MATERIAL AND TECHNIQUE

In all, twelve species of mosquitoes (Diptera: Culicidae) constituted the research material for the present investigations. Out of them, six species belong to the genus Anopheles, four to Culex and one each to Aedes and Armigeres. These species are as follows:

1. Anopheles (Cellia) stephensi Liston.
2. Anopheles (Cellia) culicifacies Giles.
3. Anopheles (Cellia) turkudsi Liston.
4. Anopheles (Cellia) willmorei James.
5. Anopheles (Anopheles) giorni Giles.
7. Culex (Culex) bitaeniorhynchus Giles.
8. Culex (Culex) spicattus Theobald.
9. Culex (Culex) pipiens Nee.
10. Culex (Lutzia) furcatus Wiedemann.
11. Aedes (Finlaya) pseudoscutatus Giles.

The vital information regarding the seasonal prevalence of each species, area of distribution, various natural breeding places, diurnal and nocturnal adult resting sites, feeding habits and relation to malaria and other diseases was gathered from the publications of Christophers (1933),
Barraud (1934), Horsfall (1935), Covell (1944), Russel et al. (1943), and Kuma (1929). The places of collections of the various species listed above are shown in the map (Fig. 2).

Anopheles stephensi is widely distributed throughout India, Pakistan, Burma and China (Williams, 1941), Bahrain, Iceland (Persian Gulf), Iran and Iraq. It is commonly found in houses, cowsheds and suchlike constructions and breeds under natural conditions in pools, river and stream beds, irrigation channels, wells and miscellaneous water collections with clean water and small pools having lots of algae. This species is an important vector of malaria in rural conditions but lately it has assumed the status of an urban vector breeding in overhead tanks of the buildings, leaking water meters and other household collections. For the present studies a wild population of A. stephensi was collected from the village complex of Nadesshib, 20 km. South-east of Chandigarh.

Anopheles culicifacies is prevalent throughout the Indian region, Baluchistan, Pakistan, Ceylon, Burma, Thiland and China. In India it is abundant in the foothills of North-western Himalayas, but is not found beyond the moderate altitudes. A. culicifacies breeds in clean fresh water pools along the streams often with sandy margins and little vegetation, irrigation channels, rice fields, shallow
wells and pools in stream/river beds. The adults of this species are found resting in cowsheds and human dwellings. In most of the regions it is exclusively rural in its prevalence (for example, Union Territory of Chandigarh).

With regard to its relation with disease *A. culicifacies* is the most important vector of India, especially North-west region, where it is particularly notorious as the causative agent of the regional malaria epidemics of great severity and wide range (Covell, 1944; Pal, 1945). Presently it was collected from the various localities around Chandigarh, Nahan (Himachal Pradesh), Jammu and Dehradun (Uttar Pradesh).

*Anopheles villiersi* is abundant along the Himalayan foot hills between the altitudes of 2,000 - 8,000 ft. The larval breeding is common in the beds of hill streams, whereas the adults are found inside houses of the villages. Its role of a malaria carrier is doubtful but can be judged from the accounts of its type form *A. megulatum* which is not a vector of any significance in India. But it is an important vector in Malaya, Java and Philippines. For the present investigations its larvae were collected from Kotla nulla at Solan (H.P.) and very rarely from Nadesahib.

*Anopheles turkuddi* is prevalent in India where it is restricted to the North-Western regions including Gujrat.
It breeds in pools with excessive algal growth and in stream beds, whereas the adults rest inside cowsheds. It is a vector of very limited importance. For the present work, a few specimens could be found in April and May from the villages Burj and Nadasahib near Chandigarh, because of its very restricted seasonal prevalence.

