Spermatogenesis in the Mosquito, *Aedes dorsalis* (Meigen)¹

Asit B. Mukherjee² and Don M. Rees³

Received January 14, 1969

Introduction

Although mosquitoes have been studied intensively from the standpoint of disease transmission, taxonomy, biology, ecology, and control, relatively less work has been done on the spermatogenesis of mosquitoes. Most studies on meiosis and other phases of spermatogenesis have been done with the *Culex pipiens* complex (Callen and Montalenti 1947, Grell 1946, Moffet 1936, Stevens 1910, and Whiting 1917). A list of other species which have been studied in this aspect has been provided by Mescher and Rai (1966). As far as can be determined no work on spermatogenesis of *Aedes dorsalis* (Meigen) has been published. It is the lack of information on this subject that directed our interest in working with this particular species.

Materials and methods

The specimens used in this study were collected in Utah from June to August, 1966. Third to fourth instar larvae were collected in the field and reared in the laboratory at 25°C. Chromosome preparations were made from the testes-tissue of both the fourth instar larvae and the pupal stages of mosquitoes. The testes are located on the sixth abdominal segment and can be recognized as white or yellowish oval organs. The testes were fixed in 1 part glacial acetic acid and 3 parts absolute alcohol for 20 minutes and were stained with aceto-lactic-orcein for 10 minutes before squashing.

Microphotographs were taken from temporary preparations using a Zeiss photomicroscope and 35 mm black and white film. Pienaar's (1955) preservative (6 parts of methanol, 3 parts of chloroform and 2 parts of propionic acid) was used for preserving the specimens for future use.

Results

In this investigation different phases of both the spermatogonial and meiotic divisions were studied. It was found that in the fourth instar larval

¹ This study was supported in part by a University of Utah Graduate Research Fellowship awarded to the senior author.

² Present address: Columbia University, College of Physicians and Surgeons, Department of Human Genetics and Development, 630 West, 168th Street, New York, N.Y., U.S.A.

³ Department of Environmental Biology, University of Utah, Salt Lake City, Utah, U.S.A.

stage the testes were still immature, and only a few spermatogonial divisions could be found. The testes of very early pupae showed actively dividing spermatogonia and early primary spermatocytes. Young pupae contained many stages of meiotic divisions in the germ cells. Older pupae contained mostly sperm.

The diploid chromosome number is six and the chromosomes are all metacentric (Mukherjee *et al.* 1967). The first pair of chromosomes is smaller than second and third chromosome pairs. The sex chromosomes are not identifiable morphologically.

Spermatogonial division

Only three double chromatin threads are clearly seen in the prophase stage (Fig. 1) although the diploid number is six. There is close synapsis of the homologous chromosomes along their entire length. In the next stage

Figs. 1-12. Spermatogonial and meiotic divisions in the testes of *Aedes dorsalis*. 1-2, early prophase. The homologues are in intimate pairing. 3, middle prophase. The homologues start to unwind. 4, late prophase. 5, metaphase. 6, anaphase. 7, very early prophase I.

Figs. 8-13. Prophase I. 8, pachytene. 9-12, diplotene.

the double nature of each of the three threads are more clearly visible (Fig. 2). In the later stages, the association becomes less intimate and the homologues start to separate (Figs. 3, 4). Thus the mitotic bivalents consist of four chromatids each. The centromeric region divides longitudinally and each chromatid has its own centromere. At metaphase the chromosomes become shorter and thicker (Fig. 5). At anaphase the chromosomes become V-shaped (Fig. 6).

Meiotic divisions

It is well recognized that the classical meiotic process consists of two cell divisions that are known as division I and division II. The different divisional stages are designated as prophase I, metaphase I etc., and those in division II as prophase II, metaphase II, etc.

Some investigators including Whiting (1917), Breland *et al.* (1964) have pointed out that the two testes of an individual mosquito may have cells at different divisional stages. This was observed in our study of *Aedes dorsalis*.

Prophase I, in many cases, can be divided into the following steps in sequence: leptotene, zygotene, pachytene, diplotene, and diakinesis. There has been considerable discussion as to whether or not the early prophase stages, especially leptotene and zygotene are clearly visible in Diptera. This discussion is also applicable to meiosis in mosquitoes. Previous publications are generally in agreement that the chromosomal strands are double when they are first visible under the microscope. The first stage of prophase when the chromosomes can be clearly seen has been interpreted as the pachytene stage (Fig. 8). Thus in *Aedes dorsalis* the visible leptotene and zygotene stages can not be distinguished. The homologues in the early stages are wound tightly around each other. The amplitude of the gyres on one side of the centromere is, in most cases, larger than that on the other side. As the prophase stage proceeds, the bivalents begin to contract and unwind. This unwinding has been found to proceed at different rates on either side of the centromere (Fig. 10). From the pachytene stage the chromosomes pass through diplotene (Figs. 9-12) and into diakinesis (Fig. 13). At diplotene, centromeric repulsion starts and the points of contact between chromosomes correspond to the locations of the chiasmata. The two chromatids of each homologue are not clearly visible during meiotic prophase. Usually one or two chiasmata are observed in each bivalent. At diakinesis, the paired chromosomes assume ring-like structures and appear very contracted and held together at points of chiasmata.

