

these males appeared as vigorous, lived as long, and mated as readily as normal males when they were not in competition.

#### References Cited

DAVIS, A. N., GAHAN, J. B., WEIDHAAS, D. E., and SMITH, CARROLL N. 1959. Exploratory studies on gamma radiation for the sterilization and control of *Anopheles quadrimaculatus*. J. Econ. Entomol. 52:868-70.

KNIPLING, E. F. 1960. The eradication of the screw-worm fly. Scientific American 203(4): 54-61.

LABRECQUE, G. C. 1961. Studies with three alkylating agents as house fly sterilants. J. Econ. Entomol. 54:684-9.

LABRECQUE, G. C., MEIFERT, D. W., and SMITH, CARROLL N. 1962. Mating competitiveness of chemosterilized and normal male house flies. Science 136:388-89.

LINDQUIST, A. W. 1961. New ways to control insects. Pest Control 29(6):9-19, 36-40.

ROTH, LOUIS M. 1948. A study of mosquito behavior—an experimental laboratory study on the sexual behavior of *Aedes aegypti* (Linnaeus). The American Midland Naturalist 40:265-352.

WEIDHAAS, D. E. 1962. Chemical sterilization of mosquitoes. Nature 195:786-787.

WEIDHAAS, D. E., FORD, H. R., GAHAN, J. B., and SMITH, CARROLL N. 1961. Preliminary observations on chemosterilization of mosquitoes. N. J. Mosq. Exterm. Assoc. Proc. 48:106-9.

## MAINTENANCE OF A LABORATORY COLONY OF *ANOPHELES MACULATUS* THEOBALD BY ARTIFICIAL MATING

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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 mosquitoes failed to mate in one-foot, two-foot and six-foot cube cages. However, the technique of induced copulation devised by McDaniel and Horsfall (1957) for *Aedine* mosquitoes 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 with 5 percent glucose solution in an insectary at 27° C. and 70-90 percent R.H. They are separated by size at the pupal stage with a reasonable amount of accuracy, as more than 95 percent of the larger pupae have been shown to be males. Two days after the emergence of the females, the glucose is removed the morning and a guinea pig is substituted the same evening, and left overnight. Fed females are collected in individual tubes the following morning. The males 3-6 days old, are caught with a fine pipette attached to a suction device. They receive no anaesthesia but are pinned laterally through the thorax using a mirror pin fixed into the end of a 6" long soft wooden stick. Several males are pinned at a time, and after removing the head and legs, are lined up ready for use. The female mosquito is anaesthetised by placing a tube, with cotton wool soaked

in ether, on the top of the tube containing the fed female. When fully anaesthetised, the female is tipped out on her back onto a clean white table. 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 the abdomen 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. The male and female remain *in copula* 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 macroscopically at a room temperature of about 26–28° C.

Using the above 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 percent. 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.

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 15th 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.

#### Literature Cited

- BAKER, R. H., FRENCH, W. L., and KITZMILLER, J. B. 1962. Induced copulation in *Anopheles* mosquitoes. *Mosq. News* 22(1):16–17.
- CARAVAGLIOS, NICOLA. 1961. Accompiamento artificiale senza decapitazione del maschio in *Anopheles labranchiae* Fall. *Riv. Parasit.* 22(2): 149–151.
- MCDANIEL, I. N., and HORSFALL, W. R. 1957. Induced copulation of Aedine mosquitoes. *Science* 125:745.