Notes on the Male Germ Cells of a Beetle, Leptinotarsa decemlineata [1]

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ABSTRACT

The morphology of the male germ cells in *Leptinotarsa decemlineata* (Say) as studied by correlative microscopy (bright field, phase contrast, and electron microscopy) has been presented. Since relatively little information pertaining to spermatid maturation in this species is available, these aspects of spermatogenesis have been emphasized. Techniques for locating germ cells within the adult beetle and general techniques of tissue preparation have been included.

INTRODUCTION

Studies of the male germ cells of the Colorado potato beetle *Leptinotarsa decemlineata* (Say) were made by use of the light microscope (both bright field and phase contrast) and by use of the electron microscope. Descriptions of the male reproductive tract and the female spermathecae are included. This article attempts to demonstrate the usefulness of correlative instrumentation in our studies of insect spermatozoa and to present some of the techniques utilized in our investigations. It is assumed that the reader is familiar with the terminology involved in insect spermatogenesis (cf. Breland *et al.*, ENTOMOLOGICAL NEWS, October 1968) and the paper is directed toward the entomologist for whom we feel a basic understanding of spermatogenesis could be helpful as a research tool in cytological and taxonomic studies.

Material and Methods: Adult Colorado potato beetles were collected at the University of Texas Brackenridge Field Laboratory in Austin, Texas; and were usually found on nightshade plants. The testes and spermathecae were dissected and studied with light microscopy and with electron microscopy.

Light Microscopy: BRIGHT FIELD—Dissection were accomplished by sagittally cutting the abdominal sterna; the prominent orange colored testes were removed and placed in Bauer's Fixative (1931) for twelve hours. The tissue was dehydrated in successive ethanol concentrations, transferred to an equal mixture of absolute ethanol and xylene for fifteen minutes and then placed in 100% xylene for fifteen minutes. Small pieces

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of paraffin were then added to this solvent for a period of two hours; after which the tissue was placed in plastic tissue molds and embedded in 100% paraffin. Sections were cut at approximately ten microns, mounted on egg albumen smeared slides, and stained with safranin-fast green following the technique of Jensen (1962). Cover slips were then mounted on the sections with Canadian balsam. The slides were then examined on a Ziess microscope utilizing the bright field objective.

PHASE CONTRAST—For phase contrast microscopy a small portion of the testes was removed and placed on a slide with a small amount of Belar's insect saline (Breland, 1961). After gently teasing the tissue with insect pins, a cover slip was added and moderately squashed to facilitate spreading, and sealed with Kronig cement. A Zeiss phase contrast microscope was used to examine the various stages of sperm development and photographs were taken using Kodak contrast process ortho 4 × 5 film with a Leitz camera.

Electron Microscopy: For electron microscopy the male Colorado potato beetles were placed on dry ice, which temporarily immobilized them so that the testes could be excised. Before the testes were actually removed, the body cavity was filled with glutaraldehyde to reduce the amount of tissue damage. After excision the tissue was cut into approximately $\frac{1}{2}$ mm cubes, placed in $2\frac{1}{2}$% glutaraldehyde in Sorensen's buffer for one hour and then placed into 1% osmium tetroxide in Sorensen's buffer for one hour. After staining in 0.5% uranyl acetate overnight, the tissue was dehydrated rapidly in successive concentrations of ethanol placed in 100% acetone for an hour and then embedded in a plastic mixture of 70% dodecyl succinic anhydride (DDSA), 20% Araldite 6005 and 10% Epon 812 with one drop from a capillary pipette of accelerator DMP-30 added per milliliter of plastic.

A Sorvall (Porter Blum MT-1) microtome with either a glass or diamond knife was used to cut sections 500–800 A thick, which were spread with toluene vapors. The sections were placed on grids and were then post-stained with lead citrate (Reynolds 1963) for five minutes and examined on a Siemens Elmiskop I electron microscope.

