Organization of the oocyte nucleus in Silphid beetles (Silphidae, Coleoptera-Polyphaga)[1]

B. Matuszewski, P. Hoser, G. Hoser and M. Michalak

Department of Cytology, Warsaw University, Krakowskie Przedmieście 26/28, P–00-927 Warszawa (Poland), 1 November 1976

Summary. In all the Silphid species examined, the oocyte chromosomes were found to contract to form the karyospheres. Despite this, as the oocytes grow the germinal vesicles increase also very considerably. Production of numerous RNA-containing nucleolus-like bodies was found to be characteristic of the Silphid oogenesis. Experiments with ^{3}H-uridine demonstrated that only the karyosphere actively synthesized RNA, while the nucleolus-like bodies remained inactive in this process.

The connection between the degree of RNA synthesis taking place in the oocyte nucleus and the size of the nucleus is not identical in all groups of insects. Thus, in cases of limited RNA synthesis in the oocyte nuclei, such nuclei are most often of small dimensions. On the other hand, the increase in volume of nuclei observed in certain insects with polytrophic ovaries does not always indicate the activity of these nuclei in RNA synthesis. For instance, in Carabidae the diameter of germinal vesicles may be as much as 350 µm, but the level of RNA synthesis is very low in them[2,3]. It is also not entirely clear whether in the case of Dytiscid water beetles oocyte nuclei of Cybister not active in RNA synthesis are significantly smaller than the oocyte nuclei of Dytiscus active in this process[4]. Finally in those insects with meroistic ovaries,in which intensive extrachromosomal RNA synthesis takes place in the oöcyte nuclei, such nuclei invariably attain exceptionally large dimensions[2,4–11].

The present contribution is concerned with certain interesting features of the organization of oocyte nuclei in Silphidae. The oocyte nuclei in these insects were observed[12] to attain extremely large dimensions and an unusually polymorphic form, and to contain structures which could be interpreted as multiple nucleoli.

Material and methods. Studies were made of the ovaries of adult females of the following species: Silpha sinuata, S. thoracica, S. atrata, Necrophorus humator, N. vespillo and Necrodes littoralis. The ovaries were fixed in Bouin-Allen's fluid or Carnoy's fluid. The paraffin sections (4–7 µm thick) were stained in Heidenhain's iron hematoxylin or in Azure B (pH 4.0), and methyl green pyronin (pH 4.7) with RNase treatment as a control. A few females of S. thoracica and S. sinuata were injected with ^{3}H-uridine (spec. act. 2.5 Ci/mM, 15–20 µCi/individual) and the ovaries removed and fixed after interval of 1, 1.5 or 6 h. Stripping film (Kodak AR 10) was applied and the preparations exposed at 4 °C for 4 weeks.

Results and discussion. In all the species examined, the oocyte nucleus greatly increases in volume during the previtellogenesis, and simultaneously becomes markedly elongated (figure 1). For instance, in advanced stages of previtellogenesis the oocyte nuclei of Silpha and Necrodes attain the length of approximately 500 µm and 580 µm respectively. In Necrophorus the late previtellogenic oocytes are about 1 mm long, and their nuclei attain the length of up to 950 µm.

Within the species studied, it is possible to observe a graduated sequence of changes to which chromosomes

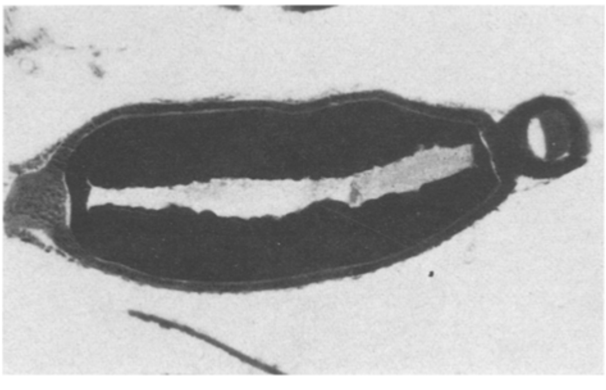

Fig. 1. Necrodes littoralis. Longitudinal section through oocyte in late previtellogenesis showing enormously enlarged germinal vesicle of highly irregular shape. Heidenhain's hematoxylin. ×90.