*Anopheles giae* is an alpine species recorded only from high altitudes, usually 6,000 ft or above (Himalayas, Nilgris and other hills of South India). It is a wild species which normally does not frequent village houses. *A. giae* breeds in fresh water springs, marshy places, shallow trickling hill streams and water collections in the gardens. Nothing is known about its capability to act as a transmitter of malaria in alpine climates. For the present investigations, a massive hunt was carried out for it along Jammu-Srinagar high way and all along the banks of the Lidder river at Pahalgam (9,500 ft) and Gulmarg (9,000 - 9,500 ft). Its larval breeding was seen in discarded flower beds in the premises of a hotel at Gulmarg. Some adult specimens and larvae were collected from salon.

*Anopheles petitesentis* is also a rare species belonging to the *Anopheles hyrcanus* group. Considered earlier as a synonym of *hyrcanus* it has an oriental distribution only. Presently it was collected from Dehradun and the hills near Chandigarh, where it appears only in the
months of April and November.

*Culex bitaeniorhynchus* has a distribution similar to that of most *Culex* species and breeds in open woody pools where its larvae are found amongst green algae to which they resemble in colour. Like *C. fatigans* it is also responsible for the transmission of filariasis. In the present studies it was collected from Burj in the foot hills of Kalka (Haryana).

*Culex spicatus* is widely distributed from Punjab to Assam and southwards up to Kerala and Madras in India. Outside India it is available only in Ceylon. The larvae were available in water rich in algal growth and that collected in hoofmarks.

*Culex pimocticus* is common from Himalayas to the hills in south India, Burma, China and Ceylon. Although it is a hill species yet occasionally larval breeding has been seen in the planes where its availability is the same as that of *C. spicatus*. For the present requirements, its larvae were collected from the river Gheger near Chandigarh.

*Culex fuscatus* is common over large parts of India including Andamans, Burma, Ceylon, Africa, and Central and South America. The sources of larval breeding are natural pools and domestic collections of water. Its larvae are predacious and devour the larvae of other mosquitoes. Its
larvae were procured from Panchkula and Pinjore (Haryana).

*Aedes pseudopesteiatus* is a common species of the sub-genus, occurring from the sea level to 7,000 ft, but its distribution is restricted only to India with some extension into Ceylon and Burma. The breeding is common in tree holes, rock pools, drains and household collections. During the present investigations, it was collected from sources along Kalka/Simla highway (H.P.).

*Arminoecus subalbatis* is also a common species of the genus *Arminoecus* prevalent all over India, Ceylon and Burma, having a thinner distribution in other countries of the region. The larval habitat of the species constitutes tree holes, bamboo holes and often very foul domestic collections of water. For the present investigations it was collected from the village Gagal near Lharamsala in Kangra hills (H.P.) The larvae were found breeding in an abandoned rain filled canister and coal tar drum with very foul water.

**Collections**

For the present investigations on the chromosomes of mosquitoes the various species covered here in were collected from natural environment without procuring any laboratory bred stocks. Figure 2 indicates the places on the map of the North-west Indian region and the names of the species collected from there.
The devices used for the collections included a hand made mouth aspirator (suction tube), torch, 8X magnifying hand lens for on the spot identification of the species, glucose, cotton and a field collection cage made of mosquito net cloth 15 x 15 x 15 cms. on each side with an outer case of plywood with slightly larger dimensions (Barraud, 1929). For the collection of larvae and pupae, a nylon sieve, dropper, plastic jar and a petri-dish were required.

The collection time varied with each species, but most of the collections were made at dawn, whereas the rest of the day was utilized in searching for the larval breeding sources. *Anopheles stephensi* and *Anopheles culicifacies*, when abundant, could be collected at all hours of the day. The most favourable villages and the cattle sheds in them were those which fell towards a water source and generally along the perimeter of the village. This was probably due to the fact that the adults of the species had no obstruction in their flight path from resting sites to breeding places. As there had been periodic spray operations by the staff of the Anti-malaria department the best abode for most of the species was the newly constructed thatched hut or the one without a fresh spray of insecticides. The specimens were collected one by one and immediately transferred to the collection cage without injuring the insect. Although the night collections were also made with the aim of procuring the species while they were taking the blood meal
from the body of the cattle, but in such a collected lot, most of the specimens were either ill fed or starved. After the collection was over a wet pad of cotton (sometimes soaked in 10% sugar or glucose solution) was placed at the bottom of the inner collection cage and the entire collection was brought back to the laboratory. Immediately on arrival each specimen was transferred to the test tube and identified individually with the help of an 8X magnifying loop lens. The various species were identified by using identification keys given by Christophers (1933), Barraud (1934), Puri (1950) and Wattal and Kaira (1967). Periodic identifications were also done from the entomologists of the National Institute of Communicable Diseases, New Delhi.