Observations and Discussion

Historical: The male germ cells in *Leptinotarsa decemlineata* were originally investigated by Stevens (1906) and by Wieman (1910). These early studies emphasized meiosis and were not concerned with spermiogenesis (the maturation of the spermatid into the mature spermatozoon). To the best of our knowledge no investigator has published any aspect of spermiogenesis in this common beetle which has prominent reproductive organs, is easily identified, and is widely distributed.

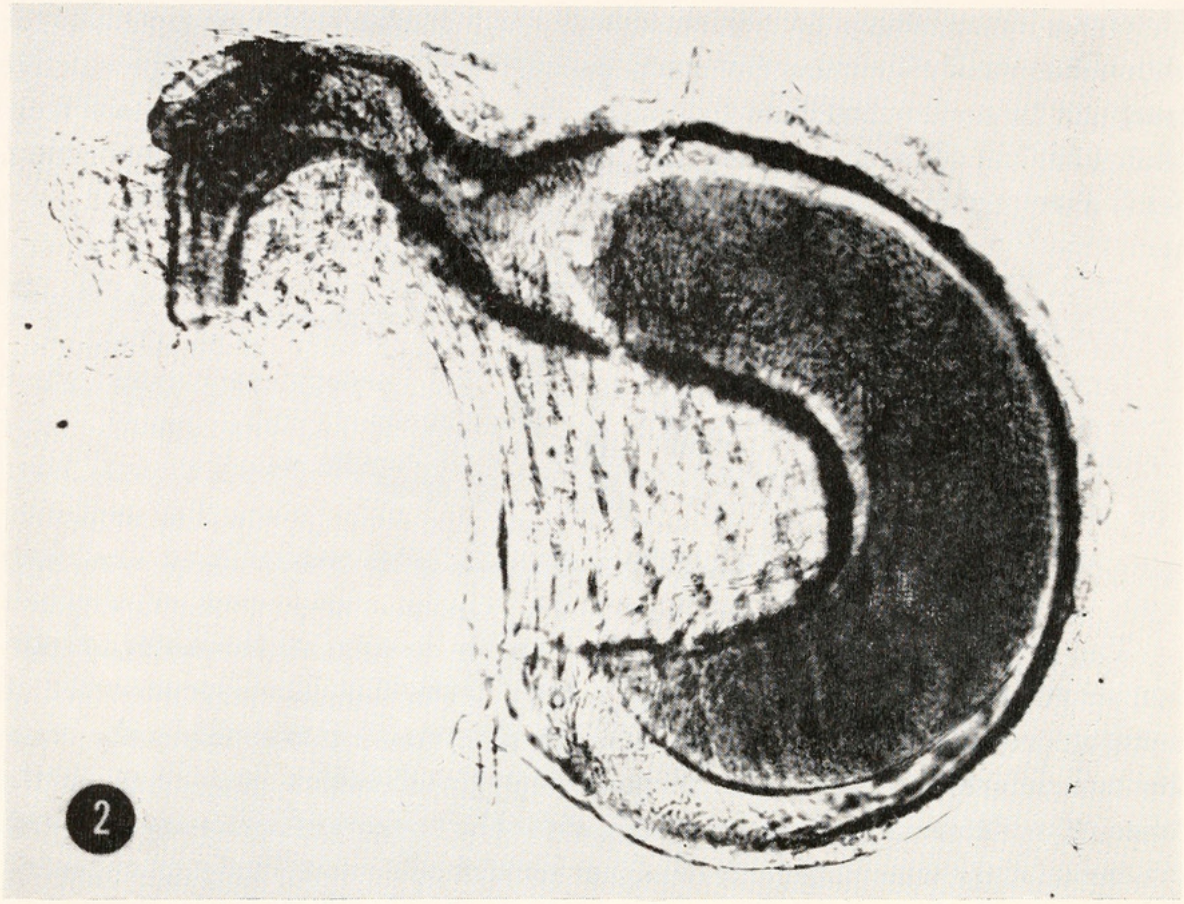
Gross morphology of the male reproductive tract: Upon opening the abdominal cavity in male Colorado potato beetles, the entire reproductive tract can be seen. Attempts to remove the entire reproductive system were successful but efforts to photograph these dissections proved disappointing. Such a system is perhaps best explained by line drawing as in Fig. 1.

