



WHO/Mal/380 ✓
21 February 1963

ORIGINAL: ENGLISH

a 63299

MAINTENANCE OF A LABORATORY COLONY OF ANOPHELES MACULATUS
THEOBALD BY ARTIFICIAL MATING¹

by

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In our investigations into the susceptibility of Malayan Anopheles to the monkey malaria parasite Plasmodium cynomolgi bastianellii, A. maculatus was selected as the control mosquito because it was found to be highly susceptible. Originally, clean adults were raised in the laboratory from eggs laid by wild caught females. This proved to be rather tedious involving much time and labour and a laboratory culture was clearly desirable.

Attempts have been made at various times to colonize A. maculatus in Malaya and elsewhere without success. In recent attempts, the mosquitos failed to mate in cages of one foot, two feet and six feet cube dimensions. However, the technique of induced copulation devised by McDaniel & Horsfall (1957) for Aedine mosquitos has been applied with success for some anophelines. Reports of induced mating of A. labranchiae by Caravaglios (1961) and of A. punctipennis, A. quadrimaculatus, A. freeborni and A. albimanus by Baker et al. (1962) suggested that our problem might be solved along similar lines.

The technique was simplified from those previously employed. The males and females are allowed to emerge separately in one foot cube cages and maintained on 5% glucose solution in the insectary at 27°C temperature and 70-90% relative humidity. They are separated by size at the pupal stage with a reasonable amount

¹ This paper will be published in "Mosquito News". The Editor of WHO/Mal appreciates the author's permission to release it in advance in this series of WHO documents.

of accuracy, as more than 95% of the larger pupae have been shown to be females. Two days after the emergence of the females, the glucose solution is removed in the morning and a guinea pig is substituted the same evening and left in the cage overnight. Fed females are collected in individual tubes the following morning. The males, three to six days old, are caught with a fine bore, blackwalled pipette¹ attached to a device producing gentle suction. They receive no anaesthesia but are pinned laterally through the thorax using a "minuten pin" fixed into the end of a six inch long, soft wooden stick. Several males are prepared at a time; after removing the head and legs, they are lined up ready for use. The female mosquito is anaesthetized by placing a tube, with cotton wool soaked in ether, on the top of the tube containing the fed female. When fully anaesthetized, the female is tipped out on her back onto a clean white glazed tile. Copulation is achieved by bringing the pinned male down at about a 45° angle to the female, so that the male and female are in the venter to venter position with the tips of their abdomens in close proximity. Responsive males open their claspers wide, bending the tip of the abdomen towards the female genitalia at the same time. When copulation is effected, the pair can be lifted up together, with the female firmly clasped by the male. There is no difficulty in lifting the mated pair if the union is correct. They are then placed in a small paper drinking cup which has a small flap about half-an-inch square cut near the base and the male is removed from the pin at the edge of the flap, thus allowing the mated pair to drop into the cup; a rubber band keeps the flap closed. The male and female remain in copulo usually for about one to two minutes. Ten pairs are placed in each cup. Two days later, the females are removed for egg laying and are kept in individual tubes with moist filter paper at the bottom or are transferred to paper cups prepared in the same way. The whole mating procedure is carried out without the aid of a magnifier at a room temperature of about 26-28°C.

Using this mating technique, the percentage of females fertilized in the first five generations was 85, 100, 83, 71 and 82 respectively, giving an average of 85%. These figures include those that lived to lay viable eggs, together with a few females which were moribund and failed to lay but were subsequently found with sperms in their spermathecae.

¹ A calibrated pipette used for haematology is actually used. [Editor]

Each female lays about 80-100 eggs. Thus, if about 10 females were mated per day, they should provide sufficient eggs to produce a daily supply of about 300 adult females. The colony is now in the fifteenth generation and there seems to be no reason why it should not be maintained indefinitely. When properly organized, a series of 50 females can be mated in 60-75 minutes. This includes the time taken to catch the fed females and to prepare the males and is thus much more rapid than has previously been described. The feeding of virgin females on guinea pigs is now much easier and the only limitation to the size of the colony is the time required for mating and the problems associated with the subsequent larval development.

Some success has also been achieved in mating other Anopheles species. In particular, several wild-caught unfed A. letifer which had survived exposure to high doses of DDT were subsequently fed and adults raised from the eggs. A few of these adults were mated and, though we were unable to establish a colony, this small measure of success suggested that the mating technique might prove useful in studies on insecticide resistance.

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