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ORIGINAL ARTICLE

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The telotrophic ovary known from Neuropterida exists also in the myxophagan beetle *Hydroscapha natans*

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Abstract The ovary structure of the myxophagan beetle, *Hycdoscapha natans*, was investigated by means of light and electron microscopy for the first time. Each of the two ovaries consists of three ovarioles, the functional units of insect oogenesis. The ovary type is telotrophic meroistic but differs strongly from the telotrophic ovary found among all polyphagous beetles investigated so far. All characters found here are typical of telotrophic ovaries of Sialidae and Raphidioptera. Both taxa belong to the Neuropterida. As in all telotrophic ovaries, all nurse cells are combined in an anterior chamber, the tropharium. The tropharium houses two subsets of germ cells: numerous nurse cell nuclei are combined in a central syncytium without any cell membranes in between, surrounded by a monolayer of single-germ cells, the tapetum cells. Each tapetum cell is connected to the central syncytium via an intercellular bridge. Tapetum cells of the posterior zone, which sufficiently contact prefollicular cells, are able to grow into the vitellarium and develop as oocytes. During previtellogenic and early vitellogenic growth, oocytes remain connected with the central syncytium of the tropharium via their anterior elongations, the nutritive cords. The morphological data are discussed in the light of those derived from ovaries of other Coleoptera and from the proposed sister group, the Neuropterida. The data strongly support a sister group relationship between Coleoptera and Neuropterida. Furthermore, several switches between polytrophic and telotrophic ovaries must have occurred during the radiation of ancient insect taxa.

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Introduction

Insect ovaries consist of morphologically and physiologically discrete entities, the ovarioles. Their structure and physiology has been investigated intensively in the past (i.e., Brandt 1878; Snodgrass 1935; Telfer 1975; King and Büning 1985; Büning 1994). In general, each ovariole is divided into two parts: a posterior part, called vitellarium, in which oocytes undergo all stages of growth and egg shell production and an anterior part in which germ cells undergo differentiation. This article focuses on the anterior part only. Here, germ cells and somatic tissues develop and thereby determine the final ovariole type. This anterior part of the ovariole is called the germarium. In most insects, the ovarioles are held in position in the abdomen by tree somatic tissues, the envelope surrounding the ovary, the envelope surrounding each ovariole, and the terminal filament that anchors each ovariole anteriorly. We distinguish two basic ovariole types. In the first type, each germ cell enters the prophase of meiosis in the germarium and differentiates as oocyte, growing finally into a giant egg (Fig. 1a). Therefore, all constituents of the egg cytoplasm, except yolk, are produced under control of the oocyte nucleus. This ovarian type is called panoistic.

In the second type, germ cells undergo a series of synchronized mitotic events in the germarium before meiosis is induced. The mitotic siblings remain connected by intercellular bridges, which are remnants of arrested cleavage furrows during cytokinesis. Thus, a syncytial cluster of sister cells emerges (Giardina 1901; Knaben 1934; Hirschler 1945; King 1970; King and Büning 1985; Büning 1994, 1996, 1998). During these steps of germ cell multiplication, a female-specific program of germ cell differentiation is initiated and results in one or several oocytes within each cluster. Finally, a physiological entity, the follicle, is formed governed by intrinsic and extrinsic factors (Fig. 1; Büning 1994; de Cuevas and Spradling

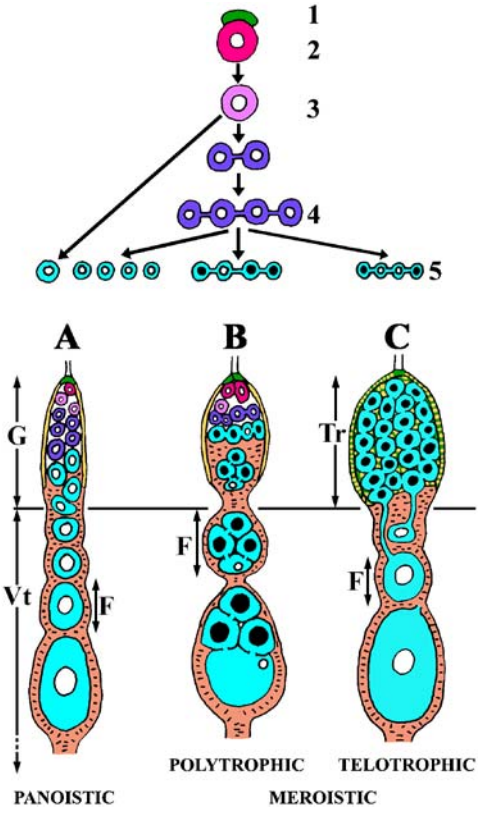


Fig. 1 Key events in germ cell cluster formation and their impact on ovary types. Somatic apical cells (cap cells) (*1*, *green*) interact with germ cells, maintaining the latter as stem cells (*2*, *red*) while keeping their anterior position. Stem cells undergo differential mitoses and give birth to cystoblasts (*3*, *purple*). Cystoblasts transform directly to oocytes or undergo a special program of mitoses, generating clusters of cystocytes (*4*, *blue*). Cystocyte clusters (*5*, *turquoise*) can split or can undergo a special program of differentiation generating nurse cells (*black nuclei*) and oocytes. **a** Panoistic ovarioles are generated by stop signals of cystocyte mitoses or by cluster splitting in the germarium (*G*). Growth of oocytes proceeds in the vitellarium (*Vt*). Meroistic ovarioles make use of germ cell clusters in addition with nurse cell-oocyte differentiation, taking place in the germarium that is ensheathed by somatic inner sheath cells (*yellow*). **b** In polytrophic meroistic ovaries, only one cystocyte develops as oocyte, all others become nurse cells. Each cluster will be ensheathed by follicular cells (*brown*) and develops as a separated unit, the follicle (*F*), consisting of the oocyte chamber and its own nurse cell chamber. **c** In telotrophic meroistic ovaries, the character of apical cells (*green*) is extended to inner sheath cells and interstitial cells (*green* + *yellow*) by which all nurse cells will be kept in a terminal tropharium (*Tr*). As in panoistic ovaries, each follicle consists of the oocyte chamber only

1998; González-Reyes and St Johnston 1998; Rübsam et al. 1998; Deng and Lin 2001). Such insect ovaries are called meroistic.

