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Germ cell cluster in the panoistic ovary of Thysanoptera (Insecta)

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Summary. Germ cell clusters are found in the germarial region of ovarioles of *Parthenothrips dracenae*. Cluster mitoses are synchronized, at least initially. The intercellular bridges are filled with fusomal material, which can fuse to form polyfusomal aggregates which in turn form small rosettes. All cells develop into oocytes. Oocytes become isolated by a secondary detachment process. Intercellular bridges, together with fusomal material and cell membranes, survive for some time as isolated bodies. Phylogenetic consequences are discussed. The data provide strong evidence for a secondary panoistic ovary in thysanopterans, since cluster formation in ovaries of primary panoists has not been shown.

A. Introduction

In the ovarioles of most insects, oocytes are assisted by nurse cells during the previtellogenic growth phase. These ovaries are called meroistic ovaries. The nurse cells are the sister cells (cystocytes) of the oocyte. They develop from a mother cell, the cystoblast, by synchronized mitotic divisions, followed by incomplete cytokinesis. Among this developing germ cell cluster, only one of the two oldest cells will become the oocyte, all others will develop as nurse cells. A special cytoplasm, the polyfusome, arises between the remaining intercellular bridges or ring canals. Very often the intercellular bridges remain tightly together and the cluster cells form a rosette. These events have been known about for a long time (Giardina 1901; Hirschler 1945; King 1970; Telfer 1975; King et al. 1982). Since all meroistic ovaries can be derived from this basic plan, we have called it the basic type of meroistic ovary (Büning 1985, 1988; King and Büning 1985). The basic type is a character complex that can possibly be used for sister group analysis between Paraneoptera and Holometabola (Hennig 1969; Kristensen 1981). In his excellent review of the insect ovary structure, Telfer (1975) suggested that the appearance of panoistic ovaries may be ancestral or may have deviated, for example by reduction of mitotic cycles during cluster development, combined with an early decay of nurse cells. Here we report the first finding of cluster formation in a panoistic ovary and its impact on phylogeny among paraneopterans.

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B. Materials and methods

The ovaries of *Parthenothrips dracenae* (Heeger, 1854) were investigated. The species was grown on *Philodendron* sp. Nymphs and imagoes were prepared for light and electron microscopy using standard methods. Serial sectioning was performed as described elsewhere (Büning and Sohst 1988).

