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THE DNA IN PRIMARY SPERMATOCYTES OF *DROSOPHILA VIRILIS*

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ABSTRACT

The Feulgen reaction was utilized to examine the distribution of DNA in the primary spermatocytes of *Drosophila virilis*. The chromosomes existed as distinct Feulgen-positive bodies throughout the growth phase of the primary spermatocytes. The sex chromosomes are associated with the nucleolus until it fragments. No stages identifiable as leptotene, zygotene, pachytene, or diplotene were observed during meiotic prophase. Chromosome disjunction occurred normally; however, some chromosomes did lag in their movement to the poles in anaphase I.

INTRODUCTION

Numerous light microscope studies of spermatogenesis in *Drosophila* have been conducted (Stevens, 1908; Metz, 1926; Guyenot and Naville, 1929; Cooper, 1950; and Clayton, 1957, 1962). These studies have been concerned with the sex chromosomes, somatic pairing, meiotic chromosome behavior, and the various details of spermatogenesis. More recently, ultrastructural studies with the electron microscope (Ito, 1960; Kaufmann and Gay, 1963; Meyer, 1968; Hess and Meyer, 1968; Tates, 1971; and Stanley et al., 1972) have helped to further clarify the various aspects of spermatogensis in *Drosophila.*

The growth stage of the primary spermatocyte has been found difficult to seriate, and the various events associated with prophase of the first meiotic division have been subject to varying interpretations. Guyenot and Naville (1929) and Huettner (1930) reported the occurrence of a leptotene stage during prophase of *Drosophila* spermatogenesis. In addition, Huettner (1930) also described a stage he termed synaptotene. However, various other investigators (Metz, 1926; Cooper, 1950; Clayton, 1957, 1962) did not observe such stages as lepotene, zygotene, pachytene, or diplotene in prophase of the primary spermatocyte.

Huettner (1930) reported a loss of Feulgen stainability as growth of the primary spermatocyte occurred, whereas Cooper (1950) and Perreault and Gay (1962) found no loss of Feulgen stainability during the growth phase of *Drosophila melanogaster* spermatocytes. They did find that the DNA became diffuse during the growth stage but continued to exist as distinct Feulgen-positive bodies. With the exception of prophase of the primary spermatocyte, there is fairly general agreement concerning the meiotic divisions and spermiogenesis with regard to the various *Drosophila* species. This study examines the distribution of DNA in the primary spermatocytes of *Drosophila virilis.*

MATERIALS AND METHODS

The *D. virilis* stock (University of Texas strain 1801.1) used in this study was maintained and processed for histological examination as described by Beck and Clayton (1977). Deoxyribonucleic acid was stained using the Feulgen procedure outlined by Humason (1967). Tissue sections were hydrolyzed for 6 minutes at 60°C in 1.0 N hydrochloric acid prior to being stained with the standard Schiff's reagent. Following hydrolysis, the sections were stained for 2 hours in Schiff's reagent prepared according to the procedure outlined by DeTomasi (1936). After staining with the Schiff's reagent, the sections were washed in three changes of sulfite bleach for two minutes per wash. The sections were then counterstained with either 0.5% fast green in 95% ethanol for 10 seconds or 0.25% aqueous aniline blue for 30 seconds. The counterstain must be kept light in order not to mask any DNA staining.

RESULTS

The spermatogonia form a compact mass of small cells lying at the cephalic end of the adult testis. Each nucleus is surrounded by a thin layer of cytoplasm and possesses a centrally-located nucleolus at interphase. The DNA of the spermatogonia stains conspicuously with the Feulgen reagent, appearing as a tangle of darkly-staining material in the resting stages (Fig. 1). The spermatogonial chromosomes form a flat equatorial plate at metaphase consisting of ten rod chromosomes and two dot chromosomes in *D. virilis* (Fig. 2). The somatic association of homologous chromosomes is evident during metaphase of the spermatogonial division but appears to be less intimate than in either prophase

or anaphase. An intimate somatic pairing of the homologous chromosomes seems to occur during anaphase so that in most cases only the haploid number of chromosomes is ascertainable. Since the chromosomes are paired during the spermatogonial stage, homologous chromosomes may enter the primary spermatocytes in a paired condition. However, due to the nature of the distribution of the DNA in the early primary spermatocyte, definite confirmation of this suggestion could not be made.