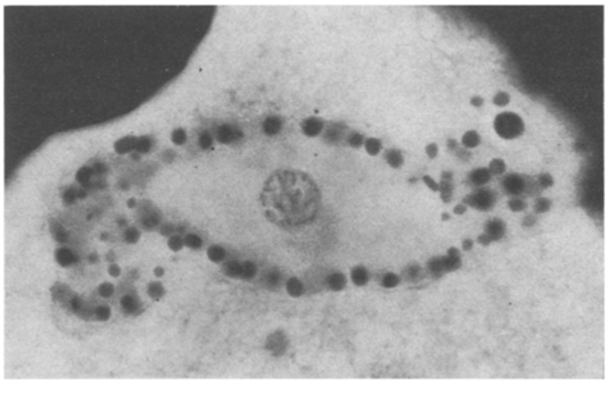

Fig. 2. Silpha sinuata. Section through a karyosphere capsule. Karyosphere of a very loose structure, in which discrete chromosome threads can be distinguished, is seen to be encapsulated by the homogenous material of its capsule. The surface of the capsule is studded with many nucleolus-like bodies of varying size. Methyl green pyronin. ×925.

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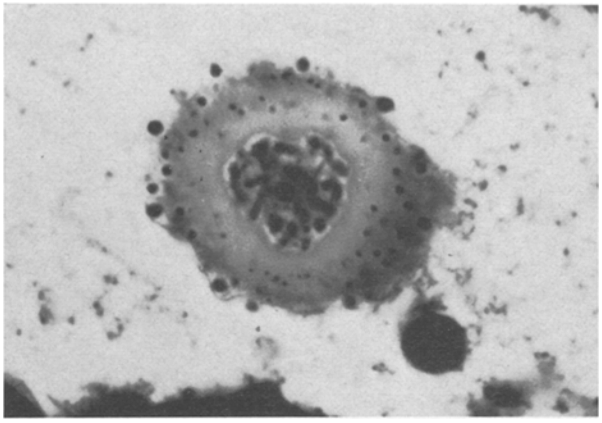

Fig. 3. Silpha thoracica. Karyosphere formed by loosely interwoven, relatively thick chromosome threads, surrounded by a faintly stained homogenous capsule. Nucleolus-like bodies in the inner part of the capsule are distinctly smaller than those in its outer part or lying outside the capsule. Heidenhain's hematoxylin. ×1200.

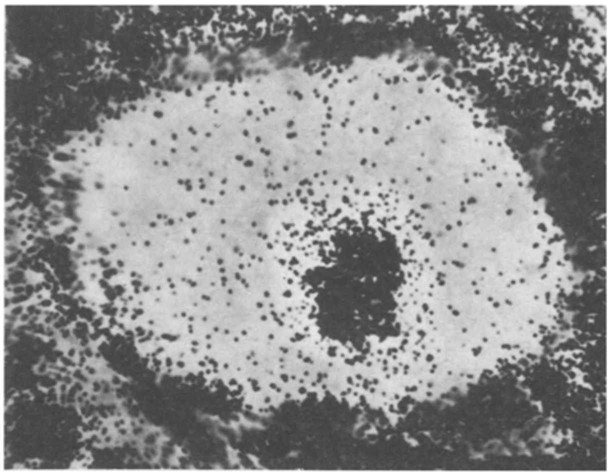

Fig. 5. Silpha sinuata. Autoradiograph of an early previtellogeni oocyte. The heavy label is localized over the karyosphere. Incubation with ^{3}H-uridine for 1 h. Methyl green pyronin. ×1000.

are subject during growth of the oocyte. Consequently it is only in Necrophorus that chromosomes, forming a compact heterochromatic karyosphere (figure 4), behave in a way typical of the oocytes of the majority of insects with meroistic ovaries. In Necrodes, S. atrata and S. sinuata, the chromosomes remain in a state of decondensation throughout the whole of previtellogenesis and the karyosphere they form is similar to an interphase nucleus (figure 2). An intermediate situation is found in S. thoracica; chromosomes are subject to considerable condensation, but the karyosphere they form is not compact and individual chromosomes can easily be distinguished in it (figure 3).

