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SPERMATOGENESIS IN *AEDES ALBOPICTUS* (SKUSE) [1]

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ABSTRACT. Chromosome behavior during spermatogenesis in *Aedes albopictus* was studied and compared with results of similar studies. Fourth instar larvae, pupae, and adults of the Mauritius strain were dissected at intervals of one hour after these stages had begun. Squash preparations stained with 2% lacto-aceto-orcein were utilized exclusively. Leptotene and zygotene stages were not observed. The chromosomes first became visibly distinct in pachytene. In early pachytene the chromosomes were usually closely paired, but some asynaptic regions were observed.

A prometaphase stretch was not seen. At anaphase I the separation of the dyads was asynchronous with the smallest pair often separating first. In prophase II polarization of the chromosome arms was observed. Separation of the chromosomes at anaphase II was synchronous, and the chromosomes were individually recognizable. The developing spermatids gradually became elliptical and contained alternating regions of light and dark staining material. The elliptical spermatids then elongated to form mature sperm.

INTRODUCTION. Work on spermatogenesis in mosquitoes appears to have been neglected when compared to the literature available in such areas as taxonomy, genetics, control, and ecology. Most early studies involving meiosis or spermatogenesis utilized the *Culex pipiens* complex (Stevens 1910, Lomen 1914, Taylor 1914, Whiting 1917, Moffett 1936, Grell 1946, Callan and Montalenti 1947, and Jost 1971). Other earlier studies included the following species: *Culex tarsalis* (Stevens 1911); *Anopheles punctipennis* (Stevens 1911); *Culiseta incidens* (Stevens 1911); *Corethra plumicornis* (Frolowa 1929); *Anopheles maculipennis* (DeBuck and Swellengrebel 1935); and *Theobaldia longiareolata* (Callan and

Montalenti 1947). Most recent studies include the following species *Anopheles stephensi* (Rishikesh 1959); *Aedes aegypti* (Akstein 1962); *Culiseta inornata* (Breland *et al.* 1964); *Aedes aegypti* (Krafsur 1964); *Aedes aegypti* (Mescher and Rai 1966); *Aedes dorsalis* (Mukherjee and Rees 1970); and a very brief description of meiosis in *Aedes albopictus* (Jost 1971). In addition, Krafsur and Jones (1967) described the process of spermiogenesis in *Aedes aegypti*.

MATERIALS AND METHODS. All specimens utilized in this study belonged to the Mauritius strain of *Aedes albopictus* currently maintained at the Mosquito Genetics Laboratory, Georgia Southern College. The colony originated from specimens collected by the second author in Mauritius during March of 1970. Eggs of this strain were hatched in deoxygenated water. Larvae and pupae were raised in distilled water in white enamel pans (23 cm x 35 cm x 5 cm) at

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a temperature of 27° C (±1° C) and ambient relative humidity. Liver Powder NF (Nutritional Biochemicals Co.) was supplied daily as food. Rearing procedures for this species closely paralleled the procedures described by Craig and VandeHey (1962) for rearing *Aedes aegypti* with the exception that larval population size was restricted to 250 or less per rearing pan.

Fourth instar larvae, pupae, and adults were dissected at regular intervals of one hour after these stages had begun. Testes of larvae and pupae were dissected in their own tissue fluids with an added drop of water. Adult testes were dissected in *Aedes aegypti* saline (Hayes, 1953).

Fat cells surrounding the testes were removed, and the testes were allowed to stain for fifteen minutes in a drop of 2 percent lacto-aceto-orcein (Breland, 1961). After staining, the preparation was gently squashed.

The slides were sealed with fingernail polish, and photomicrographs were taken using a 35 mm camera and Plus-X Pan, black and white film. Agfa-Gavaert Brovira F-6 print paper was used to obtain high contrast prints.

All measurements were made using a Hacker Ocular Micrometer, and each measurement represents an average of three measurements.

RESULTS. The testes are elliptical organs lying in the region of the sixth abdominal segment and are covered by a brownish layer of fat cells. Each testis is colorless and divided into cysts. The two testes of an organism were usually of different sizes and were often in different stages of development. It was common to find one testis with many cells undergoing meiosis while the cells in the neighboring testis were resting primary spermatocytes.

