocyte differentiation	88,480	3450	85,030
Pupal	≈1200	Midvitellogenesis	121,850	45,210	76,640
Imago	≈1200	Egg/early embryo	138,910	138,910	12,380 not integrated
Imago before birth of L1	≈1200	Fully differentiated L1 larva	650,000	—	—

cytoplasm. During these events, the last molt to the female imago occurs.

The cytoplasm of the germ cells that enter prophase becomes enriched in vacuoles. Some time later, cell membranes of some nurse cells degenerate, and large areas of syncytial cytoplasm develop, containing nurse cell nuclei, while other nurse cell membranes persist (Figs 2F, 5C). The syncytiyal areas, directly bordering the oocyte then fuse. The incorporated nurse cell nuclei persist for a short while, but later degenerate. Yolk platelets now mix with the integrated regions of the syncytial nurse cell compartments. This process continues until the vitelline membrane, secreted by follicle cells, inhibits yolk uptake as well as fusion of the last nurse cell areas. As revealed by a cap of unintegrated nurse cell compartments, the resulting egg becomes polarized in its anterior–posterior axis by the above mechanism of euplasmatic growth (Fig. 1A).

In order to test which cystocyte will be the final oocyte, two whole follicles and portions of several others were analysed by ultrastructural serial sectioning. No intercellular bridge could be detected, but, at least one part of oocyte membrane next to the nurse cells had already begun to degenerate, and this membrane could have had an intercellular bridge (Fig. 5C).

Apoptosis of clusters occurs in some follicles throughout oogenesis (Fig. 1C). During the transition stage, in which the rosette configuration is lost and differentiation of oocytes becomes apparent, the proportion of apoptotic follicles increases to about 20% of the total number of follicles counted from median sections through females (Table 1). By contrast, at the time of rosette formation and fission, the percentage of apoptotic follicles is much lower (2%).

Chorionogenesis is the last event during oogenesis. However, chorionogenesis is suppressed or absent in *E. tenuicornis*. Only a vitelline membrane is secreted. Fertilization follows after conception, and synchronous embryogenesis starts immediately (Fig. 1A). Because eggs are small and rich in euplasm, the embryo passes through the early stages of development shortly. During organogenesis, the embryo remains in the remnants of the still-existing follicle cells. A measurable growth of the embryos occurs and the fat body of the mother decreases (Fig. 1A, B; Table 1). Thus, during this developmental period, one can assume that nourishment of the embryos is via the hemolymph. The L1 larvae leave the maternal organism via the brood canal and are ready for a new infection cycle.

DISCUSSION

Reports on female gonad development of Strepsiptera are rare (Cooper, 1938; Silvestri, 1941; Baumert, 1958; Gu *et al.*, 1992) and there seems to be only one electron microscopical analysis describing late stages of oogenesis (Kathirithamby *et al.*, 1990). Baumert (1958) reports the light microscopic investigation of the various stages of oogenesis in *E. tenuicornis*, which agreed with the gross morphology shown here. However, his interpretations must be partly revised: his "Mesodermballen" are equivalent to rosette stages of clusters that split, but do not fuse in the 3rd-larval stage. Because the development of germ cells is synchronized during larval stages, this fission of rosette stages is the only mechanism by which the number of germ cells can be enhanced, from 10–20 in the gonad anlage of the first instar, to about 1500 eggs found in adult females. The electron microscopical analysis of the *Xenos moutoni* De Buysson ovarian structure by Kathirithamby *et al.* (1990) shows that some aspects of late oogenesis are comparable to those found in *E. tenuicornis* in the present investigation. The youngest stage analysed by Kathirithamby *et al.* (1990) is that of reorganization of follicles, which is the transition stage, i.e., the rosette stages are missing. Their next stage analysed is a follicle in midvitellogenesis, showing oocyte and nurse cell assembly in which cell membranes are still present. Both stages, as well as the fully developed egg, were also found in *E. tenuicornis*. Differences between these two species may exist in the amount of membrane reduction, which is not reported by Kathirithamby *et al.* (1990).

