LABORATORY COLONIZATION OF ONE MOSQUITO SPECIES AND CYTOGENETIC ANALYSIS OF TWO GENERA AND FOUR SPECIES IN THE MYRICK MARSH FLOODPLAIN OF LA CROSSE, WISCONSIN

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ABSTRACT

The objectives of this study were: to gain basic information about mosquito chromosome number and morphology; to find out how and to what extent the chromosomes of genera and species found in a geographical area differ from each other and from those areas, genera and species previously examined; and to establish a laboratory colony of a field collected species. Myrick Marsh was chosen as the sampling area because of its annual inundation with the La Crosse River flood waters and its corresponding diversity of vegetation. Such conditions were deemed favorable for mosquito production. Twenty sites were selected based on accessibility and unique habitats. Collection was by the standard dipper method. Larvae were used for karyotyping while pupae were used for colonization. Initial colonization of aedine species was unsuccessful while a field collected Culex pipiens was established and is presently maintained in the laboratory. In preliminary karyotyping attempts the squash technique first developed by plant cytologists and adapted for mosquito studies by Osmond P. Breland in 1959 was used. This method proved ineffective. A second method using colchicine pretreatment was tried. The results improved but the karyotypes were not usable for proper analysis. This latter method was then modified, tried, and was successful. Analysis of the chromosomes of Aedes cinereus, A. excrucians, A. vexans, and Culex pipiens showed uniformity in chromosome number, the diploid chromosome number being six. The chromo-

somes were numbered I to III according to total length; I being the smallest, III the largest. With the exception of chromosome I in Culex pipiens, all chromosomes demonstrated metacentric centromere positioning. Heteromorphic sex chromosomes were not found in the four species examined. Although the chromosome number was uniform, species and genera identification were readily accomplished by using a ratio method of analysis (I/II + III). The ratios found in this study were comparable to those of other investigators for some of the same species.

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INTRODUCTION

Recent investigative results implicated the mosquito Aedes triseriatus (Diptera: Culicidae) populating La Crosse's surrounding wooded areas as the vector for the La Crosse strain of the arbovirus California Encephalitis (Thompson et al. 1972, Watts et al. 1973a, 1973b). Such results exemplify the pestilent nature of mosquitoes. Because of their pestilence, the bionomic, taxonomic, physiologic and vector control aspects of mosquitoes have been extensively studied (Breland 1961, Mukherjee et al. 1966, Rai 1963). However, very little is known about the cytology of Culicidae. Out of a total of 2,680 species belonging to 33 genera and 120 subgenera of this family, cytogenetic information is available for about 120 species (Breland 1961, Aslamkhan and Baker 1969).

Breland (1961) and Kitzmiller (1963) pointed out the importance of mosquito cytogenetic studies. Information relative to chromosome morphology, chromosomal variation, and details of meiosis and mitosis might give an insight into karyotype evolution in mosquitoes; understanding the response of mosquito chromosomes to irradiation, sterilants, and genetic manipulation has already proved invaluable in abatement experimentation (Kitzmiller 1972, Laven et al. 1972, Mc Donald and Rai 1971, Rai et al. 1973).

One objective of this study was to gain more information about chromosome number and morphology. Another objective was to find out how and to what extent the chromosomes

of genera and species found in a specific geographic area differ from each other and from those areas, genera and species previously examined.

A third objective of this thesis was to establish a laboratory colony of a field collected species to facilitate cytogenetic studies during the winter months and to provide a readily available mosquito source for future investigations.

SAMPLING AREA

La Crosse is located in Western Wisconsin at the confluence of the Mississippi, Black and La Crosse Rivers. These rivers annually swell with spring flood waters; the extent of flooding depends on spring climatic conditions and the severity of snowfalls during late winter. Within the center of the city there is a marshy floodplain area that is inundated with the flood waters of the La Crosse River.

Myrick Marsh covers approximately 1500 acres. The study area of approximately 700 acres is bounded by the La Crosse River on the north, the Burlington and Quincy Railroad on the east, the Chicago and Northwestern Railroad on the west, and the Oak Grove Cemetery, Myrick and Leuth Parks on the south (Plate 1). Much of the study area is accessible by a series of interconnecting dikes once used as a service road for municipal water pumping stations. These stations have been closed for some time and the road now serves as a walkway to the marsh and is used by hikers, joggers, bird watchers and others. Because of its accessibility and diversity of

vegetation (Craig 1975, Harris 1975) due to its inundation with flood waters, the Myrick Marsh floodplain was selected for the sampling area (Mallars 1963).

It is hoped that the present study will help stimulate interest in the cytology of the mosquitoes populating the Myrick Marsh floodplain of La Crosse, Wisconsin and encourage the application of the information gained to other investigative areas.

PLATE 1 - Aerial photo of Myrick Marsh study area. Dark snaking line in upper center is the La Crosse River.

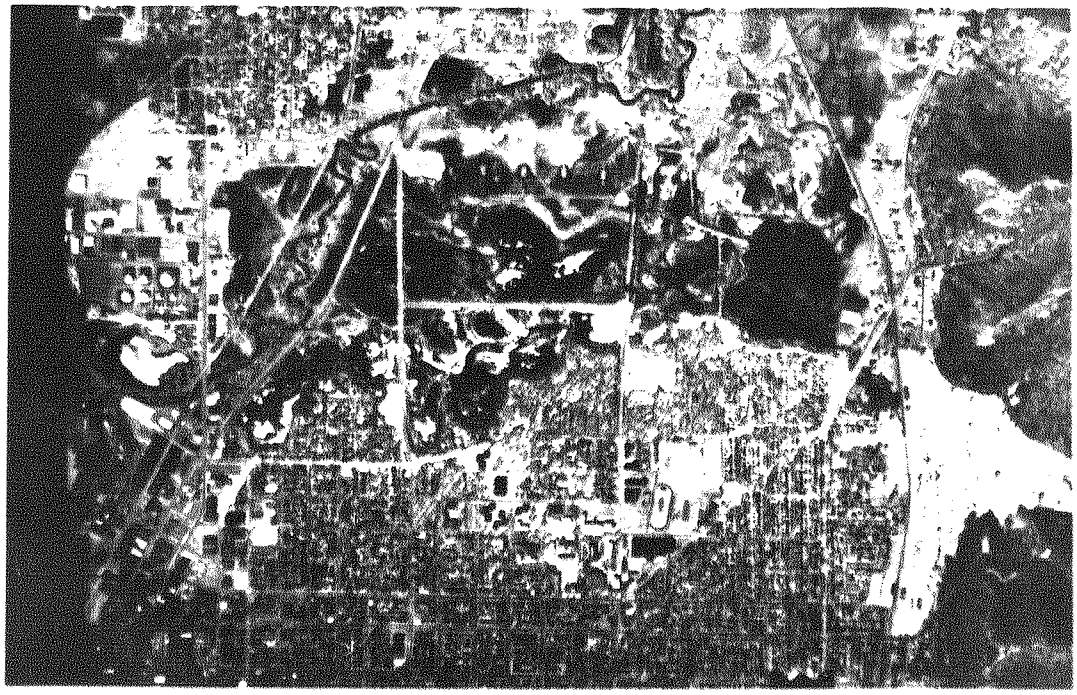

PLATE 1

LITERATURE REVIEW

The enormous proliferation of mosquito and related publications paralleling the increasing magnitude of mosquito studies is overwhelming. Any review of the literature would have to be restricted in scope; therefore, this review is limited to those publications deemed of interest or beneficial for the completion of this study.

Kitzmiller (1963) reviewed the literature on mosquito cytogenetics published from 1953 to 1972. This was valuable as a source list for background material on genetics and karyotyping. Literature on the taxonomy, distribution, and bionomics of North American mosquitoes published from 1955 through 1967 was reviewed by Carpenter in 1968. This was supplemented with a review by Carpenter (1970) of publications from 1967 through 1969. Knight and Pugh (1973) compiled a bibliography of the taxonomic publications of Giles, James and Liston.

Taxonomic background and useful keys were provided by Knight and Wonio's (1969) report on the mosquitoes of Iowa and by Ross and Horsfall's (1965) synopsis of the mosquitoes of Illinois. Both contained excellent diagrams of all stages of mosquito development plus accurate descriptions of those species found in Iowa and Illinois. They were applicable to this study since some of those mosquitoes found in the above bordering states are commonly found in Wisconsin as well. Although Smith's (1969) report of the Aedes mosquitoes of New England would seem irrelevant, it provided pertinent in-

formation on aedine larvae, especially identification by anal plates and saddle hair.

General surveys of Wisconsin mosquitoes conducted prior to 1950 can be found in Allen's (1950) thesis. The species found in La Crosse County include: Anopheles occidentalis, A. punctipennis, A. quadrimaculatus, A. walkeri, Aedes cinereus, A. excrucians, A. triseriatus, A. vexans, Culex apicalis, C. pipiens, C. restuans, C. salinarius, C. tarsalis, Culiseta inornata, and Uranotaenia sapphirina. Other informative studies have been undertaken, the most noteworthy or pertinent to this study being; the larval studies of Northern Wisconsin by Siverly (1968), the association between vertebrate hosts and mosquitoes in Wisconsin presented by Wright (1969) and Wright and De Foliart (1970), and the study of the parous rates by Morris and De Foliart (1971). Analysis by Grimstad and De Foliart (1974) of mosquito feeding compared to the relative densities of the plants flowering at the collection sites revealed definite preferences. Grimstad and De Foliart's study suggests the sequence in time and occurrence of the dominant plants in floral populations provided a continuous vector source for mosquitoes, and flower age and color determined relative attractancy to mosquitoes. In their study, ox-eye daisy (Chrysantheum leucantheum), yarrow (Achillae millefolium), common milkweed (Asclepias syriaca) and goldenrod (Solidago spp.) were dominant plants; each mosquito species changed its preference from one host to another at a specific point in the pre-

ceeding host's flowering cycle. The study also indicated that females preferred the glucose-rich composites (ox-eye daisy, yarrow, and goldenrod), while the males preferred the sucrose-rich common milkweed. A total of 3,752 mosquitoes belonging to 23 species representing 5 genera was collected in Grimstad and De Foliart's study.