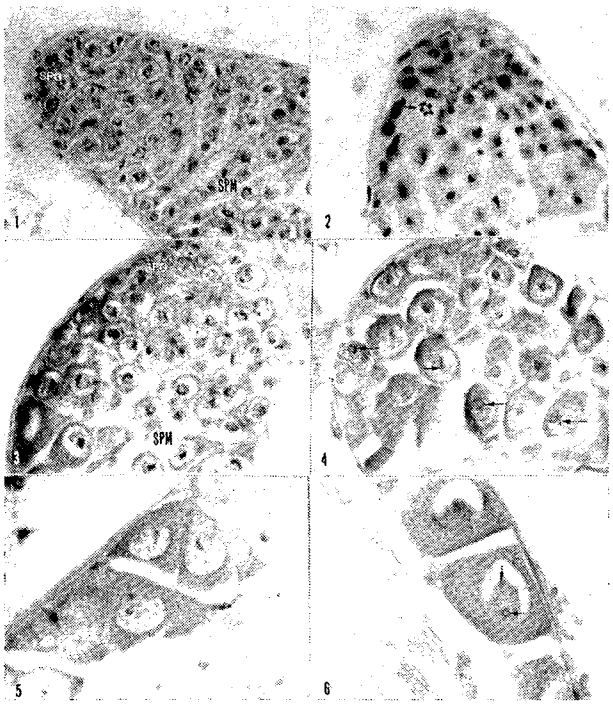

FIG. 1-6. *Growth of Spermatocytes. Fig. 1, Spermatogonia (SPG) and early primary spermatocytes (SPM). Feulgen; Aniline blue. X970. Fig. 2, Spermatogonial metaphase showing somatic pairing. Feulgen; fast green. X970. Fig. 3, Spermatogonia (SPG) and early primary spermatocytes (SPM). Feulgen; aniline blue. X970. Fig. 4, Early growth primary spermatocytes. Arrows indicate location of DNA. Feulgen; Aniline blue. X970. Fig. 5, Mid-growth primary spermatocytes. Feulgen; Aniline blue. X970. Fig. 6, Advanced growth primary spermatocyte. Arrows indicate location of DNA. Feulgen; Aniline blue. X970.*

The early primary spermatocyte has undergone very little growth and is still about the same size as the spermatogonium. The early primary spermatocyte possesses a centrally-located nucleolus with the DNA lying in close association with the nucleolus (Fig. 3). However, with the exception of the nucleolus and the DNA, the nucleus appears to be empty. As growth of the primary spermatocyte proceeds, some of the DNA begins to extend outward as projections from the nucleolus but remaining attached to it, and there is a movement of the nucleolus from its centrally-located position to one adjacent to the nuclear membrane (Fig. 4). In addition, Feulgen-negative material begins to become evident within the nucleoplasm. The primary spermatocyte continues to enlarge with some of the DNA becoming separated from the nucleolus and scattered within the nucleoplasm as small bodies of Feulgen-positive material (Fig. 5). In the later growth stages, the DNA appears more compact and stains somewhat more intensely than in the earlier growth stages (Fig. 6). Some DNA is still found in association with the nucleolus even in these advanced growth stages (Fig. 7) and probably represents the sex chromatin or portions thereof. This DNA becomes disassociated from the nucleolus at the time of nucleolar fragmentation. As the primary spermatocyte enters what might be termed diakinesis, the DNA is found to lie in the peripheral region of the nucleus as distinct Feulgen-positive aggregates (Fig. 8). The nucleolus has also fragmented by this time and a large amount of non-chromatic material is present in the nucleus. Some of this non-chromatic material probably represents fragments of the nucleolus. Spindle formation is also beginning to occur with the asters being prominent in the cytoplasm. The bivalents move from their peripheral position in diakinesis and become aligned along the equatorial plate in metaphase (Fig. 9). Anaphase appears to be asynchronous with some of the chromosomes apparently lagging during their anaphase movement (Fig. 10). Upon completion of anaphase, the DNA becomes compacted into a small dense mass and the individual chromosomes cannot be distinguished (Fig. 11). After the nuclear membrane re-