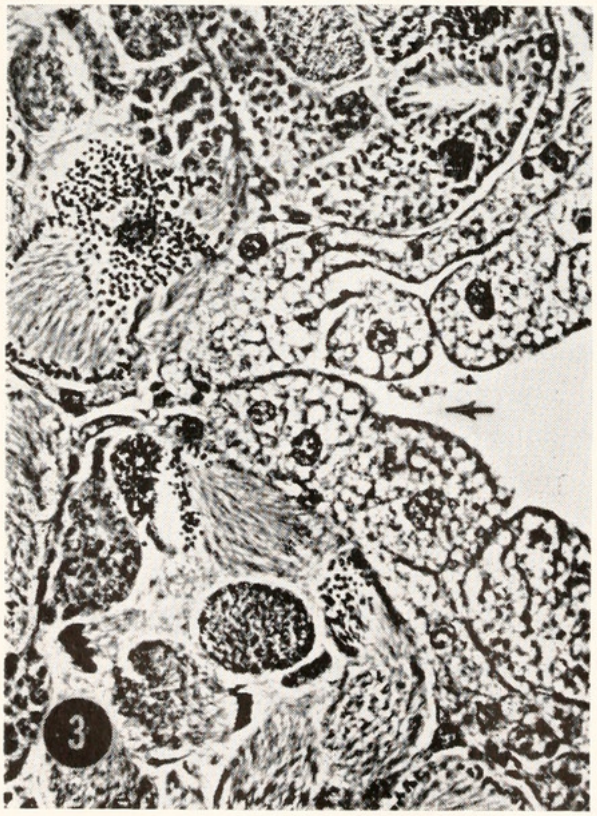
FIGURE 1. Diagram of male reproductive system.

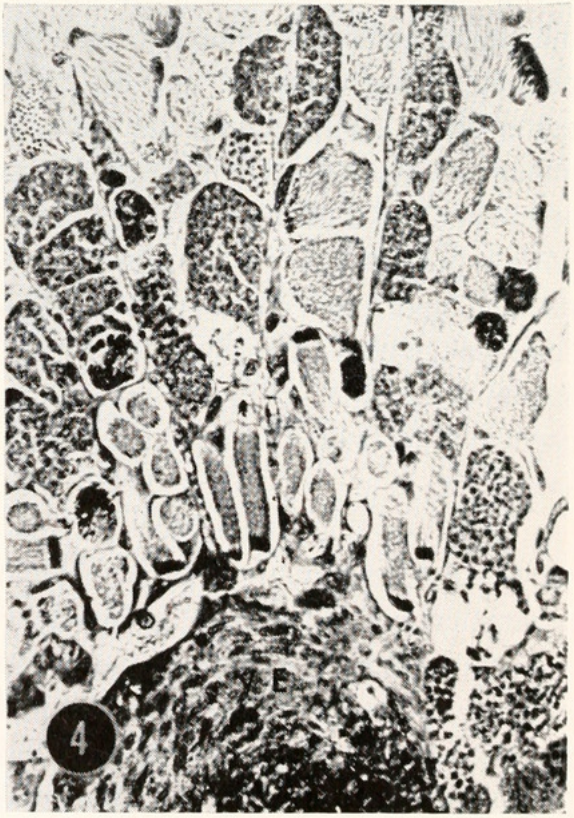
The prominent, individually bilobed bodies located in the anterior portion of the abdomen are the testes. Each testicular lobe is connected to a minute vas efferens which joins the vas deferens. Proximal to the testis the vas deferens unites with an accessory gland before joining with the other vas deferens which connect with the common ejaculatory duct. Posteriorly, the ejaculatory duct passes into the sclerotized aedeagus (penis).

Gross morphology of the female spermatheca: The spermatheca is a diverticulum of the female reproductive tract connected to the posterior portion of the common oviduct and it is the storage organ for the spermatozoa which are received during copulation with the male. In *Leptinotarsa decemlineata* the spermatheca is approximately 0.5 mm wide and heavily sclerotized. The spermatheca is a good source of mature spermatozoa which cannot always be found in testicular tissue. Attempts to observe mature spermatozoa in embedded spermathecae with the electron microscope were only partially successful due to the small size and heavy sclerotization of the storage organ. However, dissections for phase contrast microscopy were successful and frequently used when male specimens were not available.