Isolation of the La Crosse virus (California encephalitis) during 1963 from the brain in a fatal case of encephalitis in a four year old girl provided impetus for extensive mosquito studies in La Crosse County. These studies have related this disease of children to infection in hardwood deciduous-forested areas and to the mosquito Aedes triseriatus. Thompson et al. (1972) and Watts et al. (1973a, 1973b) determined the La Crosse virus overwinters and maintains itself in the eggs, with larvae and adults emerging from rain water in basal tree-holes in the forested areas, where it remains endemic from year to year. The current status of arbovirus epidemics in Wisconsin and neighboring states was evaluated in a recent Wisconsin State Laboratory of Hygiene newsletter (1975).

Concern for the pestilent nature of mosquitoes precipitates expression of need for their control. Numerous chemical and biological abatement means have been proposed. Some of the publications discussed biological means related to mosquito cytogenetics.

Excellent results have been obtained in abatement of certain Dipteran pests through the introduction of sterilized

males to targeted populations. Kitzmiller (1972) felt this could be used for mosquitoes as well. Other types of genetic control listed by Kitzmiller include cytoplasmic incompatability, recessive lethals, dominant lethals, sex distorters and meiotic drive, hybrid sterility, and chromosomal aberrations.

Laven et al. (1972) demonstrated the use of females heterozygous for a sex linked translocation in the village of Notre Dame, France. Releases were introduced into a water well with naturally controlled temperature, humidity and confinement. Five days after introduction the first egg rafts with semisterility were found. By the end of summer, sterility was found to be as high as 95%; the following April showed the first rafts with 80% translocation sterility.

Mc Givern (1974) discussed sex ratio distortion and interchange complexes in mosquitoes. Translocation homozygotes in Aedes aegypti were examined by Lorimer et al. in 1972 and Rai et al. in 1973. Further studies include the use of computer simulations to determine the potential of heterozygous translocations in population control (McDonald and Rai 1971).

In many cases abatement feasibility investigations involve the rearing of mosquitoes in the laboratory or, as with Laven et al. (1972), the locating of a desired mosquito population. The locating of a mosquito source may involve many factors. Mallars (1963) listed some of these such as observation of irrigation cycles or methods, determination of drainage patterns, visual observations of adult population

influx, and presence of desirable vegetation. Once a source is found, mosquito collection may involve use of oviposition traps (Pratt and Kidwell (1969), of light traps (Bertram et al. 1970), or of a standard collecting dipper. Interesting collection modifications were present in the literature, including one developed by Miura (1970). He described the use of a concentrator dipper to improve the estimation of the larval population density as well as patterns of distribution.

Although the mosquito may exhibit tenacious heartiness in resistance to certain abatement procedures, they are often fragile when attempts at laboratory colonization are made (Gerberg 1970, Mc Lintock 1952). Numerous articles on mosquito rearing were noted but only a few were used as information sources. Nine publications provided information on general and specific rearing and handling techniques. Mc Lintock (1952) commented on the rearing of Culiseta inornata and points out it was not a simple problem. Some of the problems involved handling techniques which were described by Chao in 1959 and elaborated later by Morlan et al. (1963) and Porter (1961).

A more expansive study was done by Belkin et al. in 1965 on the methods for collecting, rearing, and preserving mosquitoes. This was a comprehensive study that included everything from collecting, sorting, and rearing to storing, packing, and shipping. Other studies include those by Edman and Lea (1972), Grimstad et al. (1970), and Lowrie (1968).

The most informative publication was that of Gerberg (1970) printed by the American Mosquito Association as a manual for rearing techniques. Included were construction of cage rooms and cages, temperature and humidity equipment, lighting, equipment for handling all stages of development, food and feeding techniques and induced mating. All phases of mosquito rearing and laboratory colonization were completely covered. Gerberg (1970) pointed out that although references are available each colonization attempt is unique and the problems associated with each vary.

Cage construction and physical dimensions are important factors; a cage must be of large enough size to facilitate an adult population yet must be convenient to clean and to serve live animals to the females for blood meals. A number of cages were described including one by Pollard (1960), a plastic cage by Peach (1971), a disposable bioassay cage by Townzen and Natwig (1973), and one termed "escape-proof" by Mc Cray (1963).

Gerberg (1970) suggested using a quinea pig anesthetized with an intraperitoneal injection of Nembutal for the feeding of blood to the adult females. Elaborate feeders were described in the literature for presentation of nutrients or infectious material to the adults. Rutledge et al. (1964) made one of autoclavable glass with rubber tubing attached, through which was passed warm water for temperature control. The feeding surfaces tried by Rutledge were Baudrauche membrane, chick skin, Saran Wrap and Parafilm M.

Mosquito reproduction information was obtained from two excellent articles. Barr(1974) provided a comprehensive background review on the reproduction of mosquitoes and a brief review of that of biting flies. Special attention was given to follicular development, spermatogenesis, and fertilization. Jones' (1968) work concerned the sexual life of a mosquito. Both articles alluded to factors affecting copulation, insemination, and oviposition.

Wright and Venard in 1967 observed that Aedes triseriatus females with a blood meal would copulate more readily than those which had not had blood. It was also observed that the blood-fed females averaged longer times in the copulating position than did the non-blood-fed females. Baker (1962) described a method of induced or artificial copulation in Anopheles mosquitoes; Ow Yang et al. (1963) maintained a colony of Anopheles maculatus by artificial mating.

Temperature and age were shown by Horsfall and Taylor (1967) to be factors in inducing insemination. Males held at 16°C failed to transfer sperm properly about twice as often as did those held at 30°C irrespective of their ages. Horsfall and Taylor's work indicated that while insemination rates are low, a 50% level of insemination required an age of four days or more in males and more than nine days in females.

Other studies have shown that once inseminated the female will not oviposit her eggs indiscriminately. Certain species will deposit eggs on the water in rafts, others

singly into the water while hovering above it, and still others along the water line on a rough surface. In regard to Aedes triseriatus, Williams (1962) showed that the females respond to wavelengths in the blue portion of the visible spectrum at the time of egg deposition; females were attracted to the oviposition medium of greatest optical density. Williams went on to speculate on the evolutionary significance of this response to media with high optical density. He suggested that it may be a genotypic response to indications of a more favorable habitat for the larvae in terms of stability, food, or both.

Wilton (1968) also examined the oviposition site selection by Aedes triseriatus. The factors investigated were texture of the container wall, orientation of the container opening, optical density of the water, color of the container wall, color of the container background, and presence of decaying matter. He found that open-top containers received more eggs than did side-opening containers, that there was a preference for rough texture, that dark water was preferred, and that reflectance of the substrate played an important role in oviposition behavior. It was also shown that containers with a black background received more eggs than those with gray or white backgrounds. Presence of organic decay matter elicited a greater oviposition response both when no physical contact was allowed with only olfactory stimuli being present and when actual physical contact to the water surface was allowed.

Once the eggs are deposited, their development is influenced by photoperiod and temperature (Mc Haffey 1972). In nature the eggs can fail to hatch in response to a hatching stimulus as a result of seasonal conditions (diapause) or immediate, transient physical factors (deconditioning). Mc Haffey found that in testing Aedes vexans eggs exposed to a 16-hr. photoperiod, those from 14.5 hour females produced a greater hatch than did those from either the 16 or 13.5 hour females. Temperatures of 32°C or more nullified any photoperiod effect and increased the percentage of hatch over the optimum temperature of 25°C. Suboptimum temperatures of 10°C or less greatly increased the percentage of diapause.

Akov (1962) and Asahina (1964) studied food material, nutritional requirements, and feeding procedures for mosquito larvae. Clay and Venard (1972) correlated nutritional and temperature effects on photoperiodic induction in larval diapause. A low nutritional diet aided in slowing metabolism and inducing diapause. High nutritional diets induced scum formation and was implicated as a source of larval mortality. When larvae were overfed and overcrowded (Hwang et al. 1974) they demonstrated an autoregulation via synthesis of branch-chained fatty acids referred to as "larval control factors".

Diapause, deconditioning, oviposition site selection, and other variables may be assumed to be genetically regulated (Williams 1962). Numerous studies have been conducted

and articles written on mosquito genetics. Naturally occurring gyandromorphs and intersexes were discussed in the literature by Brust (1966). Eye color mutations were presented by Dennhoefer in 1971 and genetic studies of two mutants in linkage group III of Culex tritaeniorhynchus were published by Baker and Sakai in 1972.

The artificial induction of some mutations can be achieved through irradiation and subsequent inbreeding of mosquitoes (Jost and Laven 1971; Bhalla 1970, 1973). To further elucidate mosquito mutations and genetics, the chromosomes can be studied through karyotypes.

Analysis of mosquito chromosomes using the squash technique developed by plant cytologists was first performed by Breland (1959). Most of his work was with Orthopodomyia signifera, O. alba, Culiseta inornata, Toxhorynchites rutilus septentrionalis and Culex quinquefasciatus. Although many tissues were examined, brain tissue from larvae in the fourth instar stage was emphasized. Breland (1959) used brain tissue because it had been used successfully for routine chromosome surveys in Drosophila. In his studies, Breland felt the most successful studies could be made when the brain cells were in active stages of division. In mosquitoes rapid cell divisions occur intermittently, therefore it was important that the stage in the life cycle selected for study was one in which brain cells are undergoing division. This stage occurs during the last larval stage or instar just prior to pupation.

In his 1959 study, Breland observed mitotic figures in brain tissue of Orthopodomyia signifera and O. alba prepupae; the diploid chromosome number of both species was six. Breland reported finding "good salivary gland type chromosomes" in the Malpighian tubules of Toxonrychites rutilus septentrionalis; mitotic figures were found in the brain tissue from Culiseta inornata prepupae. Only two Culex quinquefasciatus larvae were reported available; therefore, the results were inconclusive.