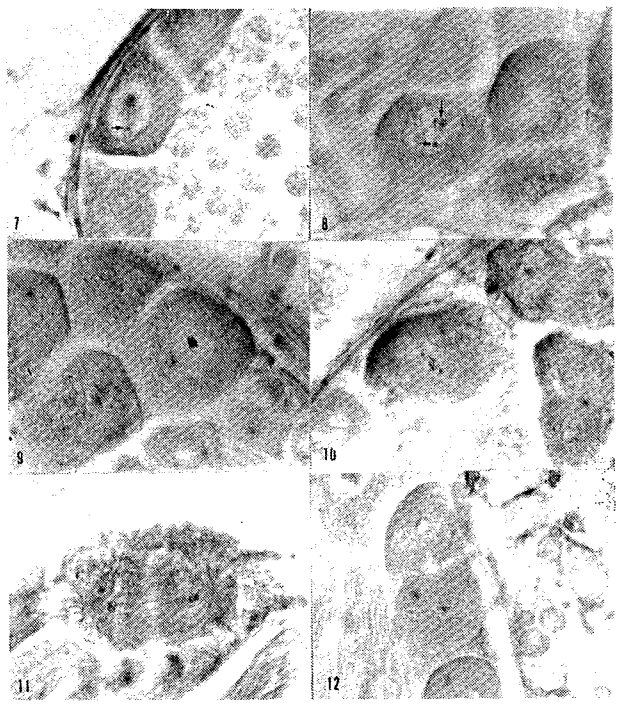

FIG. 7-12. *Spermatocytes and Feulgen staining. Fig. 7, Advanced growth primary spermatocyte showing association of DNA (arrow) with nucleolus. Feulgen; Aniline blue. X970. Fig. 8, Diakinesis. Arrows indicate location of DNA. Feulgen; Aniline blue. X970. Fig. 9, Metaphase I. Feulgen; Aniline blue. X970. Fig. 10, Anaphase I. Feulgen; Aniline blue. X970. Fig. 11, Telophase I, Feulgen; Aniline blue. X970. Fig. 12, Telophase I, Feulgen; Aniline blue. X970.*

forms, the DNA comes to lie against its inner surface (Fig. 12). Cytokinesis results in two secondary spermatocytes of approximately equal size.

DISCUSSION

The spermatogonial chromosomes of *D. virilis* were found to be associated in pairs just as had been described by Stevens (1908) and Metz (1916). This somatic association probably persists throughout the spermatogonial stage with the homologous chromosomes entering the primary spermatocyte in a paired condition. Guyenot and Naville (1929) contended that the somatic association was lost during telophase of the spermatogonial division. However, Metz (1926) concluded that the somatic pairing persisted through telophase of the spermatogonial division in *D. virilis* and that the homologous chromosomes remained in intimate association during the growth stages of the primary spermatocytes.

Initially, all the DNA of the early primary spermatocyte was in very close association with the nucleolus just as had been reported by Metz (1926) using Heidenhain's iron hematoxylin. However, with growth of the primary spermatocyte, all but one clump of DNA became disassociated from the nucleolus. This clump of DNA remained associated with the nucleolus throughout the growth stage and became freed from the nucleolus when nucleolar fragmentation occurred just prior to diakinesis. Metz (1926) concluded that the nucleolar organizer in *D. virilis* was associated with the sex chromosomes. Kaufmann (1934) also found that the nucleolar organizer was located on the sex chromosomes in *D. melanogaster*. The DNA adhering to the nucleolus, therefore, probably represents the sex chromosomes of *D. virilis*.

The DNA representing the autosomes became somewhat diffuse in the primary spermatocytes but persisted as discrete Feulgen-positive bodies throughout the entire growth period. At no time during the growth period did the DNA lose its staining capacity as had been described by Huettner (1930) in his study of spermatogenesis in *D. melanogaster*. Subsequent studies by Cooper (1950) and Perreault and Gay (1962) have also confirmed that there is no loss of Feulgen stainability during the growth stage of the primary spermatocyte. Condensation of the DNA occurred during the late growth stage resulting in a more intense Feulgen stainability.

Guyenot and Naville (1929) and Huettner (1930) described a stage during *Drosophila* spermatogenesis that they termed lepotene. In addition, Huettner (1930) presented drawings and a description of a stage he considered to be synaptotene. This study did not demonstrate any stages that could be considered true leptotene, zygotene, pachytene, or diplotene such as occurs in many insects and other animals. Other investigators (Metz, 1926; Cooper, 1950; Clayton, 1957, 1962) have also failed to observe such meiotic prophase stages during spermatogenesis in *Drosophila*. A diakinetic stage similar to that occurring in many Orthropteran species occurred at the end of the growth period in the primary spermatocyte.

Anaphase I appeared asynchronous with some chromosomes being observed to lag in their movement to the poles. Darlington (1939) found that the sex chromosomes of *D. pseudoobscura* generally preceded the autosomes in movement to the poles, whereas Cooper (1950) reported that the sex chromosomes lagged in their anaphase movement in *D. melanogaster*. Darlington (1939) suggested that this precocity exhibited by the chromosomes in the first meiotic division of *Drosophila* might be due to an error in time coordination between the sex chromosomes and the autosomes.

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