In all the species examined, the karyosphere lies within the differentiated region of karyoplasm, which may be termed the karyosphere capsule. This capsule may be homogenous and scarcely stain at all with basic stains (figures 2 and 3) or, as in the case of Necrophorus, be distinctly vacuolized and highly basophilic.

A constant component of the karyosphere capsule of Silphidae oocytes consists of structures which, on account of their shape and highly basophilic nature, can for the time being be termed nucleolus-like bodies. These bodies, as can be seen during the initial stages of oocyte growth, appear in direct contact with the karyosphere. Pictures found of this in older oocytes (figures 3 and 4) suggest that after their formation these bodies move from the interior of the capsule towards its periphery, and as they

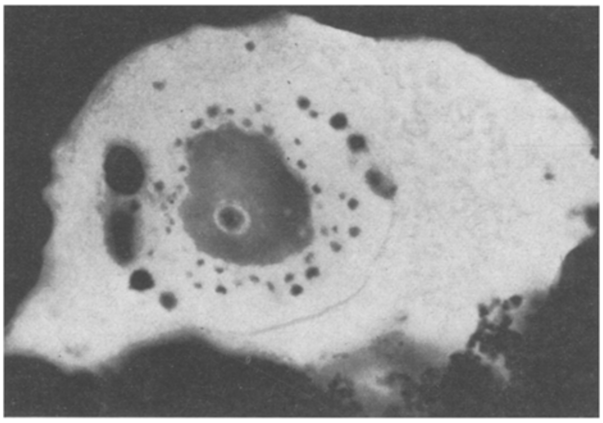

Fig. 4. Necrophorus humator. The nucleus of an early previtellogenic oocyte; a compact and deeply stained karyosphere surrounded by a capsule. The karyosphere capsule is seen to be differentiated into a distinctly basophilic inner part and an outer part which fails to stain with basic dyes and contains many nucleolus-like bodies. (This differentiation of the capsule disappears in the later stages of previtellogenesis.) Note that the nucleolus-like bodies adjacent to the inner part of the karyosphere capsule are distinctly smaller than those which lie along the border between the nuclear sap and the outer part of the capsule. Azure B. ×1200.

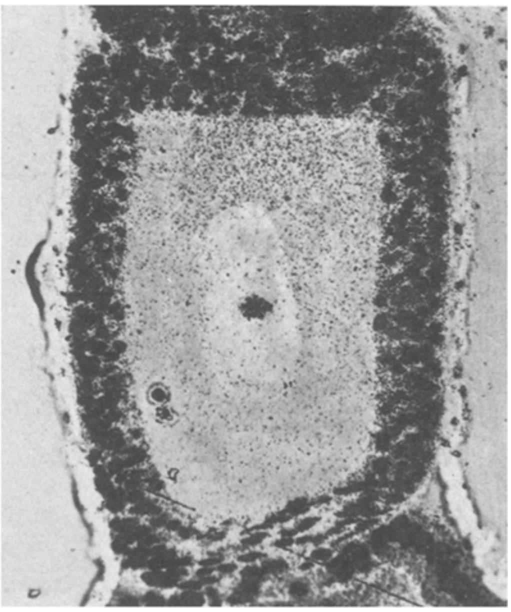

Fig. 6. Silpha thoracica. Autoradiograph of an egg follicle in late previtellogenesis. Note the labelled karyosphere in the centrally placed, elongated and largely unlabelled germinal vesicle, and the flow of labelled RNA into the oocyte at its anterior end, where the trophic cord enters the oocyte. Incubation with ^{3}H-uridine for 1.5 h. Methyl green pyronin. ×190.

do so their size gradually increases. In greatly enlarged nuclei, the position of nucleolus-like bodies is most often limited to the exterior layer or to the surface of the karyosphere capsule.