Breland in 1961 expanded his cytogenetic investigation to include prophase and anaphase chromosomes of Aedes canadensis as well as the metaphase chromosome complements of 22 other species as follows: Culex coronator, C. erraticus, C. quinquefasciatus, C. restuans, C. solinarius, C. tarsalis, C. territans, Aedes atropalpus, A. hendersoni, A. sollicitans, A. triseriatus, A. vexans, A. zoosophus, Orthopodomyia alba, O. signifera, Haemagogus equinus, Anopheles pseudopunctipennis, Culiseta inornata, Psorophora confinnis, P. discolor, P. longipalpus, Toxorhychites rutilans septentrionalis, and Uranotaenia syntheta. In all species studied the diploid number of chromosomes was found by Breland to be six (2n = 6). He also noted the chromosome complements of the species examined at metaphase were quite similar.

French et al. (1962) in his publication acknowledged the outstanding stimulus Breland provided to the study of mosquito chromosomes. French gave details of special techniques used in his laboratory that seemed to him gave better

results than those of Breland.

He stated that the key to consistantly good preparations was the use of a colchicine pretreatment which blocked cell divisions and stock-piled abundant figures on the same slide. The length of pretreatment for brain tissue analysis varied from two to four hours depending upon the species.

All dissections were made in a small drop of hypotonic sodium citrate solution which, French stated, produced a slight swelling of the chromosomes. Dissections were made on silicone treated slides placed on a cold surface. The placement of the silicone-treated microscope slide on a cold surface according to French "apparently enhanced spreading and staining" of the chromosomes.

French reported brain, testis and ovary treated as described above produced up to a 100-fold increase in usable figures per slide.

K. S. Rai published a comparative study of mosquito karyotypes in 1963. He followed Breland's (1959, 1961) protocol for the squash technique analysis of the morphology of mitotic chromosomes from brain tissues of fourth-instar larvae in 12 species of mosquitoes. Included in this study were Anopheles quadrimaculatus, Wycomyia smithii, Culex pipiens, C. restuans, C. territans, Aedes togoi, A. vexans, A. albopictus, A. aegypti, A. atropalpus, A. stimulans, and an unidentified species of Corethra. The diploid chromosome number of all species examined except Corethra (2n = 8) was six. Based on the position of the centromere and length,

individual chromosomes were numbered from I to III (I to IV in Corethra).

Rai compared the chromosomes of the different species in terms of a ratio of chromosome size; the size of chromosome I was variable in different species and so was the ratio of chromosome I to chromosome II and III. This ratio was lower in Culex than Aedes.

Rai continued his studies on mosquito cytogenetics and in 1966 published an article on the morphology of mitotic chromosomes from brain tissue of six species: Eretmapodites chrysogaster, Aedes sierrensis, A. mascarensis, A. polynesiensis, A. simpsoni, and A. vittatus. In all of these he reported the diploid chromosome number was six, the complement consisting of two relatively large pairs and one slightly shorter pair. Rai observed two chromosomal aberrations in A. simpsoni: a trisomic cell which Rai felt was probably a result of mitotic nondisjunction, and a cell displaying heteromorphism of the smallest chromosome. In this publication, comparison by chromosome ratio was not reported.

A study of the karyotypes of some mosquitoes present in Utah was conducted by Mukherjee et al. (1966). The techniques used were those described by Rai (1963). The temporary slides were sealed with nail polish. Photomicrographs were taken from temporary slides seven to ten days after preparation.

Mukherjee stated chromosome studies were made from not less than ten individual larvae in each species; eight to

ten mitotic figures were examined in each species. The smallest chromosome pair was designated as chromosome I and the largest pair as chromosome III following the convention set by Rai (1963).

Eleven species collected in Utah from April 1962 to October 1965 were described in Mukherjee's report published in 1970. The procedures used in this study were the same as in the previous study(Mukherjee et al. 1966).

Two studies pertaining to Myrick Marsh were conducted by Craig (1975) and Harris (1975). Craig investigated the ecology of the area and emphasized the animal population while Harris studied the avifauna. Both included information on the diverse habitats and vegetation of the floodplain; relevancy of such information to mosquito populations was discussed by Mallars (1963) and Grimstad and De Foliart (1974) as previously cited.

MATERIALS AND METHODS

COLLECTION SITES

Sampling sites were selected on the basis of accessibility and environmental conditions deemed suitable for mosquito population (Mallars 1963). Initially sixteen sites were chosen with four more being added for the second year's study in attempts to collect more specimens and obtain species not included in the first year's investigation. Sampling site locations are shown in Figure 1 while descriptions for each are listed in Table 1.

COLLECTION TECHNIQUES

Visual inspection of the shallow water at each site was made. Larvae and pupae were often seen at the water's surface. Standard methods of collection utilizing a small porcelin pan taped to a wooden dowel handle were employed. The collection specimens were placed either in plastic vials with the collected water or in glass containers filled with a preservative consisting of six parts methanol, three parts chloroform, and two parts propionic acid (Rai 1963). Each vial was then marked with the date and sampling site number.

Specimens used in karyotyping and colonization were identified in the laboratory as to genus and species using standard mosquito taxonomy keys (Knight and Wonio 1969, Ross and Horsfall 1965). The preserved specimens were placed in a refrigerator at $4^{o}C$ for later karyotyping. The remaining live larvae were used for immediate karyotyping while the pupae were allowed to develop into adults for colonization.

FIG. 1 - Sampling site locations. Roman numerals and dashed lines define areas described by Craig (1975).

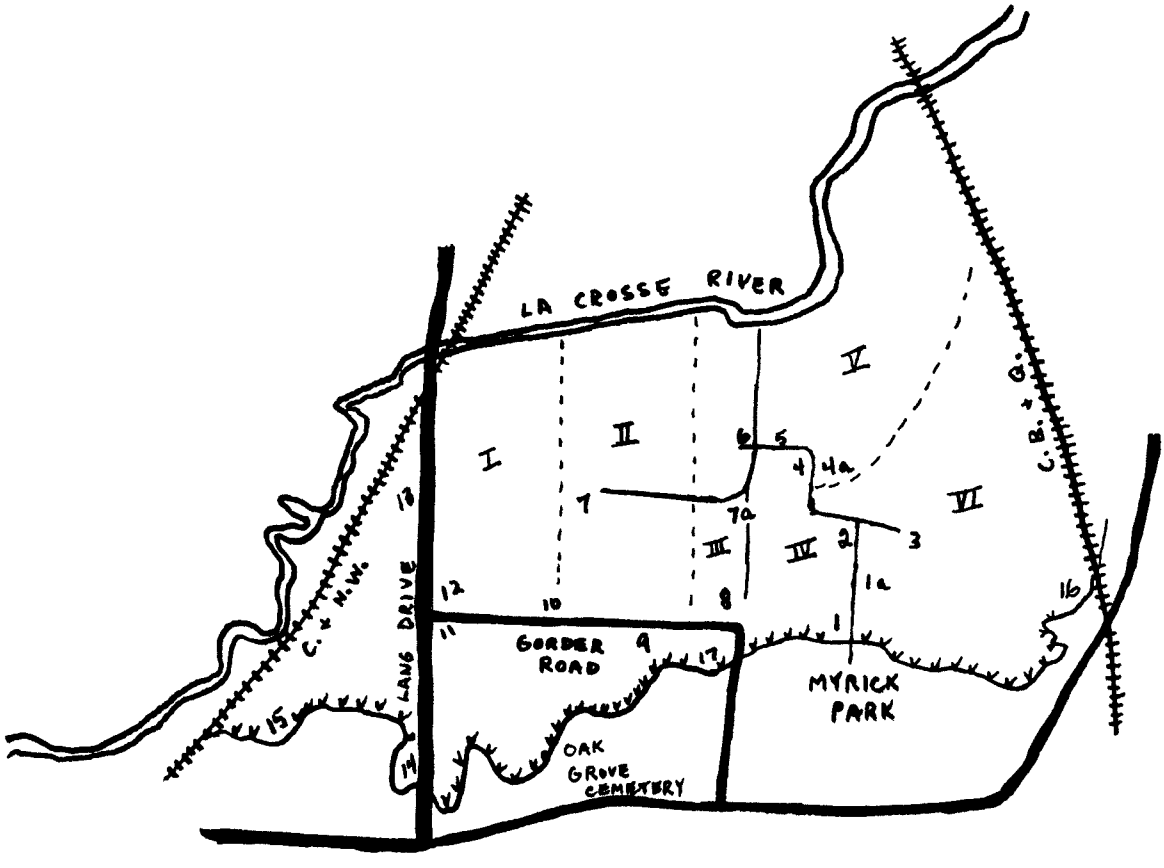
LA CROSSE RIVER
I
II
III
III
IV
V
VI
LANG DRIVE
GORDER ROAD
MYRICK PARK
OAK GROVE CEMETERY
C. & N.W.
C. B. & Q.
1
1a
2
3
4
4a
5
6
7
7a
8
9
10
11
12
13
14
15
16
17
18

FIG. 1

Table 1 - Collection Sites

Site #	Location*	Dominant Vegetation Present	Time of Day Shade Present	Comments
1.	Entrance to access road	Reed canary grass Shrub willows Marsh grass	Late afternoon Evening	Small pond of open water; overgrown with algae in late summer
1a.	Small ravine along access road	Reed canary grass Hardwoods	Early morning Late afternoon Evening	Protected by high grass and trees.
2.	Along access road	Reed canary grass Cattail	Early morning Evening	On end of large pond of standing water; protected by grass.
3.	At end of one section of access road	Reed canary grass Cattail Duckweed Hardwoods	Late afternoon Evening	On edge of open water protected by hardwoods and cattail.
4.	At turn in access road	Reed canary grass Canary grass	Early morning	Small shallow pond interspersed with grasses.
4a.	At turn in access road	Reed canary grass Canary grass Hardwoods	Late afternoon Evening	Shallow water amongst the grasses; some protection provided by hardwoods.
5.	Along access road	Reed canary grass Sedges	Evening	Open pond of water deeper than 2 and 4.
6.	At intersection of two sections of access road	Shrub willow Hardwoods	All day	Very protected by shrubs and larger hardwoods; perminant pond interspersed with hardwoods.