In the polytrophic meroistic ovary (Fig. 1b), all descendants of a single cystoblast, i.e., the whole cluster of germ cells consisting of the prospective nurse cells and a single oocyte, are kept together in the follicle. The differentiated cluster undergoes the whole process of oocyte growth and maturation in the vitellarium. In most cases, the genomes of nurse cell nuclei polyploidize and register the previtellogenic growth processes of the oocyte, whereas the chromosomes of the oocyte nucleus become silent and form a karyosome. During the growth of the follicle, the germ cell cluster is enveloped by a monolayer of somatic follicular cells, which are finally involved in the formation of the egg shell at the end of the vitellogenic growth phase.

However, a third ovarian type, called the telotrophic meroistic ovary (Fig. 1c), is found in some insect orders. In this type, all prospective nurse cells of the ovariole are kept together in the germarium, which transforms into a single anterior tropharium during reproductive phases. In the vast majority of insects with telotrophic ovarioles, the germ cell mitoses and cluster formation are confined to larval and/or pupal stages. Only in some groups of bugs, mitoses of germ cells generating additional trophocytes take place also in adult females (Huebner and Anderson 1972; Büning 1994). Developing oocytes remain connected to the tropharium via specialized anterior elongations, the nutritive cords. To date, it is widely accepted that these telotrophic ovaries have evolved four times independently. This interpretation is based on sister group relationships among insects, founded mainly on other morphological characters (Kristensen 1981; Pakaluk and Slipinski 1995; Bitsch and Bitsch 2004). The most archaic telotrophic ovary developed in mayflies, which nearest taxa are all panoistic (Ephemeroptera; Gottanka and Büning 1993). The other three telotrophic ovary types occur in taxa, the sister groups of which have polytrophic meroistic ovaries. This happened among the ancestors of the bugs (Hemiptera), among Neuropterida in the common ancestors of the order of snakeflies (Raphidioptera) and the family of alderflies (Megaloptera, Sialidae), and, finally, among the ancestors of the largest suborder of beetles, the polyphagous Coleoptera (King and Büning 1985; Stys and Bilinski 1990; Gottanka and Büning 1993; Büning 1994, 1996, 1998).

In this paper, I will report on a new telotrophic meroistic ovary, which I found among beetles of the suborder of Myxophaga. This ovary featured fundamental similarities to that special type of telotrophic ovary that was so far thought to occur exclusively among Neuropterida.

Materials and methods

Specimens of *Hydroscapha natans* were collected from shallow water in permanent rivers in Arizona (leg. D. Maddison). Eggs, larvae, pupae, and imagines live in the same habitat in shallow water, and larvae and imagines feed on blue-green and green algal mats. From this primary collection, *H. natans* has been raised successfully in the lab since 1997. Life cycle and culture conditions will be published elsewhere.

The imaginal ovaries were dissected in standard fixatives [2% glutaraldehyde in 0.1 M cacodylate buffer and 5 mM Ca Cl_2 (final pH 7.2) and finally fixed in 1% osmiumtetroxide in the same buffer] for light and electron microscopy. After dehydration, the specimens were embedded in Agar 100 (Epon 812 equivalent) epoxy resin as described elsewhere (Büning and Sohst 1988). Semi-thin serial sections were stained with 0.5% toluidine blue and

are analyzed with a Zeiss Axiophot. Ultrathin serial sections were analyzed using a transmission electron microscope (Zeiss EM 10) according to the procedure published by Gottanka and Büning (1993).

Results

Gross architecture of ovaries

Each of the two ovaries consists of three short ovarioles that open into the bulbous end of the lateral oviducts. Both lateral oviducts run into a common, central oviduct (Fig. 2a). Each ovariole is polarized, and the terminal filament is followed by a cylindrical or sometimes pear-shaped tropharium (Fig. 2b). Posteriorly, the terminal chamber continues via a broad-neck region into a short vitellarium in which oocytes undergo previtellogenic and vitellogenic growth (Figs. 4a–e). The vitellarium contains only one to two vitellogenically growing oocytes at any time.

Fine structure of somatic cells in the anterior region of ovarioles

Each ovariole is enclosed by a very thin extracellular matrix, the basal lamina, also known as the basal membrane or the tunica propria (Fig. 2c). Tightly connected to this basal lamina is a flat epithelium, the so-called inner sheath. Inner sheath cells have a homogenous cytoplasm housing moderate amounts of free ribosomes, mitochondria, and ER, which overall cause a weak contrast in electron microscopic pictures as a well as a weak toluidine blue staining in semi-thin sections (Figs. 2c,d and 4d). All other cellular constituents of the ovariole, i.e., germ cells or somatic prefollicular cells, the latter intermingling with and substituting sheath cells in the neck region of ovarioles, are highly contrasting and show numerous free ribosomes and mitochondria (Figs. 2d and 3d). Somatic cells, sheath cells, as well as prefollicular cells are flat or spindle shaped with discoidal or ellipsoid nuclei and border directly onto the extremely thin basal membrane (Fig. 2c).