Depending upon the number of specimens, the gravid females were allowed to oviposit in water filled petri-dishes or on wet filter paper kept under the glass chimneys or in breeding cages (30 x 30 x 30 cms). A few cotton pads soaked in glucose solution and hung inside the cages provided food and humidity for them. Ordinary test tube containing a strip of wet filter paper or blotting paper was found to be most suitable when only one or two females were available for egg laying. The humidity of the insectory was also maintained by using two electrically operated humidifiers and the entire process of rearing was carried out at a temperature of 25° ± 3° C and 80% ± 10% humidity. It was
observed that fully gravid females laid eggs on the very first night, while some others took 2 to 3 days.

Rearing

The rearing of the different species from egg to different stages and further colonization was carried out according to the procedures laid down by Atkin and Rost (1917), Christophers (1933), Russel and Rao (1942), Trembley (1945), Brues (1946), Rozboom (1952), McKiel (1957), Charles (1960), Clements (1963), Baker (1964a), Nettingly (1969), Hartberg and Gerberg (1971), Singh et al. (1974, 1973), Dome et al. (1974), Ansley (1976), Oguma and Kanda (1976) and Rabbani et al. (1976). According to their suggestions and several personal modifications to suit the particular species, the rearing was done as follows:

The eggs so obtained were removed from the receptacle with the help of a camel hair brush and transferred to enamelled bowls or dishes containing distilled water or chlorine free boiled and cooled water. The egg rafts were released into wax coated cork rings so as to avoid their sticking to the sides of the bowls as a result of lowering down of the water level through continuous evaporation. Sometimes hatching stimulus to the eggs was provided by adding a pinch of NaCl, CaCl₂ or KCl to the water (personal communication with Dr. V. Dhanda, Assistant Director, Virus
which takes about 6-8 days in tumor and 15-20 days in winter. In the winter months most of the larvae undergo long periods of rest (sometimes 2 months) without moulting further to pupation. This is because of the phenomenon of "Wintering of species". As for the nutrition of the larvae, several dietary combinations were tried viz., bread crumbs, powdered milk, Farex, finely powdered dog biscuits, yeast tablets, *Hymen jamaicensis* and Protinex (Syosirio, 1964). The best of these had been a mixture of 6 parts of yeast powder, 4 parts of dog biscuit powder, one part of Protinex (a rich protein supplement) and one part of Farex (Baby milk food cereal). This formula is the modification of Smith (1967) and Singh *et al.* (1974, 1975). As *Anopheles* larvae are surface feeders, small amounts of feed were sprinkled on the rearing medium to avoid putrefaction of excess feed in the dishes, whereas the scum formation which hinders the larval respiration was removed with the help of a piece of blotting paper and also by occasional changes of water. In case of species of the genera *Culex, Aedes* and *Armigeres* which are bottom feeders, the feed was first dissolved in water and a few drops of it were released into the pans. The over crowding of the larvae was avoided and rearing pans were also exposed to direct sunlight for 2-4 hours daily.
All through the rearing process the dishes were kept covered with mosquito net cloth, muslin or wire net to avoid the laying of eggs by stray mosquitoes in the laboratory. Just after pupation the pupae were picked up from the rearing dishes one by one with the help of a wide mouthed dropper and were allowed to develop into adults in the rearing cages. Freshly hatched adults of both the sexes required honey, soaked resin, grapes, pieces of apple or glucose pads as food, whereas later on the blood meal to the females was provided by keeping a fowl overnight in the cage. In spite of all the efforts none of the anopheline species, except *A. stephensi*, could be colonized beyond the first generation adults. However, some efforts were made to colonize *A. culicifacies* following the procedures of Russell and Rao (1947) and Ainsley (1975), but they proved futile. On the other hand for *A. stephensi*, the technique of French (1969) specially developed for this species under the head “maintaining mosquito colonies of *Aedes atropalpus* and *A. stephensi* with less than 5 hours labour per month” was successfully attempted. The breeding case designed by him with automatic air supply system was also fabricated for the present investigation on *A. stephensi*.