Table 1 - (Continued).

Site #	Location*	Dominant Vegetation Present	Time of Day Shade Present	Comments
7.	Western most point on access road	Shrub willow Reed canary grass Cattail Smartweed	Morning	Protected from morning sun by shrub willows and small trees.
7a.	At intersection of two sections of access road	Shrub willow Cattail	All day	Very protected and shaded by shrubs and shrub willows.
8.	Off Gorder Road at curve	Shrub willow Reed canary grass Small hardwoods	Early morning Evening	Area around this site has been used for dumping trash, grass clippings, and rip-rap.
9.	Off Gorder Road near a UW-L building	Cattail Smartweed Reed canary grass	Early morning	Protected by gravel embankment of Gorder Road; shade provided by shrub willow and small hardwoods lining Gorder Road.
10.	Off north side of Gorder Road	Cattail Smartweed Reed canary grass Shrub willows	Early morning Late afternoon Evening	Slight open water along edge of bank.
11.	At intersection of Gorder Road and Lang Drive; south side of Gorder Road.	Shrub willow	All day	Very protected by shrub willow and embankment off Lang Drive; semiperminant pond formed by outlet water from culvert.

Table 1 - (Continued).

Site #	Location*	Dominant Vegetation Present	Time of Day Shade Present	Comments
12.	At intersection of Gorder Road and Lang Drive; north side of Gorder Road	Reed canary grass	Evening	Open pond of water surrounded by grasses.
13.	Along edge on west side of Lang Drive.	Reed canary grass Smartweed	Morning	Slight open water along edge of bank.
14.	Along north edge of Leuth Park	Hardwoods	All day	Protected by hardwoods lining shoreline of the park.
15.	Near Chicago and Northwestern Railroad tracks; along edge of residential area	Hardwoods Cattail	Morning Early afternoon	Down steep embankment in open water along edge of tree covered ground; area littered with trash, old tires, and tin cans.
16.	Eastern most sampling site near railroad line	Hardwoods Cattail Shrub willow	Morning Early afternoon	Open water near sandy shoreline; protected from morning sunlight by shrubs and small hardwoods.
17.	Along edge of cemetery and marsh	Hardwoods Cattail Reed canary grass Smartweed	All day	Hardwoods line edge of cemetery; collections made along stone wall separating cemetery and floodplain.

* See Figure 1.

COLONIZATION

A colonization cage measuring 8"W x 18"H x 24"L was made from plywood and aluminum screening. The two wooden ends and bottom were painted with white enamel paint to allow easy cleaning. A door at each end enabled placement of larger objects into the cage. Two holes (one in each door) covered with a cloth sleeve provided access to the cage without loss of adults.

Protocols for colonization differ according to the genus of the mosquitoes used (Belkin et al. 1965, Gerberg 1970). Although standard procedures for different genera and species appear throughout the literature, they must be modified to achieve success as each colonization attempt is unique (Gerberg 1970). The protocols with their modifications used in this study are described below.

Aedine eggs are deposited along the water line on rough surfaces at the oviposition site (Morlan et al. 1963, Williams 1962). To collect aedine eggs in this laboratory, dark strips of semirough material were placed in glass bowls filled with distilled water. These were checked daily for eggs. After oviposition the eggs were allowed to remain in the adult mosquito cage for twenty-four hours. This initial period of conditioning is necessary in aedine eggs for proper development (Belkin et al. 1965, Clements 1963). The eggs were then allowed to dry at 80% relative humidity (RH) and 23°C and were transferred directly to moistened petri dishes and stored for future use. Even though aedine eggs

can withstand complete desication, they were stored in a moist condition to increase viability (De Foliart, personal communication).

To initiate hatching, the stored cloth strips with the attached row of eggs were placed in a 1:1000 nutrient broth prepared with sterile distilled water. (Sterilization of water rearing pans and associated equipment was for reduction of contaminating bacteria.) Hatching began within six to ten hours and usually lasted twenty-four to forty-eight hours depending on the eggs' viability. The first instar larvae were transferred by means of a pipette to clean porcelin pans filled with tap water. The larvae were allowed to mature in the tap water with finely ground Tetramin (a commercial staple food) being added as a nutritional source. Scum formation thought to be caused by anaerobic bacteria and enhanced by excess food (Gerberg 1970) was controlled through the use of an aquarium aerator and strict adherence to a feeding schedule which limited the food quantity supplied to the larvae.

Once the larvae matured to the early fourth instar stage as distinguished by physical size and coloration, some were placed in a preservative/fixative (Rai 1963) to be used for chromosome analysis. The remainder were allowed to remain in the rearing pans for pupation.

Pupae were then transferred by pipette to plastic "emerging vials" where they were allowed to mature to adulthood, emerging into the screen cage.

Adult males were the first to emerge from the pupae vials; the females usually emerged from four to ten hours later. Adults were maintained on apple slices and a small quantity of honey for their carbohydrate source.

Gravid females obtained a blood meal from access to a guinea pig (Jones 1968). The guinea pig's abdominal fur was clipped with standard electric barber's clippers and then a cream dipilatory was used to remove any remaining fur. At first the guinea pig was simply laid on top of the cage and held there while the mosquitoes attempted to feed. However, this proved to be inefficient and time consuming since the guinea pig was too active to permit many mosquitoes to feed. Those who did manage to feed took considerable time because they were interrupted by constant twitching of the guinea pig. It was decided to use an anesthetic for the guinea pig to eliminate the afore mentioned problems. Nembutal (.75 grains) was used at an intraperitoneal dosage of 0.54cc per one kilogram guinea pig body weight (Gerberg 1970).

Environmental variables such as temperature, humidity and photoperiod which affect the viability and fecundity of mosquitoes (Horsfall and Taylor 1967, Mc Haffey 1972) were mechanically regulated in the early colonization attempts by a Sherer (Model CEL25-7HL) controlled environmental chamber.

Whereas laboratory aedine eggs are deposited in rows along the water line on cloth strips of proper texture and color, Culex eggs are deposited singly or in rafts (Plate 2) on the water's surface. This eliminates the need for special

PLATE 2 - Egg raft of *Culex pipiens*.

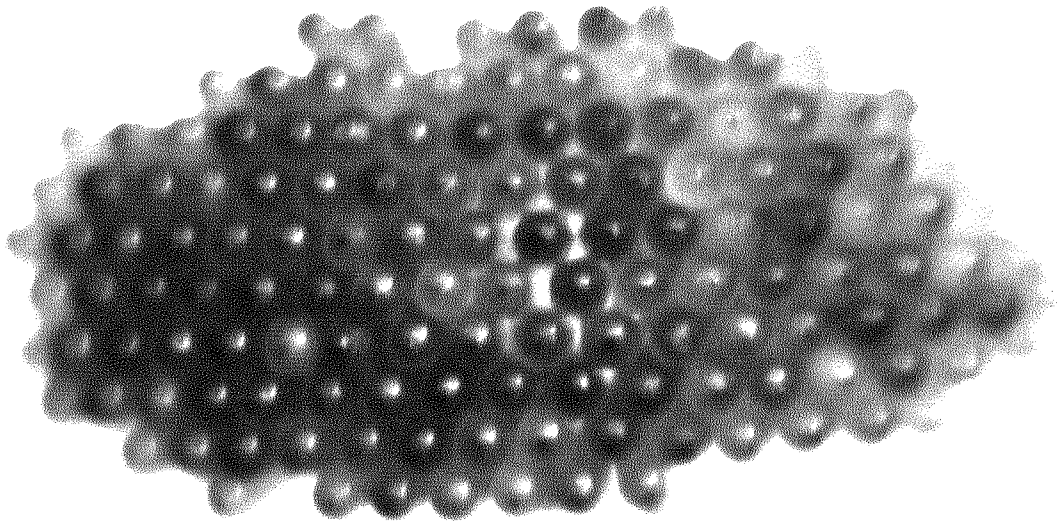

PLATE 2

attention required by aedine eggs; the same glass or plastic containers filled with tap water can provide humidity, nutrients, and serve as an oviposition site.

Culex adults were fed on honey from a small petri dish over which was placed a wire mesh to provide a platform for the feeding adults and to prevent their sticking to the honey. An anesthetized guinea pig was placed on the screen cage. Female mosquitoes obtained their blood meal by feeding on the guinea pig through the screening.

Eggs were removed a few hours after being laid and placed in plastic trays filled with either tap or marsh water. Nutrient broth was not needed to induce hatching.

Larvae from the eggs were fed Tetramin and were reared in the plastic trays used for hatching. Some larvae were allowed to mature to adulthood and were used in maintaining the colony. Others were either preserved (Rai 1963) or used immediately for karyotyping.

KARYOTYPING

Using techniques cited in the literature review, chromosomal analysis of the mosquito species found in Myrick Marsh was attempted. A third protocol, modified from the above two, and used as the final procedure is described below.

Microscope slides were cleaned with soapy water, dipped in acetone, and rinsed with distilled water. Dried slides were dipped in Sigmacote (Sigma Chemicals) silicone and rinsed with double distilled water. After air drying they

were stored at 4°C along with all of the reagents used in the final protocol.

The early fourth instar larval stage was determined by the presence of white areas (From which the pupal trumpets differentiated) located dorsally on the thorax (Plate 3). Live larvae of correct age were placed in a 0.1% colchicine (K and K Chemicals) solution and allowed to incubate for six hours at room temperature. A larva was placed in a drop of cold (4°C) hypotonic sodium citrate solution on a coated, refrigerated slide where the head was removed.

Using dissecting needles, the head was transferred to a drop of modified Carnoy's fixative (equal parts of 45% acetic acid and 95% ethyl alcohol) where the brain was dissected out. Such a procedure was carried out by placing a needle in the larva's mouth to secure the head while a second needle was placed in the mouth above the first. The second needle was pushed anteriorly causing the head capsule to split laterally.