Light microscopy of the testes: The testes are located in the anterior portion of the abdomen and are easily identified by their bright orange pigmentation. Each testis has two groups of follicles surrounded by ensheathing epithelial cells which cause them to appear non-divided and kidney shaped (Fig. 3). The follicle groups are made up of many individual follicles or sperm tubes (Snodgrass, 1935) which radiate from a central "hub" termed the cap region (Wieman, 1910). Sperm cells develop in sperm cysts within each follicle and mature in successive stages from the periphery to the cap region which is directly connected to the vas efferens (Fig. 3).







FIGURES 2–4. FIG. 2. Excised spermatheca from female, × 160; FIG. 3. Paraffin section of a testis. Note bilobed follicle groups (arrow) surrounded by ensheathing epithelial cells (EC), × 256; FIG. 4. Paraffin section revealing vas efferens (VE) and radial appearance of individual follicles, × 128.

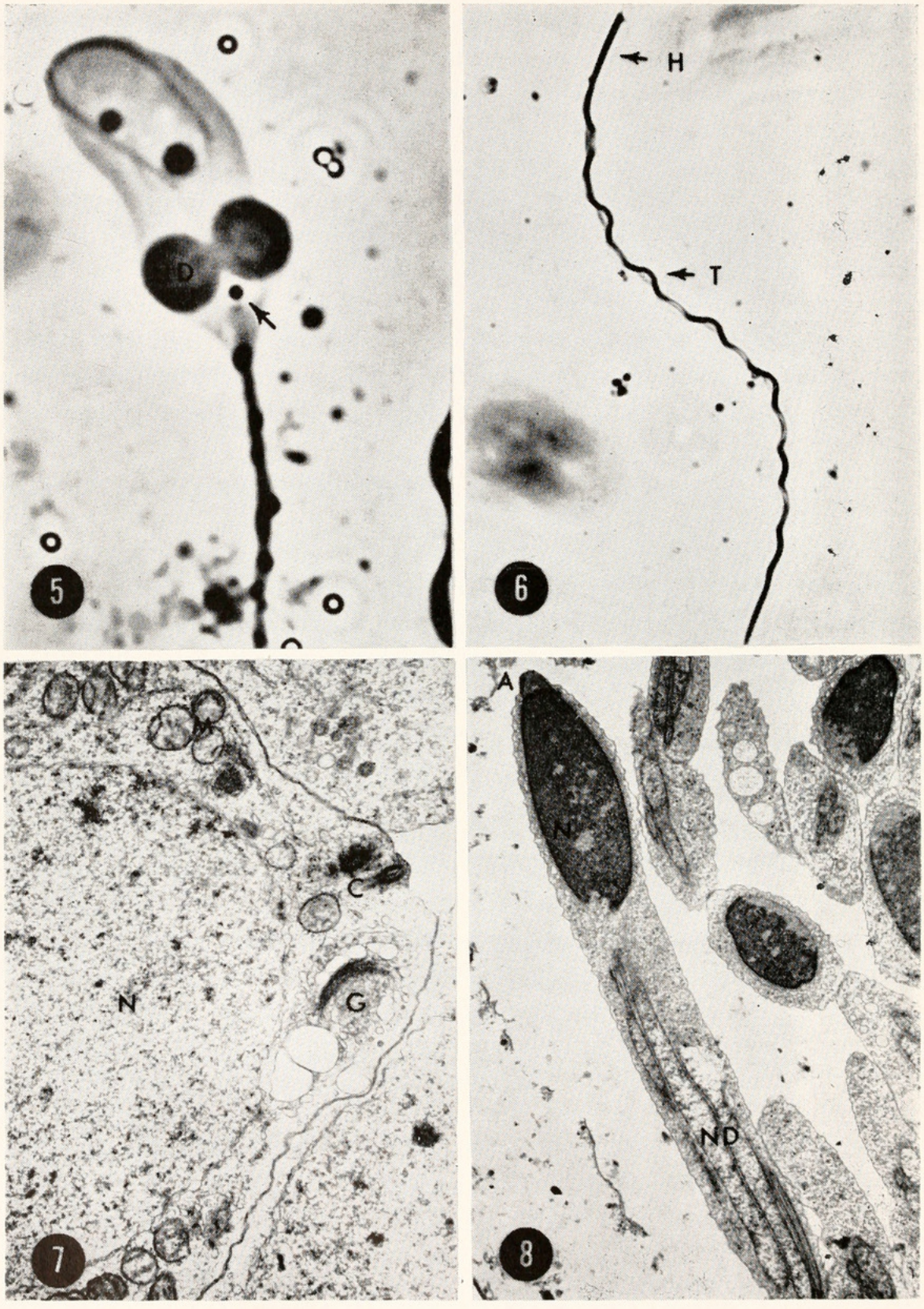
Within any given cyst the germ cells were all at approximately the same stage of development and oriented in a specific polarity with respect to their anterior-posterior axis (i.e., nuclei were all together).