**Induced copulation**

As for receiving eggs from the females from the wild
populations, there is no problem because all the species readily lay eggs in the laboratory, but problems are encountered in continuing their progenies to F₁ and subsequent generations. *Anopheles stephensi* is one species which can be colonized easily for a number of generations in a routine manner, whereas most of the anopheline species including those covered in the present work require highly specialized laboratory conditions or for most of them there are no standard procedures available. The major drawback is encountered in their mating ability in captivity. In order to overcome it one has to rely on artificial mating or the so called "induced copulation" because for the purpose of hybridization studies crossing experiments are necessary. In the present chromosomal comparisons among the species, hybridization was also attempted. In doing so the techniques of McDaniel and Horsefall (1957), McClelland (1961), Baker and Kitzmiller (1961, 1963b), Baker *et al.* (1962), McClelland (1962a), Ow Yang *et al.* (1963), Baker (1964), Kreutzer and Kitzmiller (1971, 1972), Hartberg (1972) and Kanda and Oguma (1976, 1977) were employed.

According to their procedures the adults were separated into males and females about 12 hours after emergence and placed in separate enclosures (small cage measuring 16 x 16 x 16 cm or 20 x 8 x 20 cm. or glass chimney covered with muslin cloth) and fed on glucose pads.
Males that were 1-4 days old were found suitable for this purpose. Each male was individually collected with the help of a mouth aspirator and gently blown into the fold of a cotton pad. This avoids injury or crushing of the specimens. The males were then pinned laterally through the thorax on to the tip of a dissecting needle fixed on the other end into a plastic knitting needle of about 10 cm length. After this the head, legs and wings were removed for the ease of operation. Immediately after this, 3-4 females were anaesthetized with ether and were placed on their backs on a piece of filter paper (Fig. 1). The genitalia of the pinned male were then gently rubbed over the genitalia of each female individually in a venter to venter position till copulation was complete in about 1 minute.

Preparation of the chromosomes

Detailed accounts for the preparation of mosquito chromosomes were given by Breland (1959, 1961), but French, Baker and Kitzmiller (1962) developed a standard method for the germ cell and salivary gland chromosomes of mosquitoes, to which valuable modifications were also made by Rai (1963a, 1967b) and Kanda (1964). Nicoletti (1959), Stalker (1967) and Yoon et al. (1973) gave techniques for improving the salivary chromosome preparations in *Drosophila*, which have also been found suitable for mosquitoes. Apart from this, vital information regarding the general procedures, fixatives and stains have been provided by Darlington and Le-Cour (1960). In the present investigations the procedure of French et al. (1962) formed the basis with
useful alterations at various steps. Some preparations from the brain tissue of the larvae and germ cells of the pupae were also made by air drying technique (Crozier, 1968).

Choice of the cellular material for salivary gland chromosomes: The salivary chromosome preparations were made from the well fed and healthy fourth instar larvae. The age factor of the larvae, for getting good preparations, differed from species to species. Most larval organs of the dipteran insects undergo lysis at the time of pupation due to the development of adult organs. It is because of this reason that the larval tissues have been most extensively used for studying the polytene chromosomes. The fourth instar larva can be recognised by its over all increase in body size, much swollen thorax, reduced collar between the head and the thorax, and the development of distinct "imaginal eyes" which are visible in the form of large black patches. The standard of preparation (especially for salivary chromosomes) also depends upon rich protein diet.