The brain was found to lie transversely across the head capsule with the size and shape varying with the species and stage of development. It usually had two-spindle-shaped lobes partially divided by a constriction; from these lobes nerves pass to the eyes.

The brain was transferred to the center of the slide with as little extraneous debris as possible. Excess fixative was removed with care so as not to draw the tissue into the filter paper and there become lost.

PLATE 3 - a. Prepupal stage of a mosquito showing position of one pupal trumpet designated by the arrow. This is a late fourth instar as indicated by the dark color of the trumpet. Early prepupa used for karyotyping are recognized by white areas on the thorax from which the pupal trumpets develop.

b. Pupa with trumpets indicated by arrows.

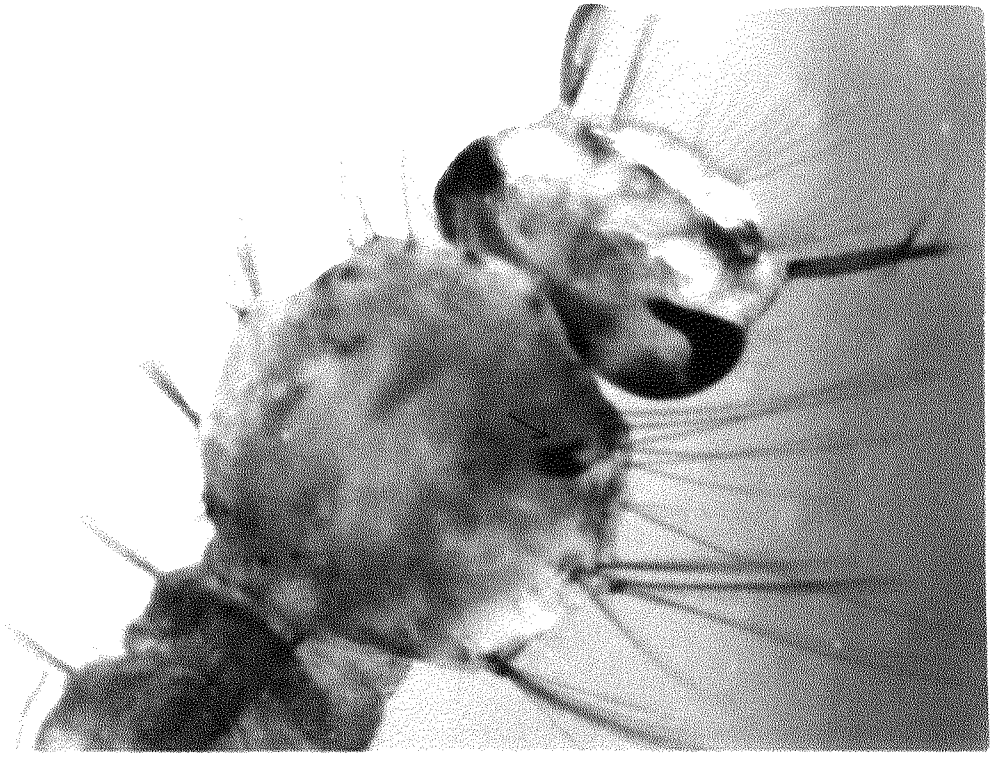

PLATE 3 - a.

PLATE 3 - b.

A drop of aceto-orcein stain was added to the brain. To prevent evaporation of the stain, a glass ring was placed around the drop of stain and a coverslip was placed on top of the ring. After staining for 30 minutes, the excess stain was removed and a cold ($4^{\circ}C$) drop of acetic acid was added. Excess acid was removed with filter paper and a clean coverslip was placed over the brain tissue. The brain was squashed and the coverslip was sealed with ordinary nail polish.

Photomicrographs were made from the temporary slides using 40x dry and 100x oil immersion lenses; the age of the slide preparations at the time of photomicrographic analysis varied from 1 hour to 2 weeks. High contrast, Panatomic-X or Kodachrome II Professional Type A film was used.

The chromosomes were studied for number, configuration, and length. Chromosome lengths in microns were determined by the ocular micrometer of the microscope; calibration of the ocular micrometer used was performed by using a standard stage micrometer.

RESULTS AND DISCUSSION

COLONIZATION

Colonization of field collected specimens was not achieved during the first year of study. During the mid and late summer of 1974 very little rainfall occurred (Harris 1975) allowing the existing vegetation and many sampling sites to dry; such conditions are not favorable for mosquito production (Hall 1972, Mallars 1963, Mc Donald et al. 1972, Woodard and Fukuda 1971). Since colonization was not the primary objective of this study, the limited number and variety of specimens that were collected were used for chromosome analysis.

During the winter of 1974 a supply of Aedes triseriatus eggs was obtained from the entomology laboratory of Dr. De Foliart, University of Wisconsin-Madison. These were used in the establishment of the aedine colonization protocol described previously. Limited colonization was achieved with many problems being encountered.

Even with the control of anaerobic bacteria, the larval mortality was high. Once hatched the larvae may have been overcrowded and produced a sufficient amount of "overcrowding" factors have lethal effects. Possibly the restricted diet maintained for control of scum formation was insufficient for proper development.

Irregular maturation and ecdysis was another problem encountered. Changing the temperature and photoperiod from the 23°C and 16 hours recommended by Dr. De Foliart (personal

communication) helped but never completely eliminated the problem. Diapause may have been induced in some larvae (Mc Haffey 1972).

It appeared copulation was taking place as confirmed by visual observation, however fecundity was low. Gravid females did not oviposit on the cloth strips provided them indicating either the texture or the color or both were unsuitable.

Changes of the factors mentioned by Wilton (1968) and cited in the review of the literature that affect ovipositioning by Aedes triseriatus females were not analyzed since the colony abruptly and completely died off after a power failure in the environmental chamber during the spring of 1975.

Colonization attempts were made with Culex pipiens collected in 1975 from site #11. Instead of rearing the larvae in an environmental chamber, they were allowed to mature in marsh water placed in plastic trays near the window of the laboratory. Thus they were at constant room tempearture with a natural photoperiod; humidity for the emergent adults was provided by water filled containers used for both ovipositioning and as a water source for the mosquitoes. By following the remaining protocol cited in materials and methods colonization was successful. Culex colonization was easier since limiting factors for ovipositioning such as surface color and texture, optical density and container configuration were eliminated. The colony still required

attention i.e. daily changing of nutrient source, cage cleaning, and egg raft maintenance. The colony is flourishing at the time of this writing (October 1975).

KARYOTYPING

During the first year of this study, chromosomal analysis attempts were made using Breland's technique as cited in the review of the literature. The results were very poor. Not only did the slides frequently stain improperly but mitotic chromosomes were not found. Even with the use of synthetic orcein instead of natural orcein as recommended by Breland (1961) the staining inconsistancies were not eliminated. Also with this technique, difficulties in bursting the cells, even when varying degrees of pressure were applied, were encountered. Perhaps a main reason for poor success was my inexperience in larval dissection and in judging the proper stage of development required for increased numbers of rapidly dividing cells.

French's (1962) protocol as cited in the literature review was used during the early summer of 1975. Until a supply of colchicine was obtained, Velban and Colcemid were used for pretreatment; the results were the same as those with Breland's (1959) technique. Difficulty in bursting the cells was again encountered. With the use of a series of sharp taps along with a 6 hr. pretreatment with colchicine, an increase in mitotic figures was noted; however, the slides were of such poor quality that chromosomal analysis could not be made (Plate 4).

PLATE 4 - a. Brain cells of *Aedes vexans* using French's (1962) protocol for karyotyping.

b. Brain cells showing chromosomes of *Aedes vexans* using French's (1962) technique.

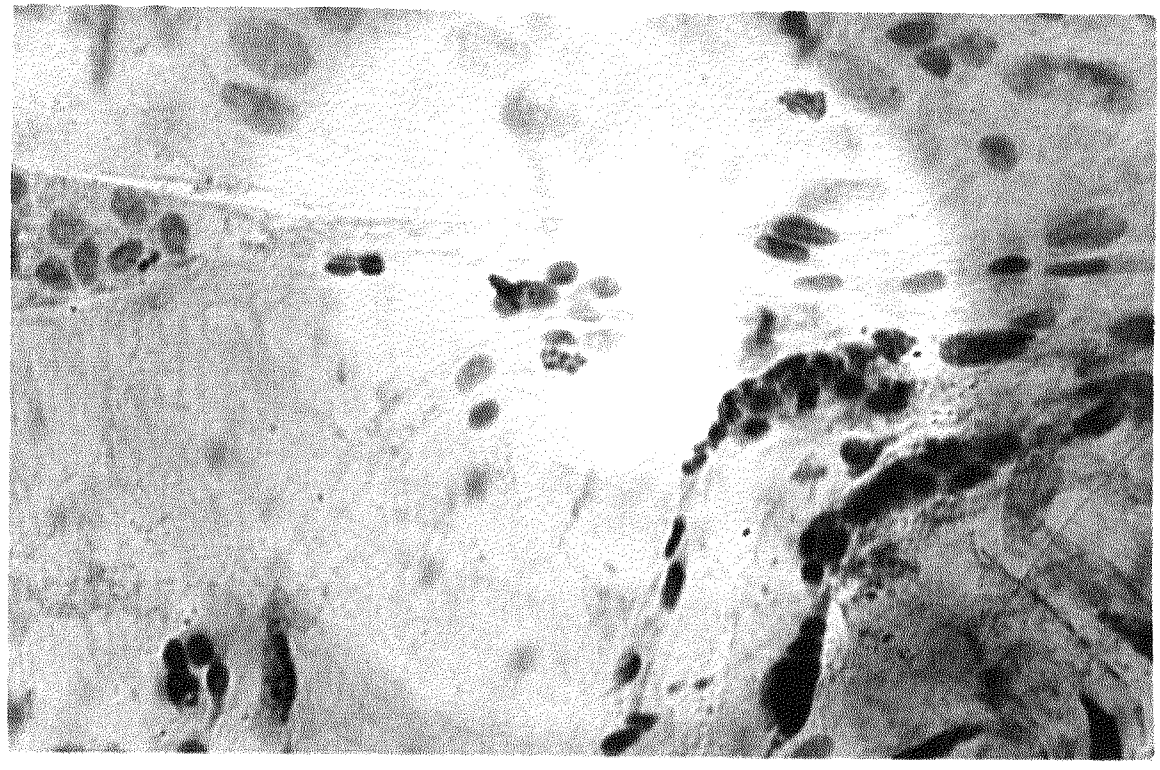

PLATE 4 - a.