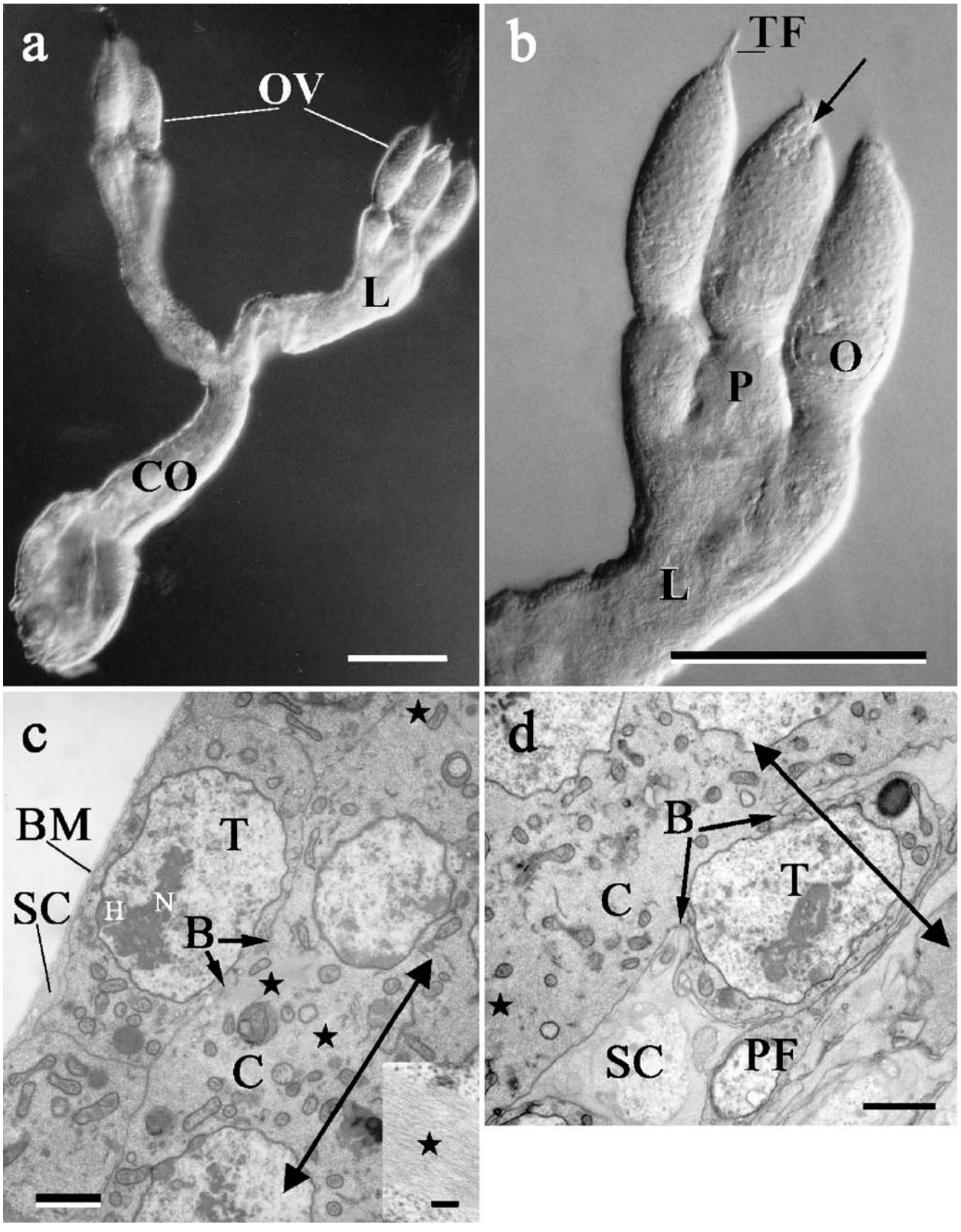
Fine structure of germ cells

Analysis of semi-thin and ultrathin sections yielded the following results (Figs. 2c–d, 3a–c, and 4a–d). The anterior region of the ovariole, i.e., the tropharium, houses numerous germ cell nuclei randomly distributed in the central area, whereas toward the periphery, the germ cell nuclei are more regularly spaced. The central area is devoid of any cell membranes and is therefore called the central syncytium. In contrast, in the periphery, single germ cells assemble into a monolayer called the germ cell tapetum. Each of the tapetum cells opens apically to the inner syncytium with one intercellular bridge (Fig. 2c) or with a wide opening, emerging from the intercellular bridge by partial membrane reduction. These openings are especially found in ovarioles dissected from egg-laying females (Fig. 2d). Intercellular bridges are decorated with electron dense material, which is greatly reduced in the wide openings. On transverse membranes of tapetum cells, intercellular bridges are never found. Thus, tapetum cells never open to each other by intercellular bridges.

The cytoplasm of tapetum cells has all the constituents found also in the cytoplasm of the central syncytium: huge amounts of free ribosomes, elongated and y-shaped mitochondria, regularly found Golgi complexes, and a sparse endoplasmic reticulum; small areas of annulate lamellae, however, occur only rarely (not shown). In addition, the cytoplasm of the central syncytium contains numerous bundles of microfilaments (Figs. 2c,d), the ultrastructure of which is identical with so-called stress fibrils of F-actin, as known from other objects. The cytoplasmatic microfilament bundles have diameters between 0.3 and 0.7 μm, whereas single fibrils have diameters of 7–9 nm, matching the known diameters of F-actin. These cytoplasmatic bundles of microfilaments build a three-dimensional meshwork in the whole central syncytium in which the germ cell nuclei are scattered randomly.

There is no apparent anterior–posterior gradient in size or morphology of germ cell nuclei in ovarioles of reproductive females. The germ cell nuclei are spherical and have a mean diameter of about 4 μm, which is the same size as that of spermatogonia during mitotic cycles (Fig. 4f), i.e., the nuclei of the whole anterior region are in the 2–4 C status. Among them are a few dumbbell-shaped nuclei, which have more or less twice the volume of other nuclei (Fig. 3a). By semi-thin and ultrathin serial sectioning of the tropharium, a total amount of about 500 germ cell nuclei was counted, of which about 220 belonged to the cellular periphery, i.e., the germ cell tapetum, whereas about 270 nuclei were located in the central syncytium. Among the latter, there are two groups indicating a doubled volume, about 10 nuclei, which have an apparently larger diameter and about 15 dumbbell-shaped nuclei (Fig. 3a). Both groups of nuclei occur simultaneously, indicating that some central nuclei divide amitotically. Especially within these nuclei, there are microfilament bundles decorated with associated proteins giving them an ultrastructural aspect that is slightly different from that of microfilament bundles found in the cytoplasm (Fig. 3a,b). Although in semi-thin sections, the spherical nuclei seem to be smooth on their surface, in electron microscopic pictures, their surface is folded (Figs. 2c,d and 3a–c). The chromatin is scattered throughout the nucleoplasm in small and fuzzy, sometimes rod-like, entities and borders a large, highly contrasting, and irregularly shaped single nucleolus. Next to the nucleolus, more or less large areas of membrane-associated heterochromatin are found (Fig. 2c).