C. Results

1. Gross anatomy

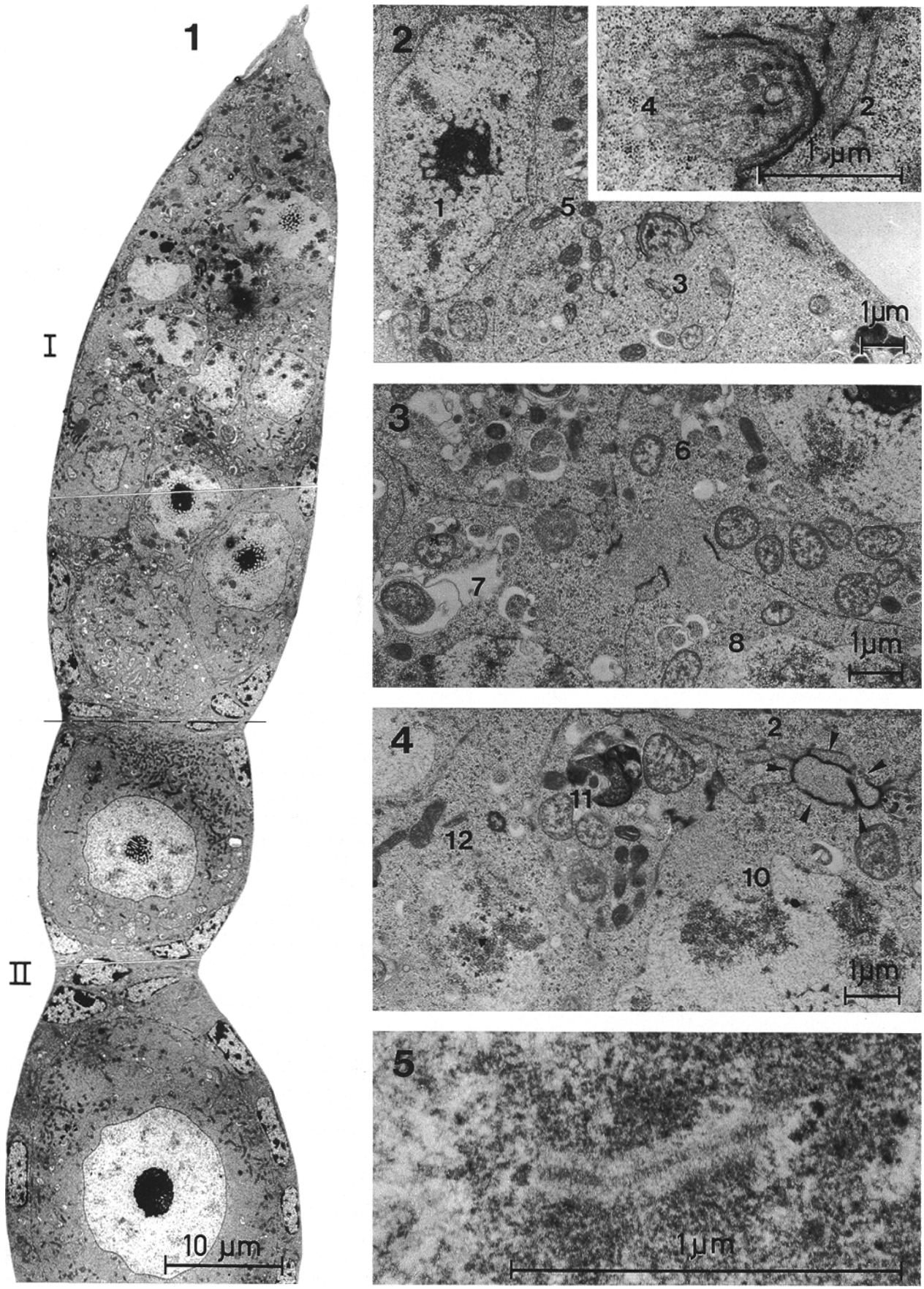
Each ovary of *P. dracenae* is composed of four ovarioles. The youngest germ cell descendants are found in the tip of each ovariole, zone I (Fig. 1). They are enclosed by a layer of flat somatic cells, the inner sheath. A tightly associated basal lamina surrounds each ovariole. A loosely connected outer envelope bearing tracheoles and muscles is also associated.

Zone II comprises previtellogenic growing oocytes, ordered in a straight line. Each oocyte is enclosed by a monolayer of follicular cells, derived from prefollicular cells which form a small tissue in the transient region between zone I and II. Somatic cells of the inner sheath, the prefollicular cells and early follicular cells, cannot be distinguished by ultrastructural criteria, but only by position. Mitoses were found in the basal area of zone I and in the follicular epithelium of zone II. All events of vitellogenesis and chorionogenesis occur in zone III (not shown).

2. Ultrastructure and cluster analysis

A complete longitudinal, ultrathin serial sectioning was prepared from a whole ovariole of a young female adult, which had already started vitellogenesis (not shown). We found 25 somatic cells in zone I, enclosing 22 germ cells, whereas 15 follicular cells surround the youngest oocyte of zone II. Thus, a high mitotic activity of somatic cells must occur at the base of zone I.

In the upper half of zone I we found intercellular bridges connecting germ cell descendants (Figs. 1–4, 6). In the tip of the ovariole only one germ cell was found without any connections to others (Figs. 2, 6, cell 1). It is followed by a 4-cell cluster showing one central bridge filled with fusomal material and two lateral bridges crossed by bundles of microtubules (Figs. 1, 2). The first bridge combines the two inner cells, while the lateral bridges connect the outer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cells to the two inner cells. The cluster forms a wide "u", lying at a 45° angle to the long axis of the ovariole (Fig. 6, cells 2–5). The following 7 cells and a small cytoplasmic compartment without a nucleus, form a second cluster. Cell 6 shows four bridges. Three of these bridges combine three terminal cells (7–9), but one bridge connects a cell (10) with two additional intercellular bridges. One of these connects the small cytoplasmic compartment (11), and the other combines a cell (12) with one additional intercellular bridge which ultimately connects the last terminal cell (13) (Fig. 6). Each bridge is filled with fusomal material, giving rise to two polyfusomes. All bridges combined by polyfusomes stick tightly together, forming two rosettes within one cluster (Figs. 3, 6). This cluster is followed by 9 isolated oocytes, whose size increases towards the bottom of zone I. Some isolated oocytes show cytoplasmic protrusions in which mitochondria, bacteria and lysosomal vacuoles concentrate. Two complexes are found, consisting of intercellular bridges, fusomes and cell membranes. No clear opening to adjacent cells exists. The first complex is situated between the 4-cell cluster and the 8-cell cluster (Figs. 4, 6). The second is found between isolated oocytes, near the bottom of zone I. All tissues are infected with bacteria.