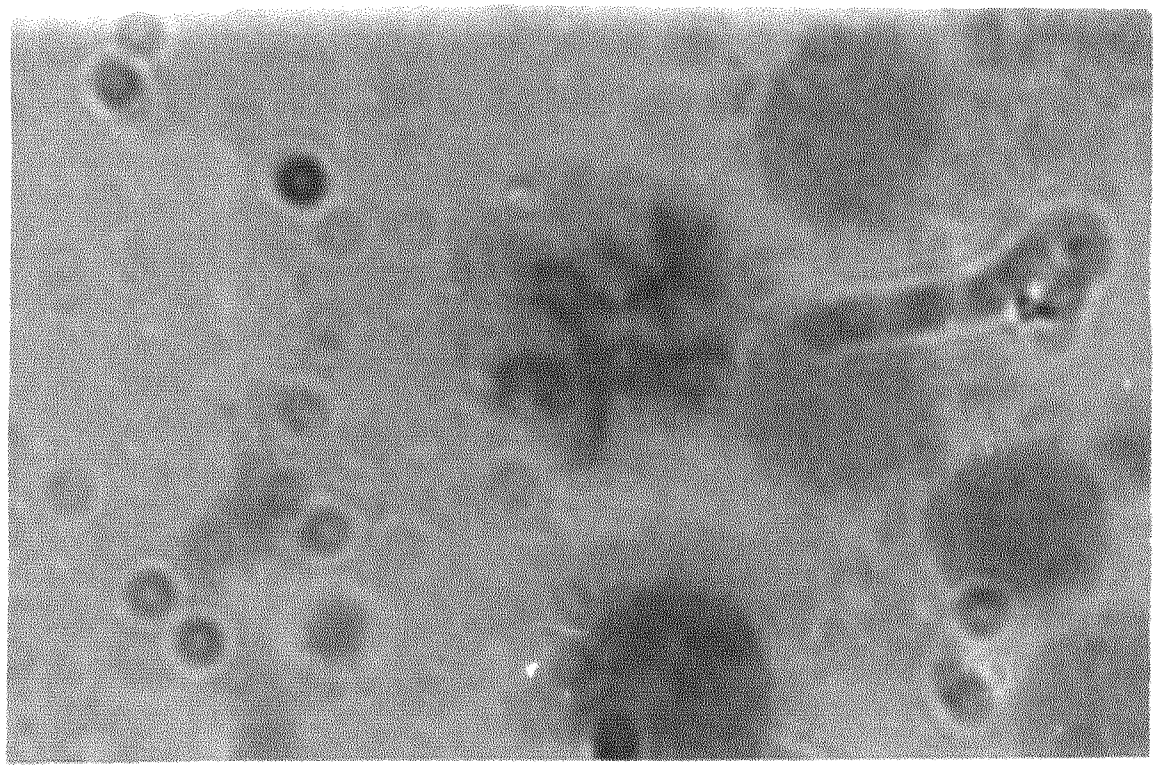

PLATE 4 - b.

By modifying the French (1962) technique as cited in the materials and methods section, success was achieved. The average number of mitotic figures per slide increased from 0 and 2, using Breland (1959) and French (1962) protocols respectively, to 10. Proper cell bursting and chromosome staining enabled good visualization of the brain cell chromosomes (Plates 5 and 6).

I agree with French's (1962) observation that the pretreatment with colchicine indeed increased the number of mitotic figures per slide. But whereas he felt that spreading and staining was enhanced by dissection on a silicone treated microscope slide placed on a cold surface, I felt that better results were obtained by using a similarly coated cold ($4^{o}C$) slide placed on a warmer ($25^{o}C$) surface. This improvement may have been due to the temperature of the slide. Such a differential may have been enhanced by the use of cold reagents all of which were kept at $4^{o}C$.

It was also felt that improved results may have been due to increased staining time; from 30 seconds proposed by French (1962) to 30 minutes.

Not only did this improve the detection of the chromosomes by better staining, but excess stain removal required the use of more 45% acetic acid which when applied cold ($4^{o}C$) to the tissue was often enough to burst the cells and increase spreading.

Using the modified French technique chromosome analysis was performed on four species in two genera of mosquitoes

PLATE 5 - a. Brain cells of *Aedes vexans* showing what appears to be cleavage or breaks in the chromosomes.

b. Brain cell chromosomes of *Aedes vexans*.

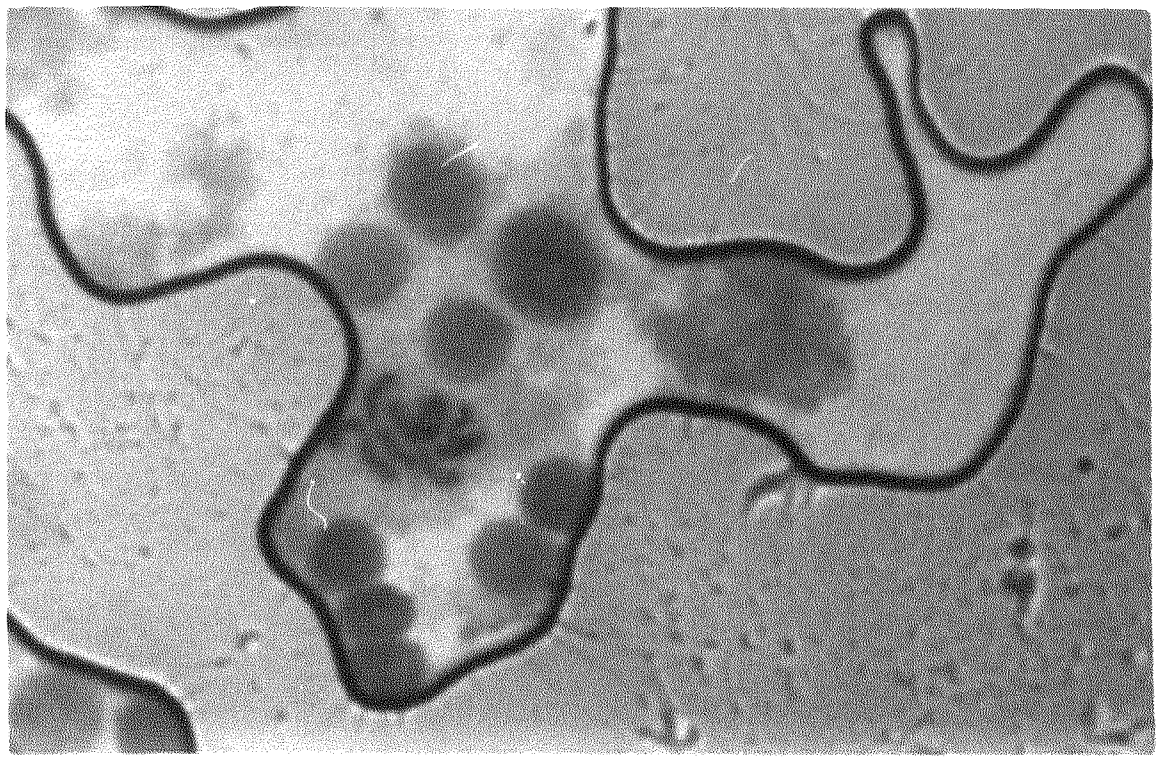

PLATE 5 - a

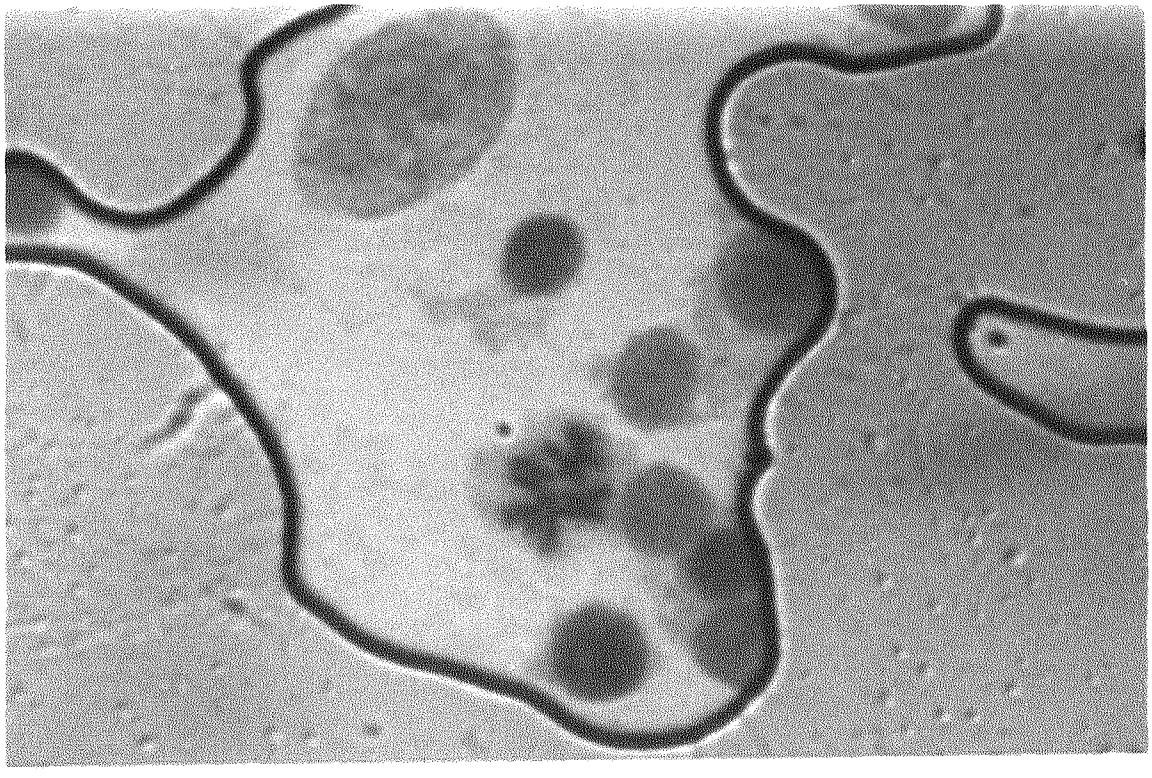

PLATE 5 - b

PLATE 6 - a. Chromosomes from brain tissue cells of Culex pipiens.

b. Cell showing anaphase chromosomes of Culex pipiens.