Nucleolus-like bodies also occur outside the karyosphere capsule, lying singly in the nuclear sap. They are larger than the bodies connected with the capsule, but like the latter exhibit a highly basophilic character (figure 3). Their relation to the nucleolus-like bodies of the capsule is not clear, but it would seem probable that they are formed from the latter after they have reached a certain size and become detached from the capsule. The basophily of the nucleolus-like bodies connected with the karyosphere capsule or lying singly in the nuclear sap as well as the capsule itself disappears after treatment with RNase.

The loose structure of the karyosphere in the majority of the species examined, the occurrence of multiple nucleolus-like bodies containing RNA and the considerable increase in volume of the germinal vesicles suggested that the oocyte nuclei of Silphidae might be very active in RNA synthesis. However, the results of initial autoradiographic studies with ^{3}H-uridine carried out on the ovaries of S. thoracica and S. sinuata failed to provide support for this suggestion. The distribution of labelling above the oocyte nuclei shows that the nucleolus-like bodies are inactive in RNA synthesis. On the other hand, with the same incubation time, the karyosphere invariably exhibited outstanding uridine incorporation, in both the early (figure 5) and more advanced stages of oocyte growth (figure 6). There can be no doubt that intensive RNA synthesis by oocyte chromosomes of both species of Silpha examined for this, as in the case of oocytes of Bruchidius[13] and perhaps Tenebrio[14], is conditioned by the exceptionally low degree of chromosome condensation. Despite the lack of comparable quantitative data, it would appear that the growth of nuclei in Silphidae oocytes is disproportionately great in relation to the extent of their activity in RNA synthesis. This growth thus most probably results not only from the action of the mechanism involved in activation of RNA synthesis in oocyte chromosomes, but also, and to a major degree, from the action of some other factor which, as in the case of the oocytes of Carabidae, is not connected with this function of the nucleus.

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Influence of an additional magnetic field on hornet nest architecture

M. Kisliuk and J. Ishay

Department of Electronics, School of Engineering, Tel-Aviv University, Tel-Aviv (Israel), and Department of Physiology and Pharmacology, Sackler School of Medicine, Tel-Aviv University, Ramat-Aviv (Israel), 13 December 1976

Summary. An additional horizontal magnetic field is lethal for adult hornets and larvae. The juvenile hornets, however, are capable of adapting to the additional magnetic field. They build combs commencing in the regions of high field intensity, and proceeding in the direction of the field intensity decrease.

It was reported by Lindauer[1] that changes in the earth's magnetic field disturb the orientation of dancing bees, and that they commence building combs whose orientation is in accordance with the changed field. Upon 10fold increase in the magnetic field, the bees built cylindrical combs that were fastened to the floor rather than to the roof as is customary[2]. There is also some evidence that ants can sense the earth's magnetic field[3], and that normal fluctuations in the earth's magnetic field influence pigeon orientation[4]. Experiments by Schneider[5] showed that orientation of the beetle Melolontha vulgaris F. is strongly affected by magnetic and electric changes. The aim of the present study was to test the effect of an additional magnetic field on comb construction by hornets.

Material and methods. Young hornets (Vespa orientalis) workers kept in artificial breeding boxes (ABBs) in groups of 5–15 individuals build combs whose dimensions are as described elsewhere[6] and which are connected by pedicles to the roof of the ABB; the cells in such combs face downwards[7,8] with a scatter of 3–14° from the vertical axis, as in normally built combs in nature[9]. Hornets before eclosion or 1–2 days afterwards (juveniles), when subjected to centrifugation, build in the direction of the resultant of the gravitational and centrifugal forces[10], whereas adult hornets (3–7 days old) tend to build in the direction of gravitation alone. These results suggested that the orientation of adult hornets during comb construction is influenced by additional physical factors apart from gravitation. Moreover, in previous experiments[11] it was found that when presented with an inverted comb, the hornets build new cells on its border whose direction is downwards. We were therefore interested to find out whether the hornets will behave in the same way when exposed to the influence of an additional magnetic field.

To test the influence of uniform and nonuniform magnetic fields on comb construction by hornets, we designed a square cross-section coil of 28×28 cm^2, with a winding length of 100 cm. The coil consisted of 1500 turns which were wound uniformly on a plywood frame and further encased in plywood. At a 40 V input voltage, the maxi-

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