The isolated germ cell on top is in interphase, as deduced from the somewhat dispersed chromatin and a large nucleolus. All other germ cell nuclei show condensed chromatin, as expected during prophase or telophase of mitosis and prophase of meiosis. The 4-cell cluster is in late telophase, showing condensed chromosomal chromatin, no nucleoli and remnants of the spindle apparatus in the new intercellular bridges. Furthermore, these cells are the smallest germ cells found in this ovariole. The 8-cell cluster is in prophase of meiosis. Chromosomes are paired and synaptonemal complexes are present (Fig. 5). These cells, as with all following detached oocytes, have one or sometimes two attached nucleoli, i.e. there is no morphological criterion for extrachromosomal rDNA replication in these oocytes. While the oocytes are detaching, the synaptonemal complexes are lost, but chromosomes are still visible during the whole period of previtellogenesis. A karyosome is not formed. Only small endobodies are found, while the nucleolus is increasing in size (Fig. 1).

Inspection of ovarioles derived from old adults reveals no clusters. All germ cells, even those at the very tip of the ovariole, are singular and in advanced previtellogenesis. Thus, stem cell differential mitosis and cluster formation have ceased during the adult phase and the ovariole starts with zone II (not shown).

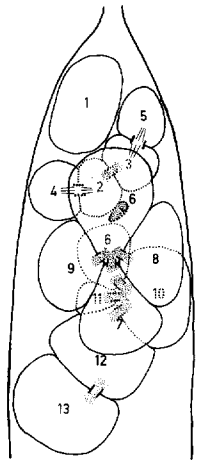

Fig. 6. Half-schematic drawing of the upper half of zone I. Cell 1 is presumed to be the stem cell. Cells 2 and 3 are the inner cells and cells 4 and 5 the outer cells of the 4-cell cluster. Cells 6–13 are the cells of the 8-cell cluster. Note the two rosette formations between cells 6–9 on the one hand, and cell 6 together with cells 10–12 on the other hand, each bearing a polyfusome. Cell 11 has no nucleus and its volume is about 6% of the average volume of the other cystocytes of the 8-cell cluster. One isolated bridge complex, enclosing fusomal material, is shown near cell 2. *Fine stippling* demonstrates the fusomal material. Bridge membranes are denoted by *thick lines*. *Broken lines* indicate cells beneath the cells indicated by *closed lines*. Cell 6 is shown in both optical levels

D. Discussion

This investigation demonstrates that stem cells stop their dividing program during the reproductive phase. Similar behaviour is known from aphids (Blackman 1978; Büning 1985), coccids (Hughes-Schrader 1925), bugs (Wightman 1973; King and Büning 1985), polyphage coleopterans (Büning 1972, 1979a, b; Kloc and Matuszewski 1977; Kozhanova and Pasichnik 1979) and megalopterans and raphidiopterans (Büning 1979c, 1980), all of which have telotrophic meroistic ovarioles. However, cessation of stem cell mitosis is also known from panoistic and polytrophic meroistic ovaries, e.g. in siphonapterans (Büning and Sohst 1988) and in boreids and sciarids (J. Büning, unpublished work).

