COLONIZATION OF *ANOPHELES EARLEI* VARGAS

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As more data have been collected and reported on the cytogenetics of North American anophelines, it has become increasingly important to rear large numbers of these species in the laboratory in order to have material readily available for crossing studies to support these cytogenetic data. Many of the chromosome maps which have been published were prepared from larvae collected in the field, because it has been extremely difficult to raise certain species in the laboratory. Some species which are needed in large numbers to continue these genetic studies are *A. punctipennis, A. crucians* and *A. earlei.* Many of the interspecific crosses between members of the Nearctic anophelines have been made (Kitzmiller, Frizzi and Baker 1967). One large and important group of crosses has not yet produced enough data for reliable interpretation. These crosses involve *earlei* as one of the members of the cross; they have not been made primarily due to the lack of adult mosquitoes in sufficient numbers to allow crossing experiments. Rozeboom (1952) has reported a short term colonization of the species. This present communication reports success over 10 generations of continuing colonization.

PROCEDURE AND RESULTS. Colonization attempts were initiated on some 20 females collected in northern Minnesota in October, 1968. These females readily took blood from a human host, and were placed in 23 x 100 mm glass shell vials. The lower third of each vial was lined with filter paper to prevent deposited eggs from sticking to the sides of the vial. After the females had passed the blood meal (on about the third day) they were lightly etherized, and one wing was pulled from the thorax. After the females became active again they were placed in a 10 x 12 inch enamel pan which was lined with filter paper. Enough water was added to cover the bottom of the pan, and the pan covered with a glass plate and placed in a darkened area. After 5 or 6 hours (occasionally females will oviposit immediately upon addition of water) these females deposited between 100–200 eggs. The larvae hatched after 2 days, and were fed on laboratory food which is composed (by equal weight) of one-third dry yeast, one-third Kellogg's Concentrate and one-third wheat-germ. The food was ground very finely so that particles did not readily break the surface tension and fall to the bottom of the pan. The larval period as later ascertained averaged 12–15 days at 24° C.

During this period the water in the pans was changed once after 7 days. As the larvae reached the later instars they were given increased amounts of food. It is best to feed earlier instars small amounts twice a day, and then to give the later instars large quantities of food. As long as the water in the pan remains clear, the larvae are not being overfed; however, if the water becomes clouded it should be changed immediately and the amount of food reduced. The pupal period lasts approximately 2 days. The adults were fed on a 10 percent sugar solution prior to further treatment. The second through fifth generations were maintained by artificial copulation. This was a slightly altered technique from that described by Baker *et al.* (1962).

Large numbers of adults were produced by this method. It was noted that both males and females in the adult holding containers (half-gallon cardboard ice cream containers) in which they were placed prior to copulation were very active. Some females were removed and fed on a human host without being artificially copulated. After these females had their wings pulled many readily deposited 100–150 viable eggs each, proving that, as suspected, mating

had occurred in the ½ gallon containers. These eggs hatched, and more adults were induced to deposit (by wing pulling) without artificial copulation. The adults of the next generations were placed in a cage 12 x 12 x 10 inches in an insectary at 26° C. and 59 percent humidity. Five days after emergence the adult females were offered a guinea pig. The females took blood only when the pig was left in the cage overnight; little or no daylight feeding has been observed. The blood-fed females were immediately removed from the cage and placed in vials, and after the wing-pulling treatment, they deposited 150–250 eggs each. At least ten generations have been produced following this procedure.

Samples of egg batches produced a hatch of 87 percent, with 70 percent adults from these larvae.

DISCUSSION AND SUMMARY. Following this procedure *earlei* can be obtained in large numbers with a minimum of effort. The procedure may be summarized as follows: 1. Place pupae in cage 12 x 12 x 10 inches (smaller or larger cages may be used), 2. Wait at least 5 days after emergence, during which time the adults are fed on a 10 percent sugar solution, 3. Feed the adult females on a guinea pig, overnight, 4. Collect blood-fed adults the day after feeding, 5. Isolate these females in shell vials lined with moist filter paper, 6. After the female passes the blood meal (2–3 days) lightly etherize her and pull one wing from the thorax. 7. Place 1 or 2 females in a 10 x 12 inch pan lined with filter paper with about ¼ inch of water, 8. Cover the pan and place it in a dark place over night, 9. Feed larvae as stated above. The length of embryonic development of this species is approximately 2 days, larval period 12–15 days and the pupal period two days at 24° C.

There are only three procedures which must be followed closely: 1. Remove adults immediately from the cage after the blood meal or within one day. This procedure will facilitate the determination of the passage of the blood meal. 2. Pull the wing from the thorax on the day the blood meal is passed. If the wing is cut oviposition is reduced. 3. Do not disturb the treated females for at least 8 hours. If disturbed within this period they usually will not oviposit.

After the wing pulling procedure is mastered the species can be maintained with little effort. As of May, 1969 we are on the sixteenth generation of this species in the laboratory and the tenth generation of non-artificially copulated animals. The colony is as strong as any of our others which do not require the wing pulling procedure. We have not been able to induce oviposition without the wing pulling treatment of the female. The species is now being maintained routinely in the Urbana laboratory.

References

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