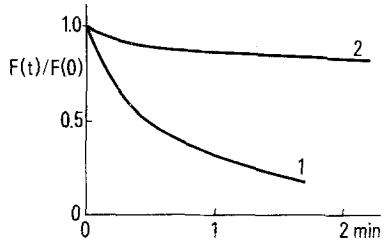
crine, the addition of dithionite did not change the observed intensity. Fading is accelerated by increasing excitation intensity Io, and as this is not specified, the figure and its like[2-6] are qualitative (Io-independent dimensionless parameters can be derived from the curves[8]). In the table, the fade retarding effect of $Na_2S_2O_4$ is reported for several fluorochromes.

The results are most encouraging for fluorescein, the mainstay of immunofluorescence, and for several cationic dyes of central importance to cytogenetics. With fluorescein and acridine orange, the intensity of fluorescence actually *increased* in time before assuming a slow downward trend ~ 5 min later. Quinacrine hydrochloride lost much of its fluorescence with dithionite, while the fading rate remained the same. This agrees with known properties of this dye[9].



Fluorescence intensity emerging from a nucleus of onion cell stained in 10^{-5} M acriflavine, placed in fluorescence microscope and excited at 405 nm with 200-W Hg-lamp by incident illumination through × 100 objective. 1: mounted in 0.01 M phosphate buffer, pH 5.5, 2: same buffer, containing 0.02 M $Na_2S_2O_4$. Absolute initial intensities were nearly equal for the 2 samples.

Rhodamine 3GO was covalently bound to DNA by the SO_2-Schiff reaction[10], also meant to imitate rhodamine-labeled antibodies. ANS, the probe of lipid-water boundaries[11] was not protected from fading. Quercetin resembles morin[12] in being a phenolic natural product which binds to a trivalent metal cation and the complex is a nuclear stain. It did not endure Io at × 100 magnification, but with Io reduced to 0.16 initial value, dithionite was definitely effective as fade retardant. Dithionite could not be substituted by sodium thiosulfate, sodium azide or propyl gallate.

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Contribution of germ-line cells to formation of the nurse chamber in egg follicles of nonpaedogenetic gall midges (Diptera, Cecidomyiidae)[1]

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Summary. One of the nuclei present in the syncytial nurse chamber in nonpaedogenetic gall midges contains lamellae characteristic only of the germ-line cells. This finding indicates that the nurse chamber in bisexually reproducing gall midges, as in the case of paedogenetic species, derives from both germ-line and somatic cells.

The egg follicle in a polytrophic insect ovary consists of an oocyte and nurse cells surrounded by a simple follicular epithelium. In typical cases, the oocyte and all nurse cells associated with it, are germinal in origin and they are joined together by a system of intercellular bridges. The oocyte-nurse cell complex is produced by a number of consecutive and synchronous divisions of an oogonial cell, frequently referred to as a cystoblast, and the cytoplasmic interconnections between all members of the complex are interpreted to arise as a result of incomplete cytokinesis[2]. Since the number of synchronous divisions which a cystoblast undergoes is species-specific, the number of nurse cells associated with the oocyte is also constant and characteristic of each species.

Although the ovaries of gall midges are polytrophic in structure, the process of formation of their egg follicles seems, however, to be essentially different from that in other polytrophic ovarioles. The cecidomyiid egg follicle contains a syncytial, multinuclear nurse chamber instead of a group of nurse cells, and the number of nuclei in nurse chambers varies considerably within each species. It has been suggested that nurse nuclei in egg follicles of Cecidomyiidae are of somatic origin and result from fusion of a variable number of mesodermal cells lying adjacent to the oocyte[3-6]. However, in paedogenetic gall midges, such as Heteropeza and Miastor, one of the nurse nuclei differs distinctly in size and morphology from the remaining nuclei found in the same nurse chamber, and it has been suggested that this nucleus is germinal in origin[7-9]. Decisive evidence in support of the view that the nurse chamber in paedogenetic gall midges derives from both germ-line and mesodermal cells has recently been obtained in developmental investigation[10], and also in ultrastructural studies[11].

In nonpaedogenetic gall midges belonging to the subfamily Cecidomyiinae, there has as yet been no observation indicating that the nurse chambers in their egg follicles contain descendants of germ cells. It has been assumed, therefore, that the nurse chamber in these gall midges is exclusively of somatic origin[6], although according to other authors it is composed exclusively of the germ-line derivatives[12]. The object of the present ultrastructural study was to clarify the origin of the nurse chambers in bisexually reproducing species of gall midges.

Material and methods. Investigations were carried out on the larval and early pupal ovaries of the following gall midges: *Boucheella artemisiae, Mayetiola poae, Mikiola fagi*

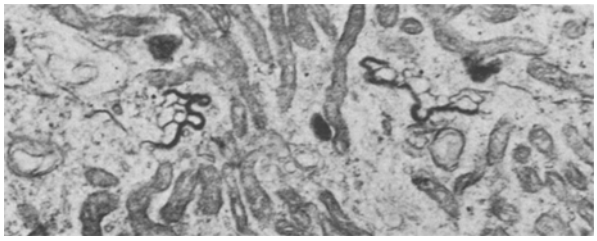

Fig. 1. *Rhabdophaga rosaria.* An intercellular bridge connecting the oocyte with the nurse chamber. An electron dense material on the inner surface of the bridge is visible. × 12,000.