At metaphase I (Fig. 14) the diameter of the chromosomes increase considerably as they contract and the chromosomes orient themselves on the equatorial plate. At this stage the chromosomes seem to show maximum stickiness, and they usually lump together.

During anaphase I the four arms of each chromosome, i.e. the two arms of the two chromatids of each chromosome are clearly visible. In some anaphase stage, the dyads of the smallest bivalent separate first.

As the chromosomes pass from anaphase I to telophase I, the arms of the chromatids are in repulsion from each other. The centromeric region is a small rectangular structure. The chromatids then become diffused in appearance as they enter the interphase stage (Fig. 17). The cells at these interphase stages can be designated as secondary spermatocytes. The chromatin mass at this stage is smaller than that in prophase I.

Figs. 13-27. Meiotic divisions in the testes of *Aedes dorsalis*. 13, diakinesis. 14, metaphase I. 15, anaphase I. 16, telophase I. 17, interphase, also called spermatocyte II. 18-19, early prophase II. 20, metaphase II. 21, anaphase II. 22, telophase II. 23-24, stages in the development of spermatids. 25-26, spermatids. 27, sperm.

During early prophase II the arms of the chromatids are polarized towards one side (Figs. 18-19). This type of chromosomal behavior was first pointed out by Breland *et al.* (1964) in *Culiseta inornata*.

A great deal of contraction takes place during metaphase II when the chromosome lengths are shortest (Fig. 20). The repulsive forces between the

sister chromatids is less manifested, and the chromosomes are H-shaped. The sister chromatids then separate at anaphase II (Figs. 21) and each resulting cell has three chromosomes, the "n" or haploid number. A nuclear membrane then forms surrounding the diffused chromosomes, and the nuclei apparently enlarge before becoming more condensed to form the spermatids (Figs. 25-26). The spermatids then become modified to form elongated sperm (Fig. 27). The detailed process of the development of spermatids into functional mature sperm (spermiogenesis) was not determined in this study.

Discussion

A phenomenon called "somatic pairing" is very characteristic among the Culicidae. This involves a permanent attraction and intimate pairing between homologous chromosomes during mitosis. The varying degrees of appearance of this phenomenon have been mentioned by Boss (1955). Somatic pairing is not found universally among all Dipterans (Bauer 1931, Metz 1938, White 1946).

During the prophase stage of the spermatogonial division the homologous chromosomes show somatic pairing. Their association becomes less intimate by late prophase. Careful examination of the early mitotic prophase shows points of contact between chromosomes forming the "mitotic bivalents." During meiosis these points of contact are the locations of chiasma formation. So far as can be determined no reliable evidence of mitotic crossing over between chromosomes in mosquitoes has been detected. However, spermatogonial chiasmata were observed and somatic pairing was shown in *Drosophila* (Cooper 1949, Stern 1936). On the basis of these observations it has been suggested by Akstein (1962) that genetic segregation in mitosis is a regular phenomenon in Dipterans.

The present study reveals that in *Aedes dorsalis* the earliest stage at which the chromosomes become visibly discrete is the pachytene. Unlike the situation reported in *Culiseta inornata* (Breland *et al.* 1964) and in *Aedes aegypti* (Mescher and Rai 1966), the chromatids of each chromosome are not very clear during diplotene in this species.

A phenomenon called the prometaphase stretch (Hughes-Schrader 1943, 1947, Cooper 1951) is found to occur just before metaphase I in some mosquitoes (Breland *et al.* 1964, Mescher *et al.* 1966). This prometaphase stretch could not be well identified in *Aedes dorsalis*. This phenomenon might be a species-specific characteristic. The authors are of the opinion that more detailed knowledge of this situation is required before attaching phylogenetic significance to this phenomenon.

In *A. dorsalis*, unlike some other species of mosquitoes, the separation of the homologues during anaphase I is quite synchronous, although in some cases the smallest bivalents is found to separate first. The latter situation might occur due to the differential pressure applied for squashing the tissue.