At the posterior end of the tropharium, about 30 tapetum cells border the neck region that mediates between the tropharium and the vitellarium. All these germ cells are in contact with a mixture of somatic inner sheath cells and prefollicular cells (Figs. 2d and 3c). Out of this tapetum cell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◀ **Fig. 2** **a** Ovary of a young female of *H. natans* few days after final moult. Ovarioles (*OV*) insert terminally on two lateral oviducts (*L*), which fuse into a common oviduct (*CO*). The *bar* represents 10 μm. **b** Each ovariole ends anteriorly with a short terminal filament (*TF*). The oocytes (O) nest in a broad prefollicular tissue, followed by a pedicel region (*P*), connecting each ovariole to the enlarged terminal region of the lateral oviduct (*L*). The *bar* represents 10 μm. **c** Transmission electron micrograph showing the lateral region of the terminal chamber. A thin basal membrane (*BM*) encloses the whole organ and is connected tightly to the superficial monolayer of somatic inner sheath cells (*SC*), which surround the tapetum cells (*T*). Tapetum cells open with an intercellular bridge (*B*) to the central syncytium (*C*). Nuclei contain one prominent area of nucleoli (*N*) as well as some membrane-associated areas of heterochromatin (*H*). *Stars* mark areas of microfibrillar bundles (*inset*). The *bar* represents 1 μm (inset bar=0, 1 μm). **d** Posterior region of the terminal chamber. Sheath cells become more and more replaced by prefollicular cells (*PF*). Double-headed arrows indicate the long axis of the terminal chamber

population, the future oocytes are recruited (see below). As a result, each female has the capacity to produce a maximum of about 180 eggs during her lifetime, provided that tapetum cells of the lateral periphery cannot be shifted into the posterior region of the tropharium, thereby replenishing the pool of presumptive oocytes. The architecture of the tropharium in which the membrane-free central syncytium is connected with the tapetum cells via intercellular bridges enables each germ cell nucleus of the region to contribute euplasmatic substances to all growing oocytes via their nutritive cords (see "Oocyte differentiation"). In some ovarioles, near the tip of the tropharium few small independent syncytial areas exist, which are not connected to the main central syncytium or to each other (Figs. 2b and 4d). In the periphery of these anterior areas, sometimes single tapetum cells are found (not shown).

Oocyte differentiation

About 30 posteriorly located tapetum cells of the tropharium remain in close contact with prefollicular cells that intermingle with sheath cells. This special population of tapetum cells represents the pro-oocytes of the ovariole. The developing pro-oocytes are enveloped by the prefollicular cells. The oocyte's opening to the central syncytium remains; thus, a short elongation of the growing oocyte occurs toward the tropharium, resembling a nutritive cord (Figs. 3c,d and 4b,c). However, in some of these nutritive cords, heavily contrasting materials and some vacuoles assemble next to the opening toward the central syncytium (Figs. 3c and 4b). These nutritive cords collapse shortly thereafter, and the connected potential oocytes degenerate. Nutritive cords linking successfully growing oocytes to the central syncytium enlarge their diameter up to 4 μm while containing the same constituents as the central syncytium (Figs. 3d and 4c). The nutritive cords contain some microtubules, most of them located next to their membranes and arranged in parallel to their long axis (Fig. 3d, inset). During vitellogenesis (not shown in detail), the germinal vesicle grows tremendously and is shifted to an anterior–lateral position, which may represent the future dorsal side of the embryo (Fig. 4e). The germinal vesicle shows some spheroid bodies, a single nucleolus and individual chromosomes, which appear to be in a lampbrush configuration (Figs. 2b, 3c, and 4a–c). A typical karyosome is not formed during the previtellogenic and the early vitellogenic growth phases.

The data presented above allow the following conclusions:

1. The ovary of *H. natans* is telotrophic meroistic and has the following characters: the central part of the tropharium, called the central syncytium, is a cell membrane-free area housing numerous small nuclei. The central syncytium is surrounded by a monolayer of germ cells, the tapetum cells. Each tapetum cell is connected to the central syncytium by an intercellular bridge. Posterior-tapetum cells, which are in contact with prefollicular cells, can grow and differentiate into oocytes. Each oocyte remains connected to the central syncytium via its anterior elongations, the nutritive cords. These main characters are identical to those known from telotrophic ovaries of alderflies (Megaloptera, Sialidae) and snakeflies (Rhahidioptera). Therefore, the telotrophic ovary of *H. natans* is of the *Sialis* type (Fig. 5a,b). Telotrophic ovaries of polyphagous beetles have a very different set of main characters (Fig. 5c; Büning 1972, 1994).
2. Oocyte nuclei remain synthetically active during previtellogenesis. The lack of an extra DNA body as well as multiple nucleoli suggest, however, that extrachromosomal amplification of rDNA does not take place in the oocyte nuclei of *Hydroscapha*. As a consequence, most of the abundant ribosomes stored in oocytes have to be produced by nuclei of the central syncytium and are transported via nutritive cords into the oocytes.

Discussion

The somatic cells of the hydroscaphan ovary

Gross morphology and ultrastructure of somatic cells as found here in *H. natans* ovaries are similar to telotrophic meroistic ovaries of polyphage Coleoptera in many respects. In tropharia of both the broad-neck region houses numerous spindle-shaped prefollicular cells, enclosing the young and arrested oocytes. In both, the posterior-sheath cells are clearly discernible from prefollicular cells. It clearly differs from the gross morphology of somatic cells found in tropharia of Sialidae and Raphidioptera. In these taxa, prefollicular cells are rare, and they are not clearly discernible from sheath cells (Büning 1979c, 1980).