Phase contrast microscopy: The morphological changes during spermiogenesis in *Leptinotarsa decemlineata* were studied with phase contrast microscopy. In electron microscopy and paraffin sectioned bright field microscopy, the tissue is usually fixed, stained and sectioned before examination (usually a lengthy process) but in phase contrast microscopy the tissue is examined immediately after excision. There are many advantages of correlating phase contrast microscopy with electron microscopy and it should be remembered that even though electron microscopy has certain resolution advantages, it does not enable the investigator to observe the living cell.

Phase contrast microscopy is especially useful for observations of flagellar motility and for observing different mechanisms by which round spermatids differentiate into long, slender spermatozoa. Figure 5 is a spermatid beginning to elongate. The mitochondrial nebenkern has split into the two apparently spherical mitochondrial or nebenkern derivatives which will elongate on each side of the axial filament. Note a possible centriole (arrow) lying directly beneath the mitochondrial derivatives. Figure 6 is a phase contrast micrograph of a mature spermatozoon which measures approximately 100 micra in length.

Electron microscopy: We have investigated many aspects of spermiogenesis in *Leptinotarsa decemlineata* with the electron microscope and a recent article concerned with the subunits in each of the 9 +9 + 2 flagellar tubules has recently been completed (Shay *et al.*). These subunits measure approximately 35–40 angstroms which represents a considerable increase in resolution when compared with phase contrast microscopy.

Figure 7 is a low magnification micrograph of an early spermatid. Note the two centrioles, early acrosome formation and mitochondria which will eventually coalesce to form the nebenkern. Figure 8 is an elongating spermatozoon which shows the acrosome, nucleus, mitochondrial derivatives and axial filament (flagellum). There have been many statements published regarding the number of centrioles in the spermatids in insects, and Friedlander and Wahrman (1966) have stated that only one typical centriole occurs in the spermatids of all species of insects that have been studied with the electron microscope. Breland *et al.* (1968) have demonstrated the existence of two centrioles in many of the insect spermatids they studied and Fig. 7 clearly shows two centrioles in *Leptinotarsa*. Shay and Biesele (1968) have stated that even though only one centriole was observed in spermatids of the cave cricket (*Ceuthophilus secretus*) there



FIGURES 5–8. FIG. 5. Phase contrast photomicrograph of elongating spermatid. Nebenkern dividing into two Nebenkern derivatives (ND) and arrow indicates possible centriole, × 1,900; FIG. 6. Phase contrast photomicrograph of mature spermatozoon (H = Head, T = Tail), × 1,130; FIG. 7. Electron photomicrograph of young spermatid. Note mitochondria (M), Golgi (G), nucleus (N), and two centrioles (C), × 9,900; FIG. 8. Electron photomicrograph of elongating spermatid. Note acrosome (A), nucleus (N), and Nebenkern derivatives (ND), × 4,500.

still remained the possibility of the existence of a second one and that perhaps they were not fortunate enough to cut sections showing both centrioles.

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