MEIOTIC BEHAVIOR OF DIPLOID AND TETRAPLOID *AEDES AEGYPTI* [1]

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INTRODUCTION. The earlier concept of polyploidy being extremely rare in animals needs revision. It has been reported in salamanders, mexican axolotls, earthworms and weevils, etc. Bungenberg de Jong (1957) gives a detailed review of polyploidy in animals. However, when compared to plants, polyploidy is still less frequent in animals. Among mosquitoes, multiplication and reduction of chromosome number has been reported in the larval intestine of *Culex pipiens* (Berger, 1938; Grell, 1946) and in the larval epidermis of *Aedes aegypti* (Risler, 1959). Moffett (1936) observed tetraploid cells with 12 chromosomes in three ovaries and two testes of *Culex pipiens*. Evidence of tetraploidy in the male germ cells of *Aedes aegypti* is presented in this report.

Meiotic behavior of diploid *A. aegypti* is included briefly for comparison. A number of workers have investigated meiosis in various species of mosquitoes, mainly in *Culex pipiens*. The earlier literature has been cited in a recent publication by Mescher and Rai (1966). However, studies on meiosis of *A. aegypti* are scant, being limited to observations on karyotypes (Sinoto and Suzuki, 1942; Akstein, 1962; Rai, 1963; Baker and Aslamkhan, 1969) and a description of spermatogenesis (Mescher and Rai, 1966).

MATERIALS AND METHODS. Mosquitoes were reared at 80 ± 2° F and 80 ± 10 percent relative humidity. Rearing procedures were similar to those described by Craig and Vandehey (1962). Testes were dissected from pupae of varying ages in distilled water and stained in 0.5 percent aceto-lactic-orcein. Temporary squashes were made following the technique described by French *et al.* (1962). Photomicrographs were taken under phase contrast microscope using 35m Ziess camera and panatomic x black and white films.

RESULTS AND DISCUSSION. *Spermatogonial divisions* (Fig. 1A-C). The diploid number of chromosomes is 6. One pair is relatively smaller than the other two. Somatic pairing so characteristic of many dipteran chromosomes begins at early prophase. During later prophase and metaphase the chromosomes contract and lie side by side. The pairing at metaphase is not so intimate as in prophase. Individual chromatids can be made out in prophase (Fig. 1A). Frequently polarization of chromosomes can be observed at later prophase (Fig. 1B). Centromeres can be made out with clarity as unstained circular areas in later prophase and metaphase (Fig. 1B, C). According to Rai's (1963) measurements, the smallest and one of the longer pairs of chromosomes are metacentric and the other long pair submetacentric.

Meiotic divisions in diploid (Fig. 1 D-P). The chromosomes appear distinctly paired at pachytene (Fig. 1D), the earliest visible stage in first meiotic division. Through a process of slow contraction and repulsion of chromosomes, pachytene leads to diplotene (Fig. 1E, F). Doubleness of each chromosome can be seen in Fig. 1E. Chiasma formation gives the diplotene its characteristic appearance. Further contraction leads to diakinesis (Fig. 1G) and metaphase I (Fig. 1H, I) where the bivalents are fully condensed

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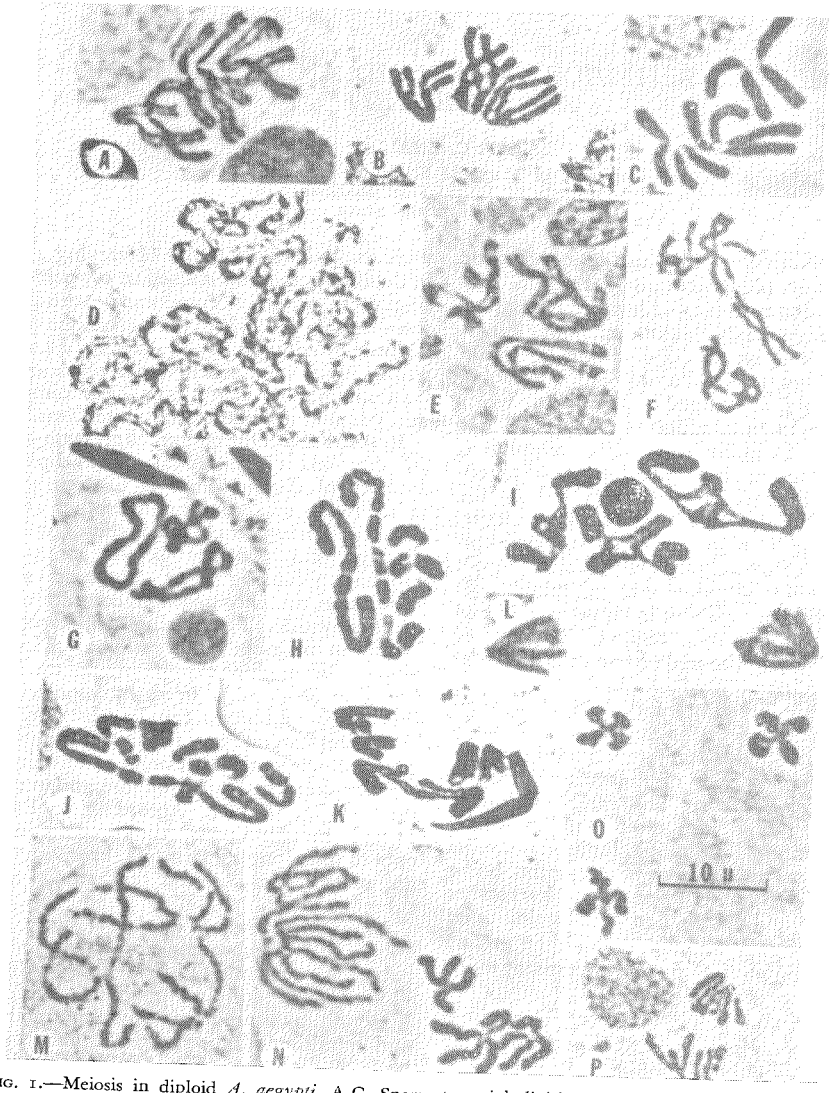