These common characters of somatic cells found in the hydroscaphan ovary as well as in the telotrophic ovaries of Polyphaga can be interpreted as having developed along a common evolutionary path. This statement can be extended

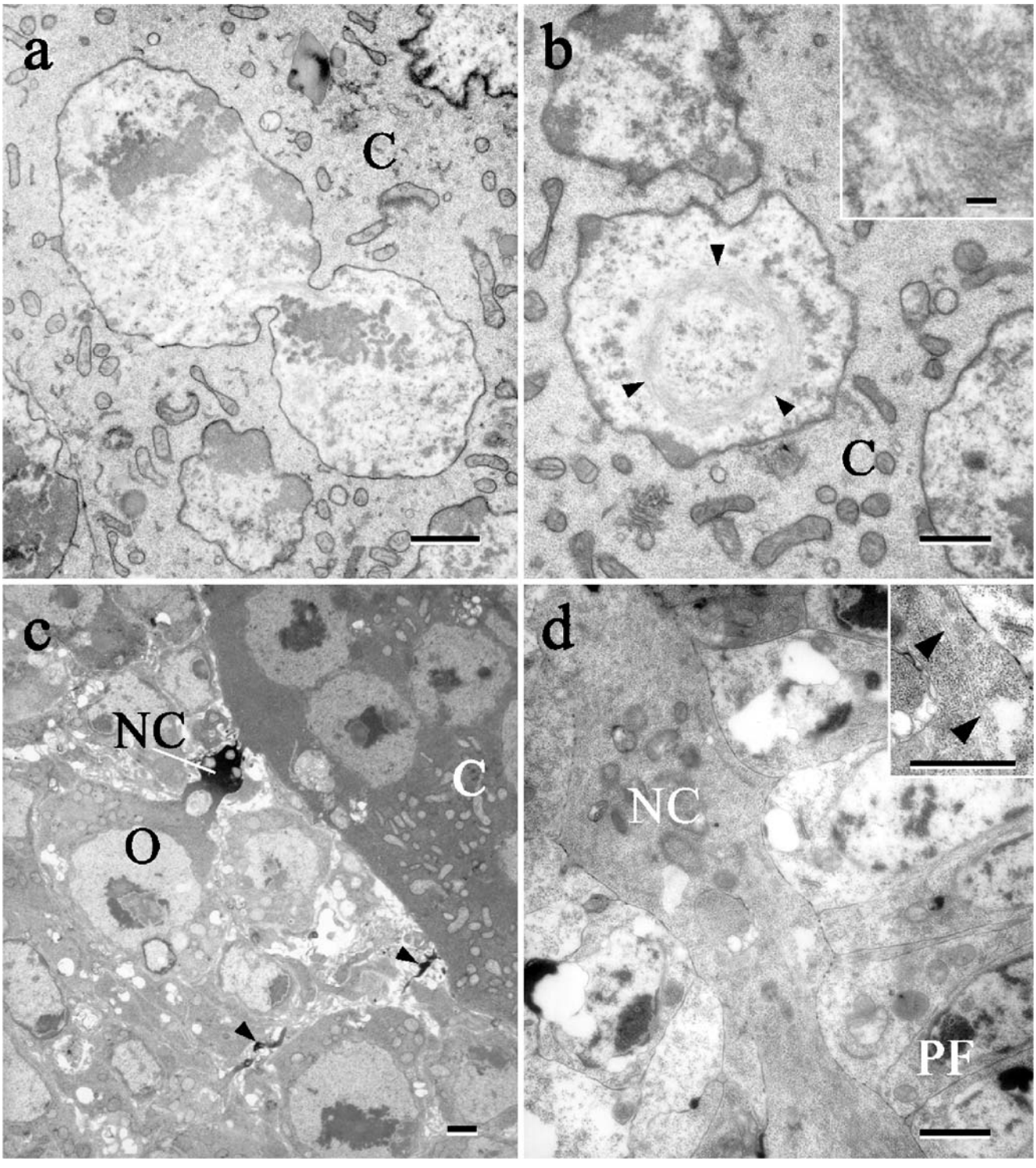

Fig. 3 **a** Dumbbell-shaped nuclei of the central syncytium (*C*). Especially here, as well as in some other nuclei (**b**), intranuclear bundles of microfibrils occur (*arrowheads*), showing slightly different morphology as cytoplasmic bundles (compare the inset of Fig 2c with inset 3b). **c** In the posterior region of the tropharium, the young, arrested oocytes (*O*) are connected via nutritive cords (*NC*) to the central syncytium (*C*). Note the intense contrasting materials in the upper part of the nutritive cord as well as in a degenerating nutritive cord (*arrowheads*). **d** Nutritive cords (*NC*) of successfully growing oocytes enlarge and contain the same substances as found in the central syncytium. Microtubules are found next to the nutritive cord membrane, most of them ordered parallel to the long axis of the nutritive cord (*arrowheads in the inset*), prefollicular cell (*PF*). The *bars* represent 1 μm (inset bar in b=0, 1 μm)