The diploid number is 6 and consists of three pairs of metacentric chromosomes. Sinoto and Suzuki (1943) reported the largest chromosome of this species to be submetacentric. However,

based on actual measurements, Rai (1963) showed that all three chromosome pairs were metacentric. Our observations concur with Rai's findings, and our measurements of 5.62, 7.52, and 8.75 microns are in close agreement with Rai's measurements of 6.2, 7.6, and 9.2 microns for chromosomes one, two and three, respectively.

Fourth instar larvae contained testicular cells in interphase and various stages of spermatogonial mitosis. From the start of pupal life onward, primary spermatocytes comprised the majority of cells present in the testes. There was much variation in the progress of spermatogenesis among different pupae, with feeding and spacing seeming to have a sizeable effect. Larvae grown in pans with populations of 250 or less and fed heavily appeared to possess larger testes, to begin spermatogenesis earlier, and to be more uniform from pupae to pupae. Larvae grown in crowded pans had noticeably smaller testes, delayed spermatogenesis, and much variation from pupae to pupae. All cells of an individual cyst were normally in the same stage of development.

Pupae from 4-6 hours old usually possessed posterior cysts in pachytene, the first stage of meiosis in which the chromosomes were visibly distinct. In early pachytene the chromosomes were usually closely synapsed, but some asynaptic regions were noted. Deeply stained chromomeres were prominent (Fig. 1).

Shortening and repulsion of the homologous chromosomes characterized diplotene. Repulsion was greatest at the centromeric region but was also prominent at the flared ends of the chromosomes. The chromomeres which had been so conspicuous in pachytene were less evident. The chromosomes appeared to be held together only at the chiasmata. The number of chiasmata per bivalent ranged from 1-2 (Fig. 2).

Diakinesis was a short stage characterized by contracted chromosomes held together at the chiasmata. Ring-shaped bivalents were common.
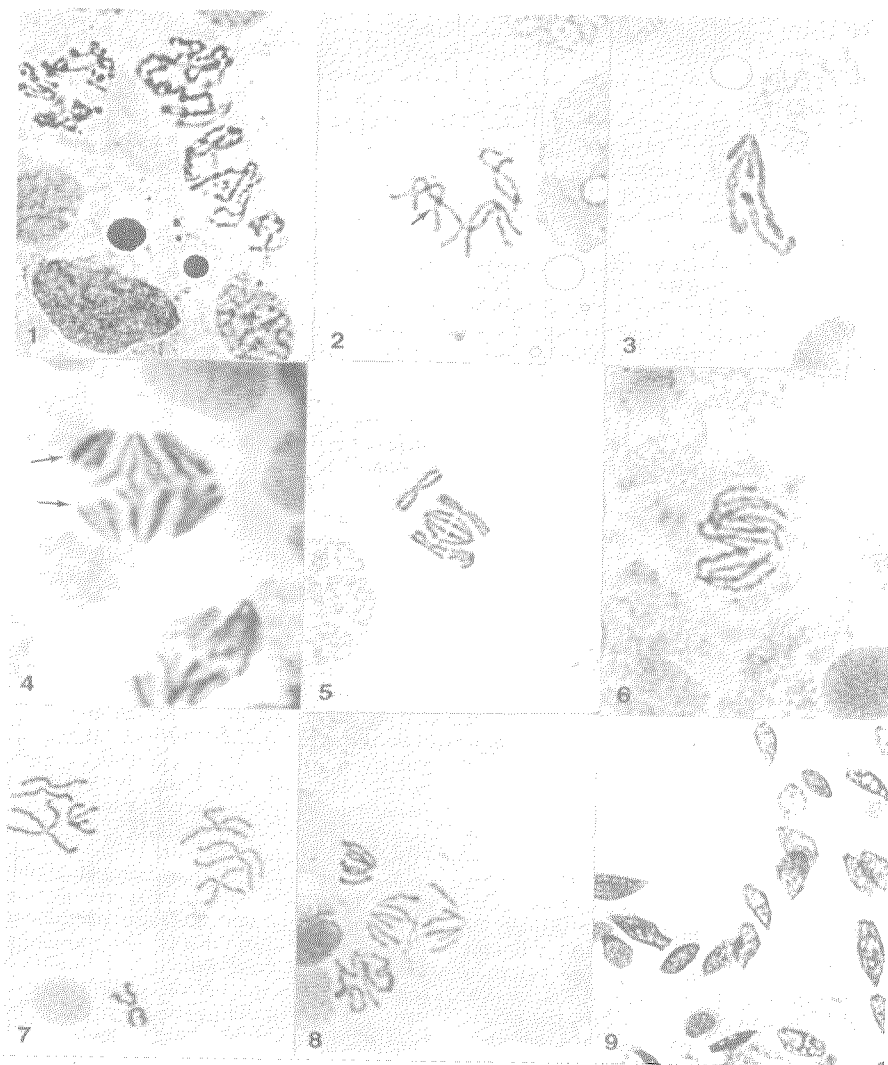
PLATE I.—First and second meiotic divisions in *Aedes albopictus*. FIG. I.—Pachytene (1000X). FIG. 2.—Diplotene (500X). FIG. 3.—Metaphase I or late diakinesis (500X). FIG. 4.—Anaphase I with precocious separation of smallest pair of dyads (1000X). FIG. 5.—Anaphase I without precocious separation of smallest dyad pair (500X). FIG. 6.—Prophase II with polarization of the chromatid arms (1000X). FIG. 7.—Early metaphase II (possibly late Prophase II) (500X). FIG. 8.—Anaphase II (500X). FIG. 9.—Spermatids (1000X).