Dissecting medium: Various dissecting media ranging from simple distilled water to Ringer's saline, Beler's saline, 45% acetic acid and dilute Carnoy's solution have been suggested for dissecting out salivary glands from the larval body, but in the present procedure 0.67% saline water was used successfully.
**Method of dissection**

For salivary glands, the fourth instar larvae were picked up from the rearing pans and immediately dissected. But when preparations were poor they were also kept at 18° - 20° for 12-26 hours prior to dissections. The low temperature treatment, in addition to improving the development of salivary glands, also delayed pupation of the larvae. The dissections were carried out under Poach and Lamb dissecting microscope using 3 X objectives and 10 X eye piece. Each larva was individually transferred to a drop of dissecting medium taken in a cavity slide and dissected with fine dissecting needles.

First of all, the abdomen was separated from the body after which one of the needles was placed over the thorax while the other was used to gently pull out the head anteriorly. In doing so the two glands lying laterally in the thorax, came out while they are still attached to the sides of the oesophagus with their salivary gland ducts. When they failed to come out along with the head, they were taken out by stretching open and teasing the thorax with the help of the needles. Both the salivary glands are shining bilobed structures visible to the naked eye as white dots.

**Fixation and staining**

The stain used for staining the salivary glands was 2% Aceto-lactio-orcein (Bridland 1961; French et al., 1962; Rea 1963a,b, 1964c). It was prepared by dissolving 2 gms of orcein (BDH, Gurr's and Loba chemie)
in equal parts (50 c.c. each) of 45% Glacial Acetic acid and 85% commercial lactic acid. This combination was thoroughly shaked and warmed for dissolving orcein and the mixture was kept overnight. Next morning it was filtered and kept as stock solution. Small amounts of this concentrated stain were taken at a time for use after diluting the same with 45% acetic acid (1:1). As the glacial acetic acid (generally used as a fixative) is already present in the stain, the use of a fixative separately was eliminated.

Preparation of temporary slides (Squashing)

The salivary gland, after its removal from the larval body, was immediately transferred to a drop of IN.HCl taken on a clean microslide. In this way the tissue was hydrolysed in it for about 30 seconds after which IN.HCl was removed with the help of a piece of blotting paper and a drop of stain was released on the gland immediately after. Sometimes the gland was taken out from IN.HCl and transferred to the stain. 5-10 minutes of staining was found sufficient for getting bright staining. However, the major modifications of the usual procedures were : (i) over staining of the chromosomes (30-40 minutes of staining) (ii) washing the glands with lactic-acetic acid (1:1) at least three times before squashing to remove the excess of stain and other dirt particles. The over staining causes the bands to darken.
and the thinner faint bands to be stained intensely, thus improving the resolution of fine details of the bands and the contrast between band and interband regions.

After staining, the gland was squashed in a drop of stain on a clean grease-free siliconised slide on which a siliconised cover slip was inverted. The squashing was done with gentle thumb pressure after keeping a folded filter paper in between the cover glass and the thumb. The excess stain that spreads out of the coverslip was removed with the help of a blotting paper after which the edges of the coverslip were sealed with nail polish. The amount of pressure needed for the squash preparation is best learnt by experience. A well sealed and stained slide can stay in this form for 5-15 days. These temporary slides were thus studied, drawn and photographed.

**Preparation of Permanent Slides**

As the salivary chromosomes are extremely fragile the preparation of the permanent slides from the temporary ones is difficult, with only 75% success. Therefore, one has to rely mainly on the study of temporary slides. The attempts to make them permanent are made only after they have been fully studied and photographed. For making them permanent two methods were adopted. In the first procedure the modification of the methods of Bhaduri and Goel (1954) and
Darlington and La Cour (1960) was employed. According to it, the temporary slides were first frozen by keeping in the freezing chamber of the refrigerator after which the nail polish sealing was either removed with the help of a shaving blade or a piece of cotton soaked in acetone. The slide, along with the coverslip in tact, was inverted over a petridish full of 1:1 mixture of normal butyl alcohol and glacial acetic acid. In this way, the coverslip gets separated and falls at the base of the petridish carrying the entire material on it or a part of it on the slide. Both the cover slip and the slide were dehydrated separately in butyl alcohol for 10 minutes. After dehydration the slide was taken out and a drop of eupera was put on it. The coverslip was then inverted over it and adjusted in the same position where it was placed at the time of squash preparation. The boundaries of the coverslip are marked before proceeding further for permanent preparation.