FIG. 1.—Meiosis in diploid *A. aegypti*. A-C. Spermatogonial divisions. D-L. First meiotic divisions. M-P. Second meiotic divisions. A,B. Late spermatogonial prophases; C. Spermatogonial metaphase; D. Pachytene; E. Early diplotene; F. Late diplotene; G. Diakinesis; H. Metaphase I, chiasmata have terminalized in 2 pairs; I. Metaphase I, one chiasma in each chromosome; J. Precocious separation of smallest pair of chromosomes. K. Anaphase I, a developing sperm lies at one pole; L. Telophase I; M. Prophase II, chromatid arms diverging; N. Polarization of arms in one and contracted chromosomes in another prophase II cell. O. Metaphase II; P. Anaphase II.

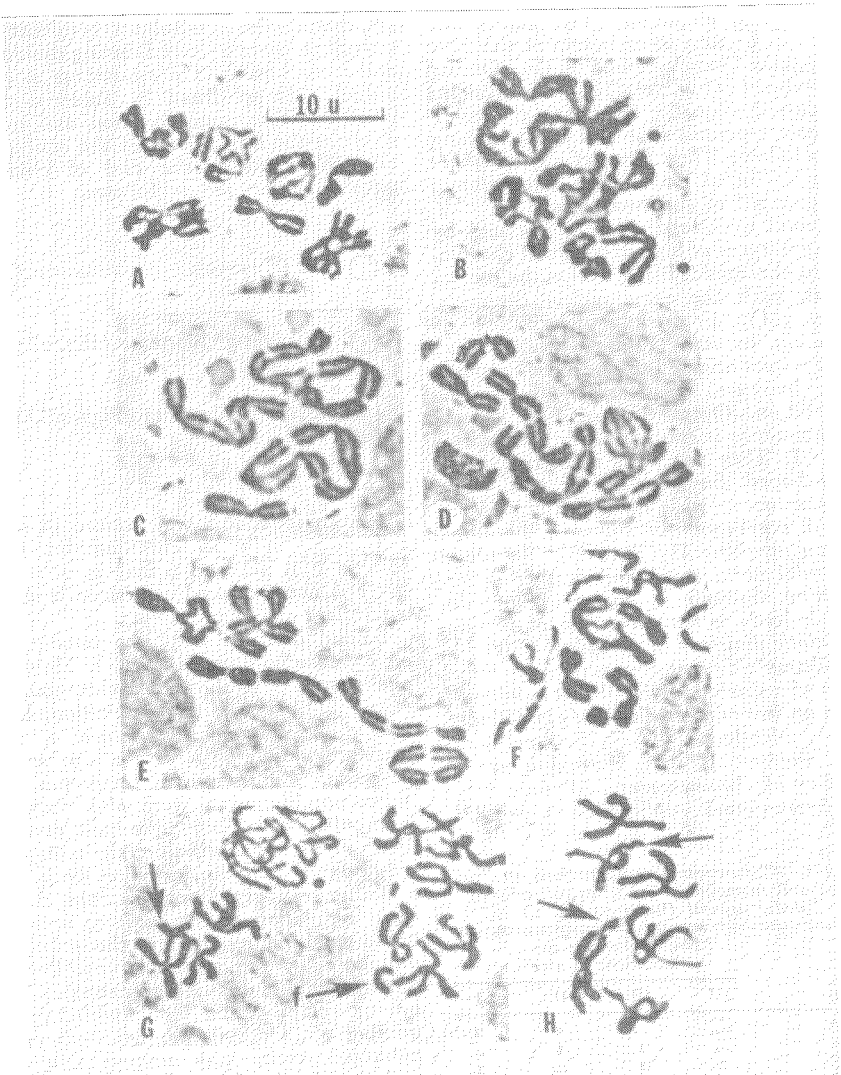

FIG. 2.—Meiosis in tetraploid *A. aegypti*. A-D. Diakinesis and metaphase I cells showing 12 chromosomes, associated as quadrivalents, trivalents, bivalents and univalents. E. A cell with 10 chromosomes; 2 chromosomes have been lost. F. A cell with 6 chromosomes, 6 chromosomes have been lost. G. Prophase II with 3 chromosomes; note fragment (f) and heteromorphic arms pointed by arrow. H. Prophase II with 6 chromosomes; arrows point heteromorphic arms.

and held by chiasmata. Two achromatic gaps can be seen in each arm of the fully terminalised long pair in Fig. 1H. During anaphase I (Fig. 1J, K) the bivalents divide reductionally; frequently the smallest pair separates first and the two long pairs follow. There is a definite telophase I stage (Fig. 1L) where the chromosomes are grouped at the two poles.