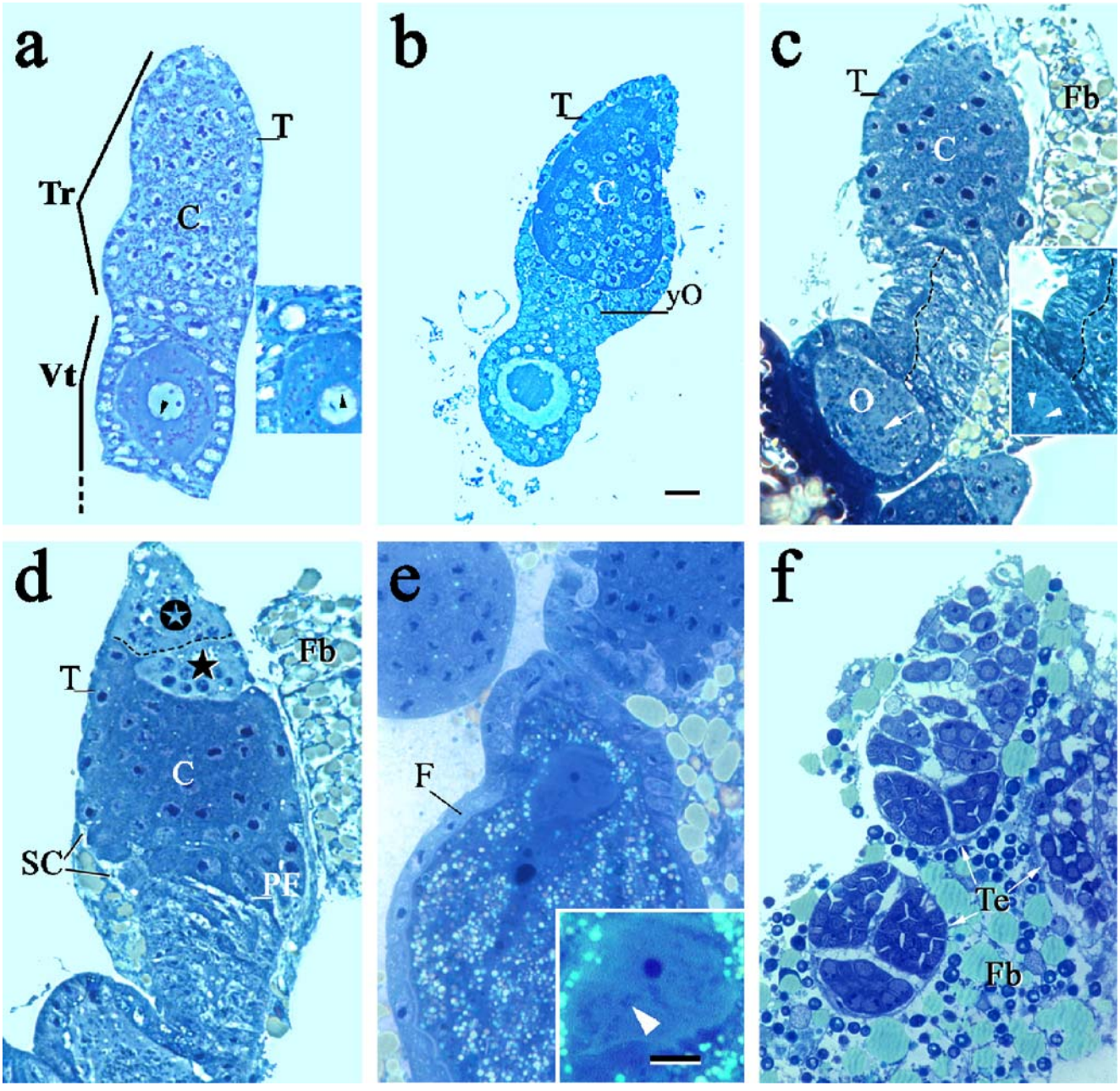

Fig. 4 Semi-thin sections through ovarioles of *H. natans*. Note the monolayer of tapetum cells (*T*) surrounding the central syncytium (*C*) of the tropharium (*Tr*). *Arrowheads* in **a**, **c**, and **e** point to single chromosomal areas in oocyte nuclei, indicating that a karyosphere has not yet formed, even when vitellogenesis has already started as shown in **e**. Note the thin nutritive cord connecting the young oocyte (*yO*) in **b** with the central syncytium. In **c**, the nutritive cord (*broken line*) has enlarged enormously. It follows a curved way through the prefollicular tissue while connecting the previtellogenically growing oocyte (*O*) with the central syncytium, vitellarium (*Vt*), sheath cells (*SC*), follicular cell (*F*). In **f**, the three testioles of a young male of *H. natans* are shown. Note the polar organization of germ cell cluster formation within the testioles (*Te*) embedded in the fat body (*Fb*). The *bar* represents 10 μm

even to the somatic tissues of Adephaga, which have a polytrophic meroistic ovary. However, interstitial cells, which form a three-dimensional net in the tropharium of polyphage Coleoptera (Büning 1972, 1978, 1979a,b), are missing in the hydroscaphan ovary and consequently have to be considered as an apomorphic character of the polyphagan type of telotrophic ovaries.

The germ cells

As it was shown, dumbbell-shaped germ cell nuclei are rare but regular components of the central syncytium of the hydroscaphan tropharium. This was not reported from ovaries of *Sialis* or Raphidioptera (Büning 1979c, 1980) and indicates that there is a need for reexamination of

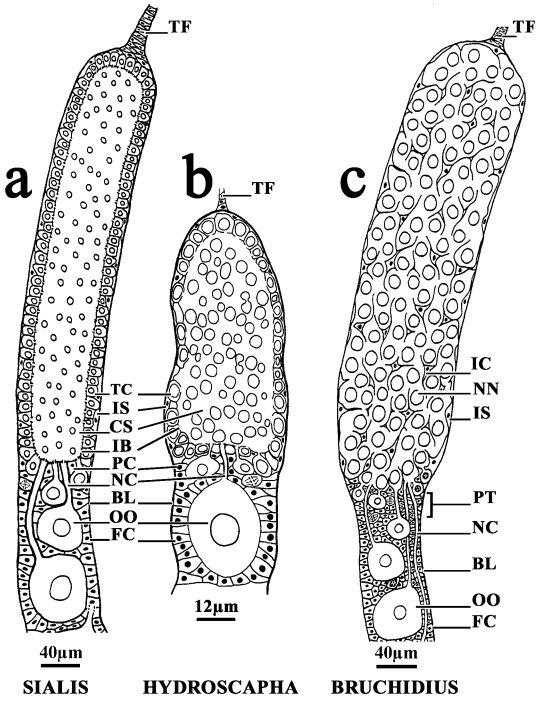

Fig. 5 Drawings of the telotrophic ovary types found among Neuropterida and Coleoptera. **a** Ovariole of *Sialis flavilatera* (Megaloptera, Sialidae; Büning 1979c, 1980). **b** Ovariole of *H. natans* (Coleoptera, Myxophaga; Büning 2000). **c** Ovariole of *Bruchidius obtectus* (Coleoptera, Polyphaga; Büning 1972, 1994). Note that most morphological characters of ovarioles from *Sialis* and *Hydroscapha* are identical, whereas the ovariole of *Bruchidius* represents a clearly separated telotrophic type. *BL* Basal lamina, *CS* central syncytium, *FC* follicular cell, *IB* intercellular bridge, *IC* interstitial cell, *IS* inner sheath, *NC* nutritive cord, *NN* nurse cell nucleus, *PC* prefollicular cell, *PT* prefollicular tissue, *OO* oocyte, *TC* tapetum cell, *TF* terminal filament

ovariole development in these species. The same is true for the occurrence of actin filament bundles, numerously found in the hydroscaphan tropharium. However, a radial orientation of these bundles toward the nurse cell nuclei does not occur. This has been found in *Drosophila*, where the position of nurse cell nuclei is stabilized by actin filament cables oriented that way during the dumping phase (Riparbelli and Callaini 1995; Guild et al. 1997). In Hymenopterans, this task is taken over by a cage of microtubules (Bilinski and Jaglarz 1999). Microtubules are common in the syncytial cytoplasm of Sialidae and even abundant in Raphidioptera (Büning 1979c, 1980). So far, it is a matter of speculation that these cytoskeletal elements might substitute in these taxa the prominent actin fibril bundles found in the hydroscaphan ovary.

