(10 SUMMARY

The present work covered the following points

1) Obtaining metaphase chromosomes from pupal testes

In this work, metaphase chromosomes from *Ae. aegypti*, *Ac. albopictus*, *Ae. triseriatus* and *Cx. pipiers* were prepared following the standard procedures of *French et al,(1962)* without pretreatment with colchicine. Good results were obtained from individuals, which were collected after 10-12 hours post pupation. Also good preparation of metaphase chromosomes were obtained when dissection was made in the morning and at spring and summer seasons as compared to the preparations made in the afternoon and at winter season.

2) Estimation and cutting DNA with restriction endonucleases

The concentration of the DNA sample was estimated by spectrophotomeric analysis at260 to280 nm using spectrophotometer. The best results are obtained when the ratio between 1.7 — 2.0.

3) Microscopy and digital imaging

By probe combination, pairs (or more) are mapped at the same time on the same slide. This is accomplished by labeling the probes with fluorochrome which emit light at different wavelengths. The *Ae. aegypti* chromosome- specific tags (plasmids p1887, p2405, and p2056) and a centric heterochromatin probe K20.1A5 were labeled with biotin-fluoroscein, the tagging plasmid p2392 which containing the chromosomal tags or landmarks was detected using anti-digoxigenin-rhodamine, and the chromosomes identification was generally done by DAPI counterstain. This allows three different probes to be hybridized and detected on one slide.

Fluorophore images were captured as grayscale and pseudocolored to match the emission wavelength : rhodamine to red, fluoroscein to green, and DAPI counterstain to blue. Images of hybridized probes can be collected with high resolution into computer memory via static charge coupled device (CCD) camera. Each fluorophore signal is generally recorded using a different set of optical filters. It is imperative that each image be obtained in precise registration in order to guarantee the correct spatial representation of each after they are merged to construct the final image.

4) Cytogenetic studies

The diploid chromosome number of all species examined is 2n=6. The centromere position of each chromosome was measured from the terminus of the short arm (p-arm) of the chromosome (pter) relative to the total length of the chromosome and reported as a percent fractional length or%FLpter. The length of the chromosomes and chromosome's arms were measured by microns. The three pairs of chromosomes of the four studied mosquito species can be distinguished by their relative length as well as the position of their centromeres.

Chromosome 1 of *Ae. aegypti, Ae. albopictus, Ae. triseriatus* and *Cx. pipiens* is the . smallest pair, chromosome 2 is the longest chromosome, and chromosome 3 is considerably shorter than chromosome 2. Chromosome 1 is metacentric in *Ae. aegypti* and *Cx. pipiens,* and slightly submetacentric in *Ae. albopictus* and *Ae. triseriatus* . Chromosome 2 is metacentric in *Ae. aegypti* and submetacentric in *Ae. albopictus, Ae. triseriatus* and *Cx. pipiens* Chromosome 3 is submetacentric in *Ae. aegypti* and *Ae. triseriatus* and metacentric in *Ae. albopictus* and *Cx. pipiens*

5) Characteristics of chromosome landmarks

Ae. aegypti pupal testes derived, metaphase chromosomes are used as DNA target in FISH hybridization with the individual components of the p2392 tagging reagent. The p1887 signal produced broad bands with the signal midpoint located at — 70% FLpter. When p2405 was used as a FISH probe, detectable signals were seen on all three chromosomes with the prominent signal on the q-terminus of chromosome 2 at — 96% FLpter, lighter signals were also seen subtelomeric on the p-terminus of chromosome 2, midway on the q-arm of chromosome 1, and subtelomeric on the p-arm of chromosome 3. The p2056 FISH signals were located on chromosome 3 at — 85.7% FLpter.

When these probes are used in hybridization with other aedine metaphase chromosomes, only the p1887 sequence produced a detectable FISH signal in *Ae. albopictus, Ae. triseriatus* or *Cx. pipiens* metaphase chromosomes. When chromosomes 2 and 3 specific probes, p2405 and p2056, were used in FISH to pupal testes metaphase chromosome spreads, signals were not seen, thus, the p2392 tagging reagent would not work as chromosome landmarks in these other aedine species . Although *Ae. albopictus* and *Cx. pipiens* exhibited one ribosomal cistron (p1887) locus per haploid genome, *Ae. triseriatus* had signals on two different chromosomes.

When the centeric heterochromatin probe (K20.1A5) is used in FISH to *Ae. aegypti* pupal testes, strong hybridization signals to chromosome 1 and 3 with a faint signal on chromosome 2 were seen. In contrast, FISH signals were not observed in pupal testes material derived from *Ae. albopictus, Ae. triseriatus* and *Cx. pipiens*