(I) INTRODUCTION

Besides the well-known painful bites inflicted by the females of mosquitoes, these insects are the proved vectors of several important human diseases. Virtually all cases of malaria, yellow fever, dengue, and certain forms of filariasis result from the bites of mosquitoes which have previously bitten persons who had these diseases ***(Metcalf and Metcalf, 1993)***.

The yellow fever mosquito, *Ae. aegypti* has received more researche than any other mosquitoes. The importance of this mosquito as a disease vector, together with its tractability for laboratory research, including genetic analysis, makes *Ae. aegypti* well suited as a model for laboratory studies ***(Severson et al., 1993 and Knudson et al., 1996)***.

Culicidae includes about 3100 recognized species. Except for the Anopheline mosquitoes, the chromosomal karyotypes of these mosquito species are surprisingly uniform (2n=6). The typical picture, with only minor variations, is three pairs of metacentric chromosomes, 2 larger pairs of about equal size and one pair considerably smaller in total length. In some species one or more of the three pairs may be slightly submetacentric. The length of chromosome pairs often vary in different species. ***Rai (1963, 1966); Baker and Aslamkhan (1969), White (1980) and Rao and Rai (1987).***

A technique for preparing mitotic chromosomes (squash technique) was developed by ***Belling (1926)***. This method was used

for detection of salivary chromosomes of *Drosophila* ***(Painter, 1934)***, for preparing mitotic chromosomes from the brain tissue of mosquitoes ***(Breland 1959, 1961)***. An improvement to this technique was made by ***French et al., (1962)*** using the squash technique for preparing mitotic chromosomes from ovaries and testes.

Fluorescent *in situ* hybridization (FISH), is currently being adapted for use in *Ae. aegypti*, and other mosquito species ***(Knudson et al., 1996)***. Standard FISH techniques were employed for visualization the position of hybridized probes on the extended DNA strands through a fluorescence microscopes ***(Parra and Windle 1993)***. Application of FISH in biological studies has expanded rapidly since introduction of the technique in the late 1970s, ***Rudkin*** and ***Stollar (1977)*** and ***Bauman et al., (1980).*** This technique has several advantages over hybridization with isotopically labeled probes i.e. spatial resolution, speed, probe stability, a variety of probe-labeling schemes are available for simultaneous detection, in different colors in the same nucleus, and the entire genome of a particular species, entire chromosomes, chromosomal subregions can be specifically highlighted depending on the complexity of the probes used probe - labeling and fluorescent reagents are commercially available, making the FISH procedure straightforward and reliable ***(Nederlof et al., 1990*** and ***Trask 1991)***.

Digital imaging is essential in the capture and manipulation of FISH images. Instead of capturing images to photographic film, a camera attached to the microscope directly converts the image into a digital format. A cooled - CCD camera allows the detection of signals

too faint to be seen by eye which can be subsequently enhanced by computer *(**Jovin** and **Arndt Jovin 1989, Jones et al., 1990)*.

The combination of FISH and digital imaging has provided a direct method to map a specific nucleic acid probe onto the total genomic DNA of organism *(**Ferguson et al., 1996)*.

Fluorescence *in situ* hybridization (FISH) physical map demonstrated the utility of FISH digital imaging microscopy and its application to physical mapping in mosquitoes *(**Brown et al., 1995)*. Although it was relatively easy to distinguish the three chromosomes (2n = 6) from each other due to size differences, the chromosomes were not easy to orient, that is the submetacentric centromeres of metaphase chromosomes made identification of the p-terminus or the smaller arm. FISH landmarks are needed so that the three chromosomes could be tagged uniquely and oriented unambiguously in future FISH physical mapping studies *(**Brown** and **Knudson 1997).***

The aim of this work is to prepare *Ae. aegypti*, *Ae. triseriatus*, *Ae. albopictus* and *Cx. pipiens* chromosomes from pupal testes. Cytogenetic studies of the four mosquito species were made to supply elementary data on the chromosome number, length and the position of centromeres. The *Ae. aegypti* chromosome -specific tags (plasmids p1887,p2405 and p2056) and a centric heterochromatin probe (BAC K20.1A5) were used in FISH to other aedine metaphase chromosome spreads.