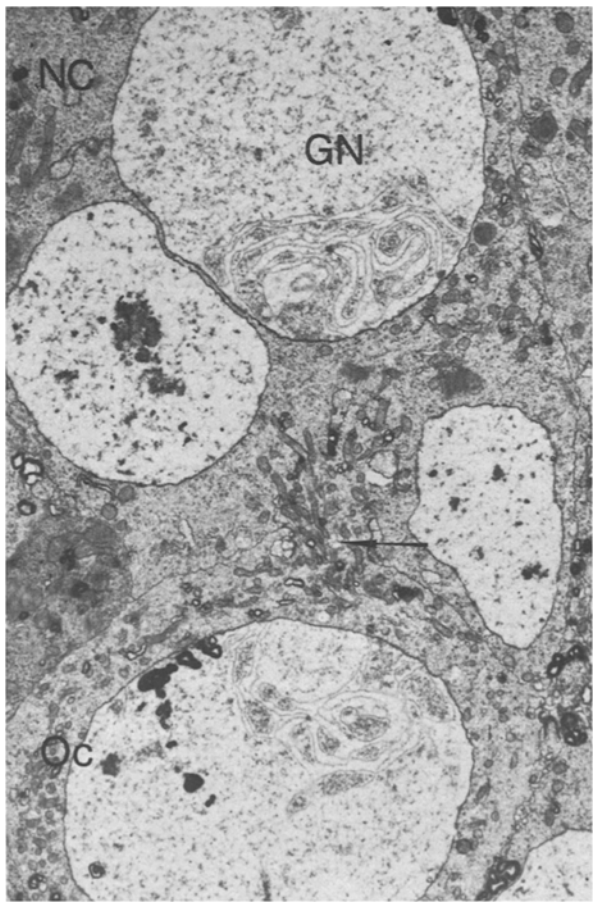

Fig. 2. An oocyte (Oc)-nurse chamber (NC) complex from a prepupal ovary of *Mayetiola poae.* An intercellular bridge (arrow) connects components of the complex. The nurse chamber contains 1 generative nucleus (GN) with prominent lamellar structures and mesodermal nuclei. × 7300.

and *Rhabdophaga rosaria.* The ovaries were fixed in cold 5% glutaraldehyde buffered at pH 7.4 with 0.1 M phosphate for 60 min, washed in 0.1 M phosphate buffer, postfixed with 2% osmium tetroxide and embedded in a mixture of Epon and Araldite. Thin sections were stained with uranyl and lead salts[13].

Results and discussion. The larval ovaries are spherical bodies, the diameter of which does not exceed 0.2 mm. They consist of 2 parts, i.e. a somatic and a generative part, which differ considerably from each other with respect to their structure. The somatic part is composed of small, closely-packed mesodermal cells, while the generative part, making up only about ¼ of the ovary, comprises 2 types of cells morphologically different from cells of the somatic part. In the generative part there is a network of mesodermal, interstitial cells with oogonial cells interspersed between them. The oogonia are large cells with spherical nuclei about 10 µm in diameter. Apart from their relatively large size the oogonial nuclei may easily be distinguished from nuclei of somatic cells by the presence of lamellar structures surrounding the set of S-chromosomes[14] which are always heterochromatic in interphase oogonial nuclei[15-19].

The first oocyte-nurse chamber complexes were observed to appear at a late larval stage in *B. artemisiae* and *M. fagi,* and at a prepupal stage in *M. poae* and *R. rosaria.* Their formation apparently takes place very quickly, since the nurse chambers were found to be multinuclear and syncytial in structure, even in the youngest complexes so far investigated. As in the case of paedogenetic gall midges[11], only 1 intercellular bridge was found between the nurse chamber and the oocyte in all the species examined in this study. The diameter of the bridge is 1.5–2.0 µm and its appearance (figure 1) is similar to that of the bridge described in egg follicles of a nonpaedogenetic gall midge *Wachtliella persicariae*[20]. The presence of only 1 bridge indicates that oogonial cells of the last generation (i.e. cystoblasts) undergo only 1 division with an incomplete cytokinesis, and therefore that the nurse chamber contains at least 1 germ-line nucleus. In fact, in every nurse chamber there is only 1 nucleus which differs distinctly from others. Shortly after an oocyte-nurse chamber complex has been formed this nucleus is found to be about 10 µm in diameter and to contain nuclear lamellae which, in gall midges are characteristic exclusively of the germ-line nuclei[14,20,21]. With respect to its size and structure it is identical, in young nurse chambers, with the oocyte nucleus (figure 2), and there is no doubt that it is a germ-line nucleus which is a sister to the oocyte nucleus. At the same time, the absence of lamellar structures in any one of the remaining nuclei in a given nurse chamber also makes it clear that all of these nuclei are somatic in origin.

On the grounds of the data presented, it may therefore be concluded that in bisexually reproducing gall midges, as in the case of paedogenetic species of these insects, the nurse chamber is of a dual origin. It contains a variable number of somatic nuclei and, as a rule, only 1 nucleus of germ-line origin.

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