A phenomenon called the prometaphase stretch (Hughes-Schrader, 1943, 1947; Cooper, 1951), which Breland *et al.* (1964) observed in *Culiseta inornata,* was not seen.

At metaphase I the chromosomes were arranged on the equatorial plate. Chiasmata terminalization resulted in either ring or rod-shaped bivalents. Chromosome stickiness did not appear to be a problem (Fig. 3).

At anaphase I the chromosomes moved asynchronously toward the poles approximately 50 percent of the time. It was common to observe the precocious separation of the smallest pair of dyads. The arms of the chromatids appeared to repulse each other as the dyads moved to their respective poles (Figs. 4–5).

Telophase I was a brief, rarely observed stage. The secondary spermatocyte was small and stained similarly to a primary spermatocyte.

Prophase II was characterized by orientation of the centromeres toward one side of the cell while the chromatid arms were oriented toward the other side. This polarity was gone before metaphase II began. Also, the arms of the chromatids seemed to repulse each other (Fig. 6).

The chromosomes were maximally contracted at metaphase II, and their arms were still flared. The chromosomes assumed the shape of small H's and X's (Fig. 7).

At anaphase II the three chromosomes moved toward their respective poles in a smooth, synchronous manner. In the majority of preparations, the individual chromosomes were easily recognizable (Fig. 8).

After a brief telophase II, the chromatin material of each nucleus became concentrated along the inner edge of the nuclear membrane. The developing spermatids gradually elongated and assumed an elliptical shape. The fusiform spermatids exhibited alternating regions of dark and light staining material as the elongation process proceeded to the formation of mature sperm (Fig. 9). Only the deeply stained head of the sperm was observed because the tail structure was apparently destroyed by the acetic acid of the staining process (Breland and Gassner, 1964).

Spermatids were seen in posterior cysts of the testes of some pupae as early as seven hours after pupation. Approximately 40 percent of pupae 11 to 14 hours old contained spermatids, whereas almost all pupae more than 14 hours old contained spermatids. Sperm were first visible at 35 hours after pupation, and at approximately 45 hours after pupation the posterior halves of the testes of most pupae contained massive numbers of sperm. The posterior halves of the testes of all adults were filled with mature sperm.

DISCUSSION. Somatic pairing is a phenomenon characteristically observed among the Culicidae and involves a relatively permanent attraction and intimate pairing between homologous chromosomes during mitosis. This attraction between homologous chromosomes modifies the pattern of mitosis and meiosis and has led many workers to question the existence of visible leptotene and zygotene stages in mosquitoes (Rishikesh, 1959; Akstein, 1962, Breland *et al.,* 1964; Mescher and Rai, 1966; Mukherjee and Rees, 1970). One worker, Jost (1971), claims to have observed zygotene stages in *Culex pipiens* and *Aedes albopictus.* In the present study no visible leptotene or zygotene stages were observed in *Aedes albopictus.* This was probably because the close pairing of the previous mitotic anaphase and telophase carried over into the early stages of meiosis. The first stage in which the chromosomes became visibly discreet was pachytene as evidenced by almost complete pairing along the length of the chromosomes.

