MATERIALS AND METHODS

A. Laboratory Rearing Techniques:

A. subpictus, A. annularis and A. culicifacies were collected from nature. A. subpictus was collected from a village, Lohara, just near the Panjab University campus at Chandigarh. A. annularis and A. culicifacies were collected from village Burj near Pinjore. The A. tesselatus stock was obtained from the Director, Virus Research Centre, Poona, India. A. maculatus was provided by the Communicable Disease Center, Atlanta, Georgia. All these species were reared in a laboratory insectary which was maintained at a constant temperature of 24.5 ± 1 °C and a relative humidity of 80%.

For mating A. tesselatus large cages (1 yd x 1 yd x 1 yd) were used. For egg laying by tesselatus wet filter paper cones were placed in petri dishes inside the cage. The other two species laid eggs directly on water or on wet filter paper.

The larval food used in the laboratory was a mixture of live yeast, wheat germ and Kellogg's concentrate. The larval food was ground finely so that it would more easily float. The male adults were fed on a 10% sucrose solution or on honey. Females were fed on the blood of guinea pigs. For hybridization and to maintain the colony of A. maculatus, the method of induced copulation was adopted and it proved to be very successful.
B. Induced Copulation:

The technique used was the same as described by Baker _et al., 1962. The adults were separated according to sex within 12 hours after emergence and placed in separate pint paper cartons with netted sleeves at 78°-80° F and 78-80% relative humidity. The males were fed on honey. The females were given only a sponge saturated with water. The feeding of females before copulation did not prove to be successful.

Males, three to four days of age, are the best to use. The males are collected with an aspirator and softly blown between two layers of cotton. The males are then pinned laterally through the thorax using a "minuten" pin fixed into the end of a 3-inch stick. The heads of the males are separated and the wings as well as legs are pulled off to facilitate copulation.

The females are anaesthetized with ether and are placed on their backs on a piece of filter paper. Copulation can be made under a dissecting microscope or any low power, wide field, lens.

The pinned male is held at a 45° angle to the female, ventral side up. They are placed in such a position so that the tips of the abdomen are in close proximity. The male responds by opening the claspers wide and bending the tip of abdomen towards the female genitalia. The males clasp the female firmly when copulation occurs to the extent that they may be lifted up together. It takes only about five seconds
to stimulate the male. The mosquitoes remain joined for 5 to 20 seconds after which the female is released by the male. The same male can be used again to copulate another female if needed.

The copulated females are then fed on blood. For induced egg laying usually one or both wings of the female are cut and the females placed in a pan of water with filter paper on the sides. Such females lay eggs within 24 hours. Females which do not lay eggs under normal conditions may thus sometimes be forced to do so.

C. Preparation of Chromosomes:

The germ cell chromosomes were prepared by using the colchicine pretreatment as described by French, Baker & Kitzmiller, 1962. Usually the early pupae were used for dissection and were given 24 hours pretreatment with colchicine. They were then dissected in 0.5% Sodium citrate solution. The squashes were prepared in aceto carmine as well as in Gomoris' haematoxylin.

For the salivary glands, dissections were made in 45% acetic acid. To remove the salivary glands, pressure was applied just posterior to the thorax, the head removed and the dissecting needles inserted under the cuticula from the anterior. The incision was made by rubbing a second needle over the inserted one. The interior of the thorax was then exposed by opening the incision further and removing the gut. After removal, the salivary glands were placed in a drop of
Lacto-aceto-orecin on a siliconized coverslip. Depending on the species, the concentration of stain and staining period vary. The pressure applied on the coverslip to spread the chromosomes also varies in different species.

The "dry-ice" method was used to make the preparations permanent. The slides were placed on dry ice for about half an hour, the siliconized coverslip flipped off with a single edge razor blade. The tissue remains on the slide. It is then dehydrated for five minutes in absolute alcohol. Euparol or Zeiss "Einschlusmittel L 15" were used as mounting media.