Fig. 1. Germarial zone (I) and previtellogenic growth zone (II) of an ovariole of *Parthenothrips dracenae*. Zone I includes stem cells, cystocytes of germ cell clusters and isolated oocytes, which have already started their previtellogenic growth, still free of a closed follicular epithelium. Zone II is characterized by previtellogenic growing oocytes with a closed follicular epithelium separating the oocytes from each other. Zone III houses one oocyte, going through vitellogenesis and chorionogenesis (not shown)

Fig. 2. The stem cell (1) is in interphase and shows a large nucleolus. A young bridge develops by incomplete cytokinesis between cells 3 and 5 of the 4 cell cluster (see Fig. 6). The *inset* shows transversely oriented microtubules stretching through the second bridge between cells 2 and 4

Fig. 3. Two intercellular bridges, connecting cell 6 with cells 7 and 8 (see Fig. 6). The bridges are in close contact. Fusomal material extends throughout the bridges, forming a polyfusome

Fig. 4. Intercellular bridge, connecting cell 10 with "cell" 11. Note the isolated fusomal-bridge complex between cells 2 and 10, enclosed by *arrowheads*

Fig. 5. Synaptonemal complex of cell 10. All cell nuclei of the 8-cell cluster are in zygotene/pachytene stages

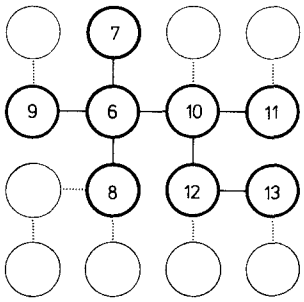

Fig. 7. One of several ways to construct the 8-cell cluster; by secondary detachment processes in a hypothetical 16-cell cluster which is built according to the 2^n-rule

Thus, a restricted number of stem cell mitoses in insect female gonads may be the more basic condition compared with the unlimited mitotic program of stem cells as found in *Drosophila* (Brown and King 1964; King 1970).

Cluster formation follows the 2^n-rule, at least initially. The 8-cell cluster shows deviations from this rule, which can be explained by asynchronous repression of mitotic activity during cystocyte mitoses (cf. cells 6 and 10 in Fig. 6). The deviation may also result from a 16-cell cluster from which 9 cells have already detached (Fig. 7). The cytoplasmic compartment "cell 11" may be a relic of a recent detachment, as indicated by its small volume of about 6% of the average volume of the other 7 cells of the cluster. Following this interpretation, the two isolated aggregates of intercellular bridges and polyfusomes are remnants of preceding detachment processes of former clusters or subclusters.

Oocyte-nurse cell determination is still an enigma and open to speculation (Bier et al. 1967; Büning 1979c; Huebner 1984; King 1970; King et al. 1982; King and Büning 1985; Kloc and Matuszewski 1977; Telfer 1975). The existence of polyfusomes and rosettes in this panoistic species demonstrates that these morphological criteria are not sufficient to explain this determination. This investigation supplements findings in aphids, where we could not find polyfusomes. However, rosettes do occur, and about 50% of germ cells of the cluster develop into oocytes, while the others develop into nurse cells (Büning 1985). A total decoupling seems to exist in *Hystrichopsylla* (Siphonaptera), in which the 2^n-rule is normal ($2^n - 1$ nurse cells and 1 oocyte), but polyfusomes and rosettes are not detectable (Büning and Sohst 1988).

Thysanopterans and Hemipterans are sister groups (Hennig 1969; Heming 1980; Kristensen 1981). The cluster analysis in both lines does not allow precise information about the ancestral ovary to be obtained. However, the change in the oocyte-nurse cell determination process to more than one oocyte per cluster, may be an apomorphic character in that hypothetical ovary. Concomitantly, this change to more than one oocyte per cluster was accompanied by a reduction of stem cells to one and stem cell mitoses to zero. This necessarily leads to a telotrophic ovary as found in Hemipterans (King and Büning 1985, their Fig. 11), or the change in oocyte determination is going on to 2^n oocytes and zero nurse cells, accompanied by a secondary detachment of oocytes, as found here in thysanopterans. Furthermore, there is good evidence for the secondary character of the panoistic ovary of Thysanoptera, since the sister group of Condylognathida (Heteroptera/Thysanoptera) are the Psocodida (Psocoptera/Phtiraptera), which have polytrophic meroistic ovaries of the basic type (Ries 1932; Büning and Sohst 1988; J. Büning, unpublished work). The complex of characters of this basic type is shared by the proposed sister groups of Holometabola and Paraneoptera, which include the above orders. Thus, the polytrophic meroistic ovary of the basic type may be an apomorphic character of paraneopterans and holometabolans. Outside these taxa, a polytrophic meroistic ovary exists in the Dermaptera, but cluster development is completely different from the basic type (Yamauchi and Yoshitake 1983). As far as we know at present, all other insect (Ectognatha) orders have primary panoistic ovaries without cluster development.

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