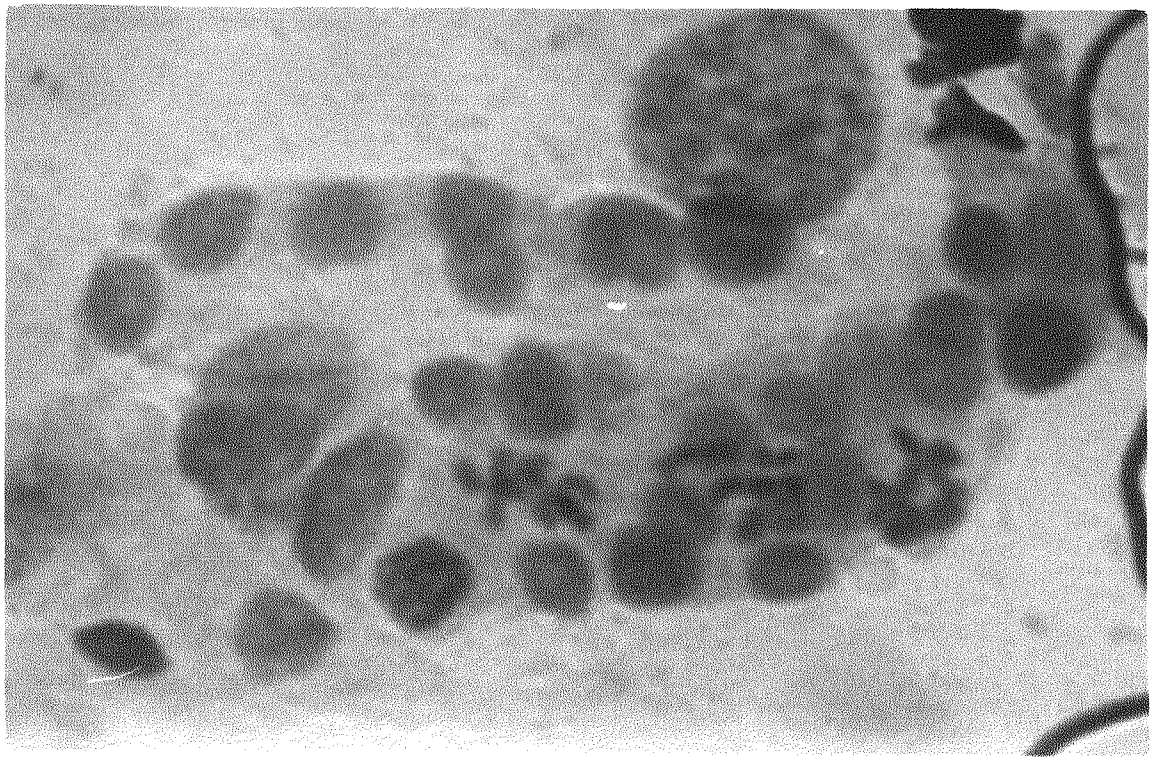

PLATE 6 - a

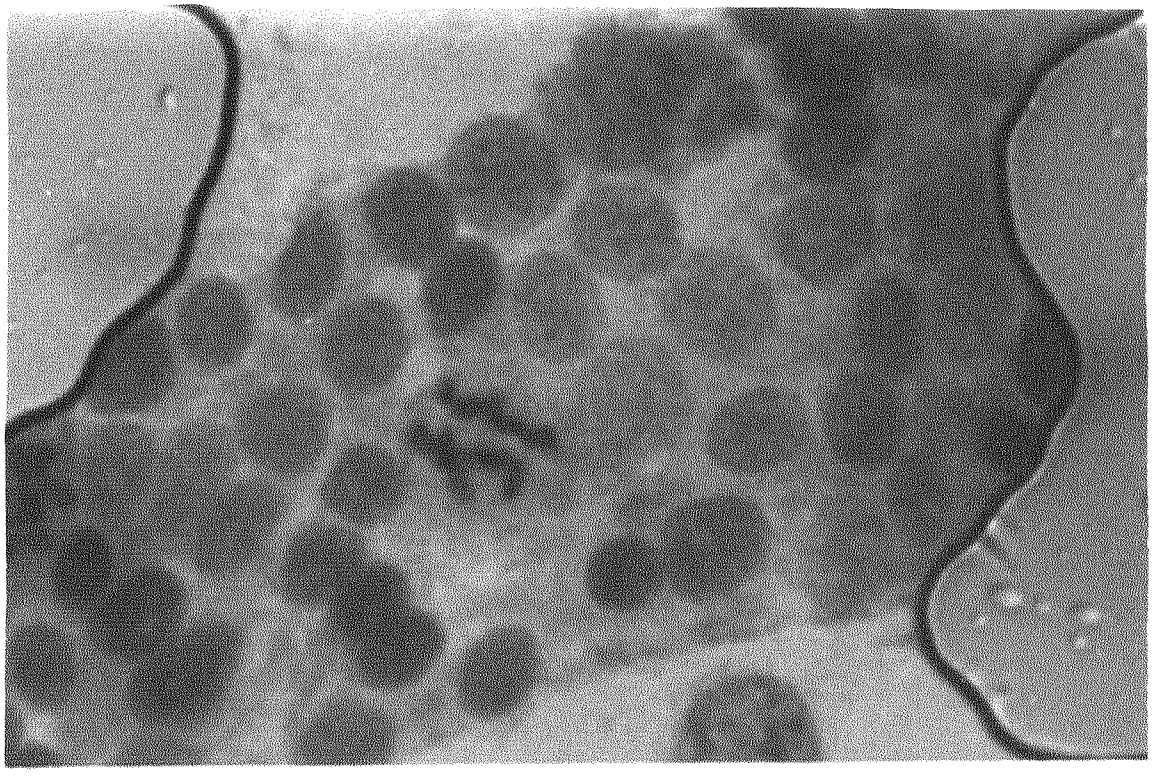

PLATE 6 - b

collected from Myrick Marsh; Aedes cinereus, A. excrucians, A. vexans, and Culex pipiens. Analysis of Aedes cinereus revealed the diploid number of chromosomes was six; all were metacentric. Lengths of chromosomes were measured in microns; the average of these measurements are listed in Table 5.

Aedes excrucians displayed longer chromosomes; like A. cinereus the diploid number was six and all displayed metacentric centromere position (Table 5).

Aedes vexans displayed the longest chromosome length (Table 5); chromosomes form this species showed more of what appeared to be breaks or chromosome cleavage (Plate 5a).

Culex pipiens displayed shorter chromosomes than those of the aedine species (Table 5); anaphase figures were found in this species more than the aedines (Plate 6b). Rai (1963) questioned whether the centromere of chromosome I of C. pipiens was metacentric or submetacentric (Table 2). If the criteria set by Levan (cited by Bianchi et al. 1972) is followed, the chromosome is metacentric; metacentric chromosomes are those with arm ratios ranging from 1 to 1.7. However, chromosome measurements of the system proposed by Levan were not employed in Rai's (1963) investigations (Bianchi et al. 1972). Therefore following previous convention I would agree with Mukherjee et al. (1966, 1970) and say the chromosome is submetacentric based on the differences found in arm lengths in chromosome I compared to those found in the other two chromosomes (Tables 2, 3, 4, and 5).

Table 2 - Measurements of metaphase chromosomes in ten species of mosquitoes (adapted from Rai 1963).

Species	CHROMOSOME I			CHROMOSOME II			CHROMOSOME III			
	Position of the centromere	Length in microns arm a	arm b	Position of the centromere	Length in microns arm a	arm b	Position of the centromere	Length in microns arm a	arm b	Ratio of length of I to II + III
Wycomyia smithii	M	2.3	2.3	M	2.9	2.9	M	3.1	3.1	0.383
Culex pipiens	SM?	1.3	1.1	M	2.1	2.1	M	2.5	2.5	0.261
C. restuans	M	1.5	1.5	M	2.7	2.7	M	3.1	3.1	0.259
C. territans	M	1.3	1.3	M	2.1	2.1	M	2.7	2.7	0.271
Aedes togoi	M	1.5	1.5	M	2.3	2.3	M	2.7	2.7	0.300
A. vexans	M	2.7	2.7	M	3.8	3.8	M	4.2	4.2	0.337
A. albopictus	M	3.1	3.1	M	3.8	3.8	M	4.6	4.6	0.369
A. aegypti	M	2.7	2.7	SM	3.8	3.1	M	3.8	3.8	0.373
A. atropalpus	M	3.1	3.1	SM	4.6	3.8	M	4.6	4.6	0.352
A. stimulans	M	3.8	3.8	SM	6.1	4.6	SM	6.1	5.4	0.342

M = Metacentric; SM = Submetacentric

Table 3 - Measurements of metaphase chromosomes of nineteen species of mosquitoes in Utah (from Mukherjee 1966).