In the second method a water soluble mounting medium developed for the salivary chromosomes of Drosophila by Milkman and Feder (1968) at Harvard University, Cambridge, Massachusetts, was also tried. The medium is made by mixing equal parts of 60% acetic acid and 30% PVP -40 (Polyvinyl pyrrolidinie (Molecular weight 28,000, or 40,000 or Plasdon-C). After fixation and staining, the gland is directly transferred to a drop of this mounting medium and
squashed by inverting a coverslip over it. The preparation hardens quickly under room temperature. Experience has shown that the spreading of the chromosomes in this medium is faulty and the stain also fades after sometime.

Choice of the cellular material for the study of germ cell chromosomes: The fourth instar larvae take about 24–48 hours for molting into pupae which were sacrificed for the study of mitotic and meiotic chromosomes from their testes and ovaries. Only the youngest pupae were suitable for preparations because the older ones had only maturing oocytes or sperm bundles. The sexing of the pupae was done on the basis of the differences in the body size and the structure of the terminal segment (genitalia).

Colchicine pretreatment: Colchicine, a commonly used alkaloid and other "mitostatic" agents prevent the formation of the spindles (Leven, 1938, 1940; Darlington and Le Cour, 1962) so that an abundant stockpile of mitotic and meiotic metaphases becomes available for study. French et al. (1962) suggested the use of 0.1% colchicine. The same concentration was successfully used by Aslamkhan and Baker (1969) for their studies on nine species of mosquitoes. For the present chromosomal analysis the freshly metamorphosed pupae were picked up from the rearing pans and put in colchicine solution. A pretreatment of 18–24 hours at 18°C was sufficient before dissections. Keeping the pupae at low temperature
also delayed their hatching into adults. The practice of pretreatment was carried out only in the initial stages, but was abandoned later because untreated material gave equally good results.

**Dissecting medium**

Pupal dissections were carried out in 0.9% sodium citrate solution because it avoids clumping of the chromosomes and causes their swelling for better results. When orcein stain was used for the squash preparations the dissecting medium was 0.67% saline which also gave good results.

**Method of Dissection**

In order to take out the gonads, the head and the posterior fan-shaped terminal segments were detached from the abdomen which was now open at both the ends. One of the needles was now placed at the anterior most abdominal segment while the other was pressed gently over the rest of the abdominal segments from anterior to the posterior sides. The entire digestive tract followed by gonads which are situated one on either side in the 6th and 7th abdominal segment get pressed out. The testes and ovaries are colourless organs surrounded by fat bodies which are removed before further treatment. When brain tissue was
required, it was taken out from the head capsule of the fourth instar larvae in much the same way as has been adopted for taking out the salivary glands. After removal the tissue was immediately transferred to the fixative already taken in a cavity block.

**Fixation and staining:** For fixation, freshly prepared Carson’s fixative made by mixing 3 parts of absolute alcohol and 1 part of Glacial Acetic acid, was used in which 3–5 minutes fixation was sufficient.