Second meiotic division is initiated by the appearance of long prophase II chromosomes, chromatids being held at the centromeres, with the arms widely diverging (Fig. 1M). Frequently polarization of arms can be seen (Fig. 1N) with the 3 centromeres oriented to one side. Polarization, however, disappears with further contraction. Overall size of metaphase II chromosomes is much smaller than metaphase I. They appear as small condensed cross-shaped figures (Fig. 1 O). The chromosomes divide equationally and are pulled synchronously towards the poles during anaphase II (Fig. 1P). Telophase II nuclei are formed and further differentiation through a process of spermiogenesis leads to the formation of sperms.

Meiotic divisions in tetraploid (Fig. 2A-H) Tetraploidy was observed in the pupal testes of a single individual that originated from an inversion stock—*In.(1)2* (Bhalla, 1970). Nearly all the dividing cells, except prophase II cells, showed increased number of chromosomes. All four types of chromosomal configurations possible, i.e., quadrivalents, trivalents, bivalents and univalents were observed during diakinesis and metaphase I. Their numbers in individual cells are listed in Table 1. The bivalents were most common and quadrivalents the least. Some of the configurations observed at the end of chiasma terminalization are as follows:

◯ and ◯′ involve 4 chiasmata (Fig. 2C);

◯ involves 3 chiasmata (Fig. 2E;

| | involves 2 chiasmata (Figs. 2C,D,F).

Chromosome loss was noticed in some cells, e.g., only 10 chromosomes were seen in Fig. 2E, and 6 in Fig. 2F. The latter is not a diploid cell, since it shows a trivalent and 3 univalents.

Second division prophases usually had only 3 chromosomes (Fig. 2G). However one prophase II showed 6 chromosomes (Fig. 2H). The latter observation, however, is not conclusive, since 2 cells squashed closely may give a similar appearance. Chromosomal fragments and heteromorphic arms were also observed in prophase II. These may have resulted from aberrant meiosis since the tetraploid was isolated from an inversion stock.

Reductional regulation, i.e., the downward regulation of chromosome number, has often been reported in the polyploid somatic cells of mosquito larvae (Berger, 1938; Grell, 1946; Risler, 1959). The presence of prophase II cells with haploid number of chromosomes, in an otherwise polyploid testis, may suggest that the typical reductional regulation also occurs in the germ cells. This might be the process by which some species compensate for polyploidy and thereby prevent the evolution or fixation of polyploid strains.

TABLE 1.—Chromosomal configurations at diakinesis and metaphase I in tetraploid cells of *Aedes aegypti* (Refer to Fig. 2)

Fig.	* No. of chromosome configurations in a cell			
	IV	III	II	I
2–A	..	..	5	2
B	..	2	1	4
C	2	1	..	1
D	..	2	2	2
E	..	..	5	..
F	..	1	..	3

* IV, III, II, I refer to quadrivalent, trivalent, bivalent and univalent respectively.

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FIELD AND LABORATORY STUDIES ON THE HOSTS AND VECTORS OF THE SNOWSHOE HARE STRAIN OF CALIFORNIA VIRUS

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INTRODUCTION. Snowshoe hare (SH) virus, which is closely related to California encephalitis virus (CEV), is endemic in certain mountainous areas of the northwestern United States and British Columbia (Burgdorfer *et al.*, 1961; Newhouse *et al.*, 1963). Antibody was found in snowshoe hares and golden-mantled ground squirrels; virus isolations were made from the blood of a snowshoe hare and from ticks removed from a chipmunk, a golden-mantled ground squirrel and a snowshoe hare. Newhouse *et al.* (1963) attempted to infect ticks in the laboratory; however, virus was not recovered from the ticks for more than 48 hours after an infected blood meal.

This paper presents additional serological information on probable SH virus infection in the Northwest. Also, experimental viremia and antibody studies were done in several species of common small mammals to determine which ones might be potentially important in the natural cycle of SH virus. Field studies on mosquitoes in Montana yielded isolations of SH virus from *Aedes fitchii* (Felt and Young) and from *Culiseta impatiens* (Walker).

DESCRIPTION OF STUDY AREAS. Mammal collections were made in several canyons in the Bitter Root Mountains, but primarily in Lost Horse Canyon, approximately 12 miles southwest of Hamilton, Montana. The mouth of this canyon lies about 4,000

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