There seems to be much variation as to when the chromosomes become visibly double. Akstein (1962) and Mukherjee and Rees (1970) working with *Aedes aegypti* and *Aedes dorsalis,* respectively, reported that the chromosomes were not visibly double in prophase I. Breland *et al.* (1964) reported that the chromosomes of *Culiseta inornata* were visibly double in

pachytene, and Mescher and Rai (1966) observed that the chromosomes of *Aedes aegypti* were visibly double in diplotene. In *Aedes albopictus* the individual chromosomes were observed to be double in pachytene, but only rare squashes showed this phenomenon.

Breland *et al.* (1964) were the first to report the occurrence of the prometaphase stretch (Hughes-Schrader, 1943, 1947; Cooper, 1951) in a mosquito, *Culiseta inornata*. Because the prometaphase stretch was also known to occur in *Boreus brumalis,* a crane fly, they were tempted to suggest that their study lent support to the theory advocated by Hinton (1958) that the Diptera were derived from the Mecoptera rather than the Neuroptera, as suggested by White (1949). Mescher and Rai (1966) reported a prometaphase stretch in *Aedes aegypti,* but Rai *et al.* (1970) indicated that such stretching was probably an artifact resulting from fixation procedures. Similarly, Mukherjee and Rees (1970) and Jost (1971) reported no prometaphase stretch in *Aedes dorsalis* and *Aedes albopictus.* We found no prometaphase stretch in *Aedes albopictus* and agree with Rai *et al.* (1970) that the prometaphase stretch is an artifact resulting from the use of fixatives.

In *Aedes albopictus* the separation of homologues during anaphase I was asynchronous in approximately half of the dividing cells observed. The smallest pair of dyads usually separated first and were often completely separated before there was any noticeable movement on the part of the larger chromosomes. Similar asynchronous separation of the smallest pair of dyads has been reported for *Culiseta inornata* (Breland *et al.*, 1964), *Aedes aegypti* (Mescher and Rai, 1966), and *Aedes albopictus* (Jost, 1971). Mukherjee and Rees (1970) found occasional asynchronous separation in *Aedes dorsalis,* but they noted that normally separation of the dyads at anaphase I was very synchronous. In *Anopheles stephensi* a different situation exists. Rishikesh (1959) confirmed the smallest pair of chromosomes as sex

chromosomes and observed that they separated last at anaphase I.

With the exception of *Anopheles stephensi* (Rishikesh, 1959) the separation of chromosomes at anaphase II is apparently quite synchronous for most species of mosquitoes. *Aedes albopictus* resembles *Culiseta inornata* (Breland *et al.,* 1964) at anaphase II in that the individual chromosomes are easily recognizable. Chromosome stickiness was never a problem in the present study.

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THE SALIVARY GLAND CHROMOSOMES OF *CULEX PIPIENS* L.[1,2]

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ABSTRACT. The salivary gland chromosomes of *Culex pipiens* L. are described and compared with previous descriptions of chromosomes of the *C. pipiens* complex. Differences which have been described among the various forms may relate to techniques of preparation rather than to real differences among the forms.

INTRODUCTION

Sutton (1942) described the salivary gland chromosomes of *Culex pipiens* L. as being three pairs, synapsed along most of their lengths. The three pairs were completely separated from each other and were without obvious chromocenters. No difference was detected between the chromosomes of females and males. A detailed

study of the banding pattern was not attempted. Kitzmiller and Clark (1952) and Kitzmiller and Keppler (1961) reported the preparation of a map of the salivary gland chromosomes for the subspecies *pipiens*. Dennhöfer (1968) described and mapped the salivary gland chromosomes of an autogenous strain of this subspecies (sometimes called subspecies *molestus*). Sharma *et al.* (1969) and Kanda (1970) described the salivary gland chromosomes of the subspecies *quinquefasciatus* (=*fatigans*). Although there are differences in the salivary gland maps which have been published to date, there