Similarly in other species of mosquitoes the smallest pair of homologous separate first. In *Anopheles stephensi* (Rishikesh 1959) the smallest pair separate last and has been established as sex chromosomes.

A great deal of chromosome stickiness is observed, particularly during metaphase I and anaphase I. Generally the chromosomes, at these periods, adhere to each other forming a single clump.

The present study reveals that the chromosome morphology and behavior pattern of *Aedes dorsalis* during spermatogenesis resemble closely that of other mosquito species studied. Differences have been pointed out when they have been detected. More detailed work on the spermatogenesis of other species of mosquitoes are required before generalizations can be made regarding species-specific characteristic.

Summary

The chromosome morphology and behavior pattern during spermatogenesis in *Aedes dorsalis* was studied. The results were compared with the findings of similar investigations in other species of mosquitoes. Chromosome preparations were made from testes-tissue of fourth instar and pupal stages by the squash technique and stained with aceto-lactic-orcein. The two testes of an individual may have cells at different divisional stages. Leptotene and zygotene stages of meiotic prophase were not distinguishable. The unwinding of the homologous chromosomes in later stages of prophase I was found to progress at different rates on either side of the centromere. The separation of the chromosomes during anaphase I was mostly synchronous. The polarization of arms of the prophase II chromosomes was observed. The repulsive forces between the sister chromatids in metaphase II were less apparent and the chromosomes at this period were H-shaped in configuration.

References

- Akstein, E. 1962. The chromosomes of *Aedes aegypti*, and of some other species of mosquitoes. Bull. Israel Res. Council 11B: 146-155.
- Bauer, H. 1931. Die Chromosomen von *Tipula paludosa* Meig., in Eibildung und Spermatogenese. Z. Zellforsch. 14: 138-193.
- Boss, J. M. N. 1955. The pairing of somatic chromosome. A survey. Texas Rep. Biol. Med. 13: 213-221.
- Breland, O. P., Gassner, III, G. and Riemann, J. G. 1964. Studies of meiosis in the male of the mosquito, *Culiseta inornata*. Ann. Entomol. Soc. Am. 57: 472-479.
- Callen, H. G., and Montalenti, G. 1947. Chiasma interference in mosquitoes. J. Genet. 48: 119-134.
- Cooper, K. W. 1949. The cytogenetics of meiosis in *Drosophila*. J. Morph. 84: 81-122.
- 1951. Compound sex chromosomes with anaphasic precosity in the male mecopteran, *Boreus brumalis* Fitch. J. Morph. 89: 37-58.
- Grell, S. M. 1946a. Cytological studies in *Culex* I. Somatic reduction division. Genet. 31: 60-76.
- 1946b. Cytological studies in *Culex* II. Diploid and meiotic divisions. Genet. 31: 77-94.

- Hughes-Schrader, S. 1943. Polarization kinetochore movements and bivalent structure in the meiosis of male mantids. *Biol. Bull.* 85: 265-300.
- 1947. The pre-metaphase stretch and kinetochore orientation in phasmids. *Chromosoma* 3: 1-21.
- Mescher, A. L. and Rai, K. S. 1966. Spermatogenesis in *Aedes aegypti*. *Mosq. News* 26: 45-51.
- Metz, C. W. 1938. Chromosome behavior, inheritance and sex determination in *Sciara*. *Am. Naturalist* 72: 485-520.
- Moffet, A. A. 1936. The origin and behavior of chiasmata XIII. Diploid and tetraploid *Culex pipiens*. *Cytologia* 7: 184-197.
- Mukherjee, A. B. and Rees, D. M. 1967. The pattern of chromosome morphology and behavior during spermatogonial and meiotic divisions in the three species of mosquitoes present in Utah (Diptera: Culicidae). *Utah Acad. Sci., Arts and Letters*. 43: part 2, 69.
- Pienaar, R. de V. 1955. Combinations and variations of techniques for improved chromosome studies in the Gramineae. *J. South African Bot.* 21: 1-8.
- Rishikesh, N. 1959. Chromosome behavior during spermatogenesis of *Anopheles stephensi* Sensu Stricto. *Cytologia* 24: 447-458.
- Stern, C. 1936. Somatic crossing over and segregation in *Drosophila melanogaster*. *Genet.* 21: 625-736.
- Stevens, N. M. 1910. The chromosomes in germ-cells of *Culex*. *J. Exptl. Zool.* 8: 207-225.
- White, M. J. D. 1946. The cytology of the Cecidomyiidae (Diptera) II. The chromosome cycle and anomalous spermatogenesis of *Miaster*. *J. Morph.* 79: 323-370.
-