The few anterior syncytial areas found in some tropharia of *H. natans*, which are not fused to the central syncytium, may represent a transient stage, which occurs regularly in the final phase of the *Sialis* ovary development (Büning 1979c). This finding, as well as the architecture of testioles, in which small, rosette-forming clusters of germ cells occur in a polarized manner (Fig. 4f), supports the hypothesis that the developmental processes leading to this ovary type are very similar in both taxa and may have common roots. However, the hypothesis strongly demands an analysis of larval and pupal gonads in *Hydroscapha* and a reexamination of *Sialis* gonads in early developmental stages using ultrastructural serial sectioning as well as immunostaining of cytoskeletal elements, which is on the way.

The germ cell architecture in functional telotrophic ovaries of *H. natans* is totally different from those reported from polyphage Coleoptera (Fig. 5; Büning 1972, 1978, 1979a,b, 1994; Kloc and Matuszewski 1977; Matuszewski et al. 1985). Instead, this ovary resembles the architecture of telotrophic meroistic ovaries typical of Megaloptera, Sialidae or Raphidioptera (Büning 1979c, 1980, 2000; Büning and Maddison 1998). In both taxa, germ cells of functional ovaries build up a central syncytium, surrounded by tapetum cells, each of which opens to the central syncytium by one intercellular bridge (Fig. 5a,b). In both, oocytes develop from basally located tapetum cells. In both, nuclei of germ cells do not polyploidize, and nuclei of tapetum cells are not discernible from those of the central syncytium. Thus, the architecture of germ cells in functional ovarioles of both taxa is virtually identical. The preliminary results concerning cluster formation processes in *H. natans* detailed above, and those assumed to occur during development of *Sialis* ovarioles (Büning 1979c; King and Büning 1985, Rübsam and Büning, unpublished data) suggest the same mode of development. In addition, preliminary results from the reexamination of the telotrophic ovary of polyphagous beetles (Trauner and Büning, in preparation) show that the early events of cluster formation are similar to those that occur during early stages in the *Sialis* ovary. The specialized characters of the telotrophic ovary of Polyphaga are established during late larval and pupal stages.

Phylogenetic considerations

The Hennigian system by which the phylogeny of species can be reconstructed has worldwide acceptance (see above and for more details Hennig 1981, 1982; Watrous and Wheeler 1981; Wiley 1981; Ax 1984) and will be applied here to ovary structures of Neuropterida and Coleoptera. How to judge the characters detailed above? First of all, the identical architecture of telotrophic ovaries found in *Hydroscapha* (Coleoptera, Myxophaga) and in Megaloptera [Sialidae and Raphidioptera (Neuropterida)] cannot be interpreted by pure chance, i.e., a so-called parallel development de novo is unlikely. There are several alternative ways of interpretation.

The telotrophic meroistic character of the *Sialis* type has been developed from a polytrophic meroistic background among the ancestors of Neuropterida and Coleoptera and is therefore regarded as synapomorphic, and, consequently, the taxa Coleoptera and Neuropterida are sister groups (Fig. 6). However, this interpretation includes the restoration of the polytrophic meroistic ovary of the basic type (Büning 1994), which exists outside the taxa in question, i.e., in lice, barklice, butterflies, flies, and other orders. Therefore, this character is plesiomorphic, but its triple

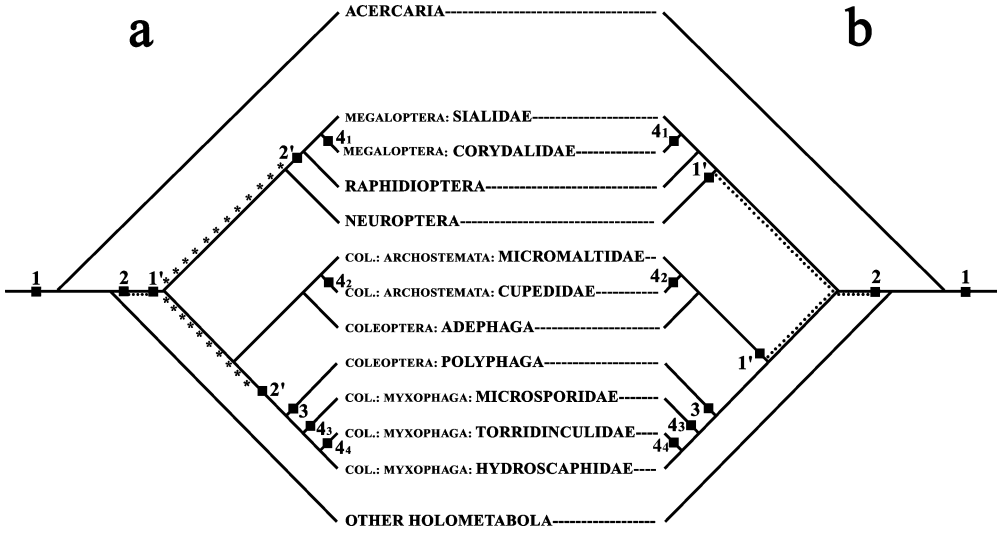

Fig. 6 **a**, **b** Two alternative phylogenetic interpretations of ovary development in the taxa of Neuropterida and Coleoptera. Note that the number of changes necessary to create the recent distribution of telotrophic ovaries among taxa is nearly identical (4 vs 5). Periods of silently traded developmental gene arrangements (..... / ****). *1*, Rise of the basic type of polytrophic meroistic ovaries. *2*, Rise of the telotrophic meroistic ovary of the *Sialis* type. *3*, Rise of the telotrophic meroistic ovary of the Polyphaga type. *1′*, Restoration of the basic type of polytrophic meroistic ovaries. *2′*, Restoration of the telotrophic meroistic ovary of the *Sialis* type. 4_1–4_4, Independent reductions to panoistic ovaries (not discussed in detail)

