INDUCED COPULATION IN *ANOPHELES* MOSQUITOES

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McDaniel and Horsfall (1957) described a method for induced copulation in mosquitoes. This method proved to be eminently successful for several species of *Aedes,* but in spite of repeated attempts by various workers, has never been successful as an operational tool in *Anopheles.* Frizzi (1958) did report some success with *Anopheles maculipennis,* but also experienced variable results.

During the course of our investigations of the genetics and cytogenetics of the anophelines of the Nearctic *maculipennis* complex, it became desirable to attempt controlled single pair matings. The induced copulation technique was again attempted, with little success. A search for ways to cut down physiological activity of the males provided the key to what is now 100 percent efficiency in the copulation of *Anopheles.* Temperature control was one approach. Males were stored in a dark cold room prior to attempted copulation. Marked success with these males led to experimental pre-treatment for 24 hours at 15° C. This cold pre-treatment is evidently the difference between success and lack of success in induced copulation of *Anopheles* mosquitoes.

Because of the widespread current interest in the genetics of *Anopheles,* the steps which we use are presented in detail. Most of these procedures are only slightly modified from those outlined by McDaniel and Horsfall, to whom credit is due for pioneering this useful tool.

Males are selected, isolated in small containers, and stored at 15° C. for an optimal period of 24 hours. Storage up to 48 hours does not impair efficiency, but neither does it increase it. Males should be at least three days old for best results. Males are removed from the cold, anesthetized with CO_2 for 10–15 seconds, then decapitated. They are affixed to a microscope slide by a smal drop of water soluble glue, ventral side up, approximately parallel to the surface of the slide. Usually three or four male are thus prepared, so that if one male i unresponsive, others are immediately available. Virgin females are anesthetized with chloroform (5 seconds or less), ther treated with CO_2 for 5–10 seconds. Fe males thus anesthetized will remain s for about 2–5 minutes. The female i picked up with a slightly curved vacuum micropipette applied to the mesonotum and in this manner she may be handle and positioned at will. We use a vacuum pump with a plastic tube containing bleeder valve for the vacuum system, bu any weak negative pressure system wil work as well.

Copulations are effected under a dis secting stereo microscope at 30X magni fication. The female is held at an angl of about 45 degrees to the male, ventra side up. Touching the male several time with the female stimulates him, as evi denced by the movement of the clasper and the characteristic reaction of his en tire abdomen. Repeated positioning of th female genitalia in proximity to the mal genitalia will result in clasping and copu lation. During copulation the male hold the female tightly, so that even a sligh movement of the operator's hands wil not pull them apart. The male remain quiescent for about five seconds then be gins to move the claspers. Transfer c the seminal fluid ensues. The mosquitoe remain joined for five to twenty second after which the male withdraws.

If a given male is not responsive, th female may be offered to one of the othe males on the slide. Between 50 and 7 percent of the males thus far attempte will copulate; therefore, almost ever group of four males on a slide will insur

success. The inseminated female is then isolated in a shell vial and handled in the usual way.

Decapitated males remain functional for periods up to an hour. A responsive male will inseminate two or three females, and four successful copulations have been accomplished with one *albimanus* male. It is best to wait about five minutes between successive attempts with the same male. Several females may be inseminated from the four males on the slide.

The entire process takes from 5 to 15 minutes, depending upon the response of the male. Our best series accomplished 12 successful copulations in one hour. All copulations have been carried out at room temperature in the laboratory, with no attempt at temperature control. Matings have been successful all through the day and night. We do attempt to maintain humidity during copulation. When males are removed from the cold, they are kept in a jar lined with moist filter paper until used. If males are used over a long period of time, any simple device to maintain humidity, such as a small square of moist filter paper laid over a male, is desirable. Our best results have been obtained with females which have had a blood meal within 24 hours prior to copulation.

The sole criterion of success has been the production of viable F_1 eggs and larvae.

We are using this technique routinely for the maintenance of species such as *Anopheles punctipennis* otherwise difficult or impossible to maintain in the laboratory. It has also been successful in 3 strains of *A. quadrimaculatus* (Allerton, Savannah susceptible, Bethesda); *A. freeborni* (Cincinnati); *A. albimanus* (Johns Hopkins) *A. earlei* (Deer Lake). We are confident that this method can be used widely for many species of *Anopheles*.

We are currently investigating salivary gland chromosome inversion heterozygotes and karyotype differences in crosses among several *Anopheles* species. With the removal of the sexual reproductive barrier, such studies are now practical. Successful crosses (at least some fertile eggs) have been obtained from the following crosses: *freeborni* x *quadrimaculatus; punctipennis* x *quadrimaculatus; punctipennis* x *freeborni*. These crosses have been successful in both directions.

We have had very gratifying and productive success with the technique outlined above. Undoubtedly there will be modifications and improvements, and individual investigators will wish to modify certain aspects, but we wish to emphasize that it does work as described above.

Induced copulation of *Anopheles* will probably be of most use to investigators concerned with the necessity or desirability of single pair matings. It will be a serviceable tool for geneticists and entomologists concerned with insecticide resistance. It will make possible the maintenance of species ordinarily poorly adapted to rearing in the laboratory. For our own purposes it will be invaluable in the study of isolating mechanisms, species crosses, chromosomal evolution, and the general evolutionary picture of the genus *Anopheles*.

Reference Cited

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