Species	CHROMOSOME I			CHROMOSOME II			CHROMOSOME III			
	Position of the centromere	Length in microns arm a	arm b	Position of the centromere	Length in microns arm a	arm b	Position of the centromere	Length in microns arm a	arm b	Ratio of length of I to II + III
Aedes cataphylla	M	4.0	4.0	M	5.4	5.4	M	6.3	6.3	0.342
A. campestris	M	4.5	4.5	M	5.6	5.6	M	6.1	6.1	0.384
A. dorsalis	M	4.5	4.5	M	5.9	5.9	M	6.4	6.4	0.363
A. increpitus	M	3.6	3.6	M	4.4	4.4	M	5.0	5.0	0.372
A. hexodontus	M	4.0	4.0	M	5.5	5.5	M	5.6	5.6	0.360
A. nigromaculis	M	3.4	3.4	M	5.0	5.0	M	5.6	5.6	0.320
A. niphadopsis	M	5.6	5.6	M	7.2	7.2	M	8.5	8.5	0.358
A. spencerii	M	6.1	6.1	SM	6.1	7.7	M	7.1	7.1	0.347
A. vexans	M	4.9	4.9	M	6.1	6.1	M	9.2	9.2	0.326
Anopheles free-borni	SMX	1.6	3.5	M	4.4	4.4	SM	5.1	5.3	0.265
	TY	2.8								0.145
Culex erythro-thorax	M	2.5	2.5	M	4.0	4.0	M	4.5	4.5	0.294
C. pipiens	SM	2.6	2.3	M	4.2	4.2	M	5.1	5.1	0.263
C. salinarius	M	3.6	3.6	M	5.7	5.7	M	6.4	6.4	0.297
C. tarsalis	M	2.7	2.7	SM	4.3	4.5	M	4.7	4.7	0.296
C. territans	M	2.7	2.7	M	4.2	4.2	M	5.4	5.4	0.281
C. thriambus	M	2.1	2.1	M	3.5	3.5	M	3.9	3.9	0.283
Culiseta impatiens	M	5.7	5.7	SM	7.2	6.9	M	7.8	7.8	0.383
C. inornata	M	4.1	4.1	SM	4.5	5.4	M	6.0	6.0	0.374
C. incidens	M	4.5	4.5	SM	6.3	5.9	M	6.7	6.7	0.351

M = Metacentric; X = X-Chromosome; SM = Submetacentric; Y = Y-Chromosome; T = Telocentric.

Table 4 - Measurements of metaphase chromosomes of eleven species of mosquitoes present in Utah (from Mukherjee 1970).

Species	CHROMOSOME I			CHROMOSOME II			CHROMOSOME III			
	Position of the centromere	Length in microns arm a	arm b	Position of the centromere	Length in microns arm a	arm b	Position of the centromere	Length in microns arm a	arm b	Ratio of length of I to II + III
Aedes implicatus	M	3.8	3.8	M	4.8	4.8	M	5.6	5.6	0.365
A. pullatus	M	3.9	3.9	M	5.1	5.1	M	5.9	5.9	0.354
A. sierrensis	M	2.5	2.5	M	3.9	3.9	M	4.2	4.2	0.308
A. varipalpus	M	2.7	2.7	M	4.0	4.0	M	4.4	4.4	0.321
Anopheles earlei	SM	1.5	2.4	M	3.1	3.1	M	4.2	4.2	0.267
	T	2.5								0.107
A. franciscanus	SM	1.7	3.1	M	4.2	4.2	M	4.8	4.8	0.206
	T	2.8								0.105
Culex apicalis	M	2.3	2.3	M	3.9	3.9	M	4.2	4.2	0.284
Psorophora signipennis	SM	3.6	3.9	SM	5.2	5.5	M	6.2	6.2	0.324
Aedes cinereus	M	2.6	2.6	M	4.0	4.0	M	4.3	4.3	0.313
A. fitchii	M	3.5	3.5	M	5.1	5.1	M	5.6	5.6	0.375
A. impiger	M	4.0	4.0	M	5.1	5.1	M	5.9	5.9	0.364

M = Metacentric; SM = Submetacentric; T = Telocentric.

Table 5 - Measurements of metaphase chromosomes of four mosquito species.

Species	CHROMOSOME I			CHROMOSOME II			CHROMOSOME III			
	Position of the centromere	Length in microns arm a	arm b	Position of the centromere	Length in microns arm a	arm b	Position of the centromere	Length in microns arm a	arm b	Ratio of length of I to II + III
Aedes cinereus	M	2.5	2.5	M	3.9	3.9	M	4.2	4.2	0.309
A. excrucians	M	3.7	3.7	M	5.6	5.6	M	6.1	6.1	0.315
A. vexans	M	5.0	5.0	M	6.2	6.2	M	9.0	9.0	0.325
Culex pipiens	SM	2.5	2.3	M	4.1	4.1	M	5.2	5.2	0.262

M = Metacentric; SM = Submetacentric.

It should be pointed out that the measurements of arm length may vary with the researcher's technique and that gradual chromosome contraction throughout prophase and metaphase makes it difficult to measure chromosomes of two species at exactly the same stage. It is also difficult to measure accurately very small chromosomes or those with bent arms.

To provide an index for comparison of species, Rai (1963) used the ratio of chromosome length of chromosome I to the total lengths of chromosome II and III (Table 2). Such a ratio would eliminate the variation of measurements found by different researchers and by the same investigator analyzing the chromosomes of cells of the same species in different stages of development. Mukherjee et al. (1966, 1970) followed Rai's (1963) example and used this ratio for analysis in his investigations (Table 3 and 4).

Such ratios were calculated in this study and are listed in Table 5. As seen in Table 6 the ratios determined by different researchers are in fairly close agreement; the difference in the calculated ratios for Culex pipiens are less than for those of the aedine species. The significance of such differences cannot be determined statistically by conventional hypothesis testing ($Ho: u_1 = u_2$, $H_1: u_1 \neq u_2$) because such a test requires knowledge of the varience of the population (ratios or chromosome lengths) and these were not provided in the publications cited. If a pooled estimate of varience is used, the population tested must be proved to be

Table 6 - Ratio of lengths of chromosome arms I/II + III.

Species	Rai	Mukherjee	Wick
Aedes cinereus	*	0.313	0.309
A. excrucians	*	*	0.315
A. vexans	0.337	0.326	0.325
Culex pipiens	0.261	0.263	0.262

* not reported

normally distributed. Again such analysis requires data not presented in the literature cited. Nevertheless, species and genera identification is readily accomplished by using Rai's (1963) ratio method of analysis.

Most studies reveal a constancy for the diploid chromosome number. This uniformity is interesting considering the variety of ecological niches occupied by mosquitoes. From the evolutionary point of view, the lack of variation in the chromosome number of Culicidae is compensated to some extent by karyotypic variability. Although the number of chromosomes has remained constant some investigators feel numerous changes have taken place within the chromosomes. Rai (1963) felt that gross changes in whole chromosomes or chromosome complements have not played any part in speciation. In Rai's opinion the possibility exists that the mechanisms of speciation may be different in different genera and that some of them particularly Anopheles may have undergone more chromosomal rearrangement than genera such as Aedes. Rai further points out that the aedines may have depended more on point mutations. More mutants are known in Aedes aegypti and fewer in Anopheles and Culex (Rai 1963, 1966).

This study did not indicate anything to the contrary. The diploid number of chromosomes was found to be six in all the species studied; nevertheless, distinct karyotypic differences among genera and species were observed (Table 5).

An interesting problem in mosquito cytogenetics is the question of sex determination. Heteromorphic sex chromosomes

have been observed in only one genus Anopheles (Rai 1966, Mukherjee et al. 1970). It has been suggested by White (1949, cited by Mukherjee et al. 1970) that the mosquito karyotype evolved from the tiploid type by the incorporation of smaller sex chromosomes into one of the pairs of autosomes. Some species of Tipulidae possess four pairs of chromosomes, three large pairs of autosomes and a pair of very small sex chromosomes (Breland 1961). The question of sex determination was not resolved in the present study. Anopheles specimens were not collected and consequently not analyzed; no heteromorphic sex chromosomes were found in the four species studied.

SUMMARY

Twenty sampling sites were selected on the basis of accessibility and location in a defined area where environmental conditions were deemed favorable for mosquito production. Sampling sites were established along the periphery of Myrick Marsh floodplain and along a man-made access road extending into the marsh. Availability of larvae and pupae of various mosquito species was dependent upon climatic conditions and the cyclic inundation and recession of flood waters during 1974 and 1975.

Colonization of field collected aedine species was not achieved; it was thought the numerous requirements by gravid females for proper ovipositioning sites precluded success. Culex pipiens collected during 1975 was colonized and is still demonstrating favorable fecundity.

Chromosomal analysis was attempted using three techniques; one by Breland (1959) who initiated the use of the squash technique in mosquito cytogenetic analysis, a second by French et al. (1962) who used colchicine pretreatment to arrest cell division, and lastly a modified French technique. The first two proved ineffective in producing desirable results: staining was inconsistant, few if any mitotic figures were found on the slides, and those that were found were not usable for analysis.

By using the third method, the diploid number of chromosomes was determined to be six in Aedes cinereus, A excrucians, A. vexans, and Culex pipiens. The smallest chromo-

some pair was designated as chromosome I and the largest pair as chromosome III.

Position of the centromere was metacentric in all chromosomes except chromosome I of Culex pipiens. The question as to the classification of this Culex pipiens chromosome seems to be dependent upon the classification system used.

Heteromorphic sex chromosomes and chromosomal satellites were not detected. Although the chromosome number was the same in all species studied, species identification was accomplished by the ratio of the length of chromosome I to the total of II plus III. These ratios adequately descriminated the karyotypes of the various species collected from Myrick Marsh and were comparable to the ratios determined by others for some of the same species.

The uniformity of chromosome number is striking considering the variety of ecological niches occupied by mosquitoes. Rai (1963) proposed such uniformity suggests gross chromosomal changes did not play a part in mosquito karyotype evolution and that point mutations and rearrangements are implicated. I feel more cytological information on different species from different geographical areas including Myrick Marsh is necessary to clearly define the process of karyotype evolution and speciation in mosquitoes.

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