occurrence among the taxa in question, i.e., in the taxon of Adephaga, in Micromalthidae, (which is a family of the taxon of Archostemata), and among Neuropterida in the taxon of Neuroptera (Scott 1938; Büning 1994, 1998) has to be valued differently as new autapomorphic entities. This restoration of polytrophic meroistic ovaries inside the taxa in question could have happened once (Fig. 6a), twice (Fig. 6b), or even three times independently (in Archostemata, Adephaga, and Neuroptera, not shown), depending on the alternative sister group relationships among coleopteran subtaxa. In both alternatives shown here, all those developmental networks of genes that are not expressed must have been preserved in a silent mode during the phase in which the other meroistic ovary type, i.e., the telotrophic one, was established among ancestors.

Does this assumption contradict Dollo's law by which complex morphological entities like organs, once reduced, cannot be restored by using the same developmental pathway (i.e., in today's terminology, the same set of genes, four-dimensional developmental programs, or same genetic and epigenetic interdependencies) as before? Of course not, but it concretizes this statement (see also Raff 1996). The repetition of such higher-ordered entities is based on a new composition of one or some ancient, i.e., plesiomorphic, developmental gene arrangements. The reduction must allow the restoration of older concepts or must be accompanied by the development of a new concept; otherwise, the organism has no chance of survival. Such new developmental networks of genes can arise from old ones in which one or a few of the genes involved will change their structural character or their spatial and/or temporal engagement. In other words, the "restored" polytrophic meroistic ovary must have changed to some extent, compared with the ancient polytrophic meroistic ovary, and these changes into a new composition must be valued as apomorphic as stated above.

Looking at the ovary types in the group of Coleoptera, it becomes apparent that among the most ancient subgroups, the Archostemata and Myxophaga, all ovary types exist (Fig. 6; Büning 2000), whereas in Adephaga as well as in Polyphaga, only one type has been found, although both groups faced a tremendous radiation of species. This can be explained by the assumption that networks of genes operating during development consist of fewer genes in the phase of their emergence. During its further evolution, the addition of further genes into the network of genes will strengthen the developmental pathways such that the loss of one gene's function will not disturb the whole concept but modulate it to some extent. Thus, in the early phase of the emergence of such a developmental network of genes, a change of only one gene may cause a switch from one type to another. Later, such changes become more and more difficult. Thus, one should postulate that during the early phase of insect radiation, such switches of ovarian types might have happened easily, but not in later times.

Such "back" and "forth" in ovarian types must have happened in ancient times (Fig. 6) and raises questions concerning the number of genes, their hierarchy, and their mode of regulation when involved in the building of ovary structures. Today, we are far from answering these questions to a satisfactory extent. The comparative studies of polytrophic and telotrophic ovaries of present species, however, indicate which developmental processes must have been modified/changed during suggested anagenesis of insect ovaries, i.e., the formation of telotrophic and/or restoration of polytrophic ones.

1. Cell–cell interactions between cystoblasts and nurse cells on the one hand and the somatic inner sheath cells on the other hand. In the polytrophic meroistic ovary of *Drosophila*, only the germ line stem cells interact specifically with the so-called cap cells of the somatic inner sheath and maintain thereby their anterior position. In all telotrophic meroistic ovaries, however, cystoblasts, cystocytes, and finally the differentiated nurse cells maintain this specific interaction with all sheath cells (Fig. 1). As the final result of this interaction, in all telotrophic ovaries, all germ cell descendants, except the growing oocytes, keep this anterior position. Consequently, following the proliferation phase of the germ cells, the whole region elongates into a single anterior tropharium.
2. Differentiation of germ cells. Whereas in the polytrophic meroistic ovary of *Drosophila*, the activities of stem cells, cystoblasts, and cystocytes are going on during the whole reproductive period, in telotrophic ovaries, these activities are shifted into embryonic, larval, or pupal stages and cease finally in reproductive ovaries. Therefore, their final number of oocytes and nurse cells is fixed (with the exception of some advanced genera of bugs, as mentioned in the "Introduction"). Furthermore, the mode of differentiation of germ cells changes. Whereas in *Drosophila*, each cluster differentiates in a stereotyped pattern into only one oocyte and 15 nurse cells, in telotrophic ovaries, the amount of oocytes in a cluster becomes variable and depends on the contact of cystocytes with the prefollicular tissue. Those cystocytes that are in contact with the prefollicular tissue succeed in developing as oocytes. All other cystocytes that are positioned in the middle or anterior regions of the tropharium will never develop as oocytes, but develop as nurse cells. In addition, in all those telotrophic ovaries in which more than one germ cell cluster develops, as is the case in Sialidae and Hydroscaphidae, cell membrane reduction between germ cell clusters is absolutely necessary to fully exploit the physiological capacity of the tropharium.
3. Formation of nutritive cords. In the telotrophic ovarioles, potential oocytes elongate their anterior parts thereby forming nutritive cords which remain connected to the nurse cells via their intercellular bridges. However, many oocytes of polytrophic meroistic ovaries develop also short anterior elongations during previtellogenic growth phases as has been described in follicles of water beetles (Dytiscidae) and named "Nährzapfen" (Bier 1965) as well as in follicles of tiger beetles (Bilinski and Jaglarz 1987) now termed as "nutritive appendix". The same structure was also found in other insect taxa developing polytrophic meroistic ovaries like Mallophaga (Bilinski and Jankowska 1987) or Hymenoptera (Cassidy and King 1972) or Neuroptera (Matsuzaki and Enomoto 1990). Thus, the potency to develop nutritive cords is a character of all meroistic oocytes.

Organisms as a whole as well as their differentiated structures like organs undergo programmed developmental changes throughout a lifetime. These changes are based on a four-dimensional network of gene activities and epigenetic entities. Only comparative developmental studies combined with additional morphological and molecular data can unravel this network and will finally allow a draft of a correct model of insect phylogeny.

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