**Staining:** After fixation, the material was hydrolyzed for 5–10 minutes first in cold 1N HCl and then for 5–10 minutes in hot 1N HCl, kept at 60°C, after which it was removed with the help of a piece of blotting paper. The tissue was then given 2–3 washings with distilled water and stained in Gomori’s Haematoxylin (Melander and Wingstrand, ’53) by keeping the material in this stain for about 45 minutes at a temperature of 60°C inside the oven. After the staining the material was differentiated in 45% acetic acid for 5–10 hours. The squash preparations were made by taking small amounts of differentiated material on a microscope, covering with the cover-slip and tapping a little for spreading the cells. These temporary slides were arranged in a slide washing dish containing a layer of cotton soaked in rectified spirit and then kept in the freezing chamber of the refrigerator for 12–20 hours.
Preparation of permanent slides: Those slides which were stained with lacto-aceto-orcein and sealed with nail polish were made permanent according to the method utilized for salivary chromosome preparations but for those slides which were stained with Gomori's haematoxylin, the following procedure was adopted. After keeping the slides in the deep freezer for 24 hours, the cover slip was lifted carefully with the help of a sharp edged shaving blade. The properly frozen material comes off along with the coverslip which is immediately put into butyl alcohol for dehydration or for 10 minutes each in rectified alcohol and absolute alcohol. It was mounted in Euparal and dried at 60°C.
The six salivary chromosome maps presented in this thesis, were constructed by a thorough study of a large number of freshly prepared temporary slides. These squashed preparations were examined under the Neopsy Research Microscope using the oil immersion objective and 10 x eye pieces. In the first instance, free hand pencil drawings were made from those slides which showed the maximum contrast between light and dark bands and other details. Preference was given to the drawings of complete complements having the chromocentre but individual chromosomes and their arms were also drawn. Each chromosomal arm was recognized by the presence of particular landmarks provided by the constancy of major bands and puffs at the free and centromeric ends. The microphotography was done with the help of an Olympus attachment having 8 x eye piece in it. The photographs were taken on a 35 mm slow OPWO film of 125 ASA and using green or yellow filter. In order to prepare the final standard maps the negatives of those complements which had minimum coiling were projected on a screen, e magnified and the outlines of the chromosomes and major band series were drawn. This gave a "skeletal map" with a known magnification in which the details were filled from the hand drawings. The finer details were incorporated by direct observations from the slides. During the inking of such maps the staining

MAPPING TECHNIQUE

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intensity of the various bands and the structural details were carefully observed. On these bases, the bands were assigned several different categories viz., dark thick, dark thin or slender, dark dotted or broken, light dotted, light thick, shaded, fused or faint, curved and discontinued etc. Such categories of bands helped in marking the various zones and subzones and clearly recognizable areas. Out of the six species of the genus *Anopheles* covered in these investigations on the salivary gland chromosomes, four belong to the subgenus *Cellia* while the other two belong to the subgenus *Anopheles*. Therefore, mapping has been done according to the scheme adopted for the already worked out species of their respective subgenera. A careful examination reveals that the bending pattern of the present six species is not exactly similar to that of the species which are selected for comparison with them. Therefore, the mapping, at places is arbitrary except in these areas on the chromosomes where convenient similarities have been found, such as the shape and bending arrangement of the free and centromeric ends. In some cases, but not in all, zones and subzones correspond to similar sets of bands and puffs in the related species. In such regions, it has been attempted to set the limits in the same places, but in areas in which homologies are doubtful the positioning of the zones and subzones is arbitrary. Accordingly, species of the subgenus *Cellia* have their salivary chromosome
complement divided into a total of 46 zones. The abbreviations 2R, 2L, 3R and 3L stand for right and left arms of chromosomes 2 and 3, whereas X - chromosome being smaller has not been separated into its right and left arms.

Accordingly, the division of the salivary chromosome complement is as follows: X - chromosome, zones 1–6; 2R, zones 7–19; 2L, zones 20–28; 3R, zones 29–37 and 3L, zones 38–46.

On the other hand, division of the complement for *A. cinctus* and *A. peditegminatus*, belonging to the subgenus *Anopheles*, is as follows: X - chromosomes, zones 1–5; 2R, zones 6–14; 2L, zones 15–21; 3R, zones 22–32 and 3L, zones 33–39.

The chromosomes were measured by the following method: the micrometer was photographed and a positive print made at the same magnification as that of the chromosome preparations. This scale was then used to measure the lengths of the individual chromosomes with the aid of a divider.
Fig. 1 Artificial mating in mosquitoes
Fig. 2. Map showing places of collection and the species collected from there.