The findings on *E. tenuicornis* oogenesis and the general line of development in insect ovaries (Büning, 1993, 1994, 1996, 1997) can be combined to give the following scenario of developmental events, characterizing the strepsipteran type of ovary:

1. Stem cell activity is restricted to embryonic stages.
2. Germ cells of the ovary anlage in L1 have the status of cystoblasts.
3. Cystoblasts undergo incomplete, synchronized division cycles, generating clusters of cystocytes, combined by intercellular bridges to which a polyfusome stretches.
4. Somatic tissues, such as an ovary sheath or ovariole sheath, do not develop. Remnants of the ovary sheath may be the few flat cells with extracellular matrix, surrounding here and there the follicles. Some somatic cells surround each cluster as a monolayer, giving rise to follicular cells.
5. During mitotic cycles, clusters form strong rosettes with a maximum of about 130–250 cystocytes.
6. Synchrony of divisions is lost during fission events, i.e., each half of the cluster follows its own synchronous clock.
7. Fission of clusters occurs regularly, by which means the number of rosettes, and finally eggs, is significantly increased.
8. Rosette formation stops in the L4 stage, the polyfusome vanishes, and each cell becomes isodiametric.
9. Several cells undergo the first steps of meiotic prophase, but only one cell, which is in contact with follicular cells, continues and becomes the oocyte.
10. All other cells serve as nurse cells, but do not polyploidize their genomes.
11. A separate previtellogenic growth phase, which fol-

lows the phase of mitotic cycles and by which euplasmatic components are produced, is reduced to the transition stage.

12. Intercellular bridges are still present and allow cytoplasmic exchange.
13. Some nurse cells, especially those starting prophase, produce many vacuoles, and begin to reduce their cell membranes. Thus, large syncytial areas are formed which later become incorporated into the oocyte.
14. The oocyte itself also produces many vacuoles which may represent a membrane reservoir for yolk platelets.
15. Simultaneously, endocytotic uptake of yolk starts, visible by means of pinocytotic clathrin-coated vesicles on the smooth oocyte surface. Vitellogenesis is reduced, resulting in small eggs.
16. Vitellogenesis is stopped by secretion of a vitelline membrane. A sculptured chorion is missing.

The developmental series of phenotypical events has, of course, an underlying genetic background. Some of these events are phylogenetically old, some are new. In Strepsiptera, as well as in other taxa (Büning, 1994, 1996, 1997), the development of the ovary can be looked at as modular, with some ancient modules (i.e., 3 or 15, 16), some that have changed only slightly during evolution (i.e., 5, 6), some that are new in this context (i.e., 7, 13), while others are silent in the female line, or have been lost (i.e., 4, 11). Generally, reductions (1, 4, 10, 11, 15, 16) are the main events composing this type of ovary. Consequently, some new inventions (7, 13) became necessary to optimize egg production (i.e., synchronous production of large numbers of small eggs) in these endoparasitic species.

The question, which of the cystocytes finally becomes the oocyte, cannot be answered as yet. Ultrastructural serial section analysis of very early stages of morphologically differentiated oocytes is in progress. Until now, it has not been possible to determine the number of intercellular bridges combining young oocytes with nurse cells. In all cases analysed to date, no intercellular bridge has been found, but at least one area of membrane reduction has been identified. This might indicate that one cystocyte of the last mitotic cycle becomes the final oocyte, but other scenarios are still possible.

The strepsipterans ovary may have developed from either a polytrophic or a telotrophic meroistic background. In the case of telotrophic meroistic foremothers, one has to assume some more reductions, in addition to those which are needed on the basis of a polytrophic meroistic ancestor. These reductions are: (1) the restriction to only one oocyte per cluster (Büning, 1994, 1996, 1997), (2) the reduction of nutritive cords, and, (3) the reestablishment of the tight connection between the nurse chamber and the oocyte chamber in each follicle. Therefore, using the rules of parsimony, the polytrophic meroistic ovary is the preferred ancestral ovary type for strepsipterans. However, the reductions and new inventions shown here do not give any hint as to at which position in the cladogram of Insecta, the Strepsiptera may be the sister group: Coleoptera (Kinzelbach, 1971) and Diptera (Chalwatzis *et al.*, 1995; Whiting, 1996) have been suggested, but any position within the taxon of Meroista (=Acercaria and Holometabola) (Büning, 1996, 1997) seems possible.

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