OPERATIONAL AND SCIENTIFIC NOTES

FIELD PRESERVATION AND STORAGE OF MOSQUITOES FOR LABORATORY STUDIES

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The simple technique for determining whether a mosquito is parous or nulliparous is by examining the state of the tracheae supplying the ovaries. The tracheoles of the nulliparous mosquito are wound into tight skeins from which run the very fine terminal tracheoles leading to the follicles. Following a blood meal the ovaries expand with the development of the eggs and these skeins become unravelled. After oviposition, the ovary contracts almost to its original size but the tracheal skeins remain unravelled. Using this criterion the parous state of a mosquito can be determined by simple dissection and microscopical examination of the ovaries as described by Detinova (1962).

The main practical difficulty arising from this technique lies in the fact that mosquitoes caught in animal-baited traps are often engorged, and must be examined within a few hours of capture before the ovaries become much enlarged and the skeins unravelled. In addition, this, and the more specific techniques for determining the number of ovarian cycles undergone by the mosquito require freshly killed material, a condition which may be difficult to fulfill if the laboratory is some distance from the field.

To overcome these difficulties the following technique was developed in connection with ecological studies on *Culex (Melanoconion) portesi* (Senevet and Abonnenc, 1941) in Trinidad where field collections were made from rodent-baited traps situated some 30 miles from the Laboratory. It has since been tried with *Simulium* and *Culicoides* and is considered to have a wide application for all types of insect dissection where "freshly killed" material is required.

Mosquitoes were removed from the traps by means of an aspirator and transferred to 1-pint Mason jars lined with damp plaster-of-paris and fitted with an inverted cone. On arrival at the field headquarters the mosquitoes were knocked down in the jars with carbon dioxide, tipped out into a petri dish, and those required for dissection picked out and while still alive placed directly into normal saline (0.9 percent NaCl) to which a very small quantity of household liquid detergent had been added. The mosquitoes were collected in batches of not more than 30 in about 5 ml. of saline in small plastic shell vials. When each batch was complete it was dropped directly onto dry ice in an insulated container; freezing took place in less than a minute. The frozen mosquitoes were then transported to the laboratory when convenient.

For storage it was found that the frozen specimens could be kept in the ice compartment of a refrigerator for about 1 week, in a commercial deep freeze at —20° C. for 2 weeks and for at least 5½ months at —60° C. or on dry ice. The upper time limit of storage at —60° C. has not been determined. For examination each vial was thawed out individually, since once warm the mosquitoes deteriorated rapidly and dissections had to be completed within 1 hour of thawing. As the material cannot be refrozen the original batch size is governed by the number of insects that could be dissected in that time. Dissections were made in fresh saline.

After 5½ months at —60° C. the blood meal of engorged mosquitoes still looked fresh and had not clotted, there was no difficulty in performing the ovarian dissections and the dilations in the ovarioles of parous females were clearly visible.

The basic technique of freezing in normal saline using dry ice should be capable of considerable adaptation to local needs. It should be of particular value in temperate and northern latitudes when material collected during times of high population densities during the spring or summer months could be stored for examination at leisure during the following winter.

The studies and observations on which this note is based were conducted with the support and under the auspices of the Governments of Trinidid and Tobago, Jamaica, Guyana and the Eastern Carribbean Territories, the Ministry of Overseas Development of the United Kingdom Government, and The Rockefeller Foundation.

Reference

DETINOVA, T. S. 1962. Age grouping methods in diptera of medical importance. WHO Monograph No. 47.

AGE GRADING OF MOSQUITOES FROM PARASITIC MITES

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A simple age grading technique which can be used by non-professional personnel would be very useful in many mosquito control operations. Gillet (1957), suggests that the presence of parasitic mites might indicate nulliparity, as these leave

[1] This work was done during the tenure of a grant from the City of Edmonton and a University of Alberta Dissertation Fellowship.

TABLE 1.—Number of parous and nulliparous mosquitoes with parasitic mites attached at George Lake in 1966.

Species	Parous	Nulli-parous	Un-known	Total
Culiseta inornata	..	..	1	1
Aedes cataphylla	..	1	..	1
A. communis	..	2	..	2
A. excrucians	2	12	1	15
A. fitchii	1	3	..	4
A. pionips	2	1	..	3
A. punctor	5	4	2	11
A. riparius	..	3	..	3
A. sticticus	..	..	1	1
Unidentified	1	..	..	1
Total	11	26	5	42
No. examined	280	449	209	983
% with mites	3.9	5.8	2.4	4.3

the mosquito during oviposition. These mites are conspicuous and remain on dried specimens, and if they are a reliable indicator of nulliparity and infest a large proportion of nullipars, would provide the necessary technique.

During the summer of 1966, I recorded the presence or absence of mites on 983 mosquitoes of 28 species taken at George Lake, Alberta. The mites were not identified to species.

These results are shown in Table 1. Only 42 mosquitoes, belonging to 9 species, were found to be infested with mites, and 4 percent of the parous females were infested. This low rate of infestation and the presence of mites on parous females, confirms the findings of Detinova (1962), that the presence of parasitic mites was not a reliable indicator of nulliparity in Russia. Though mites would appear to be ruled out as indicators of nulliparity in Alberta, the work of Corbet (1963), in Africa shows that they might be useful in certain areas. Further work on this subject would be interesting, as very little work has been done on this in North America.

Literature Cited

CORBET, P. S. 1963. Reliability of parasitic water mites (Hydracarina) as indicators of physiological age in mosquitoes (Diptera:Culicidae). Ent. Exp. Appl. 6:215–233.

DETINOVA, T. S. 1962. Age grouping methods in Diptera, medical importance, with special reference to some vectors of malaria. Monogr. Wld. Hlth. Org., No. 47:216 p.

GILLETT, J. D. 1957. Age analysis of the biting cycle of the mosquito *Taeniorhynchus (Mansonioides) africana* Theobald, based on the presence of parasitic mites. Ann. Trop. Med. Hyg., 51:151–158.

MALATHION RESISTANCE IN *Aedes sollicitans* (WALKER) FROM LANGLEY AIR FORCE BASE, VIRGINIA

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Malathion has been used for 10 consecutive years to provide satisfactory control of *Aedes sollicitans* (Walker) at Langley Air Force Base, Virginia. Each year malathion was applied by air from an Air Force UC–123 and C–47 aircraft at a dosage of 0.2–0.5 lb. technical insecticide per acre per application. The number of applications averaged about 10 per year. During the mosquito season of 1967, unsatisfactory control of *A. sollicitans* was observed after some of the spray applications. In July of the same year, Whitlaw (1967, personal communication) demonstrated resistance to malathion in both adult and larval forms of *A. sollicitans* at Langley Air Force Base.

In August 1967 and May 1968, collections of *A. sollicitans* made at Langley Air Force Base were brought to Gainesville, Florida to test the susceptibility of adults to malathion, naled, and fenthion. Adult mosquitoes from the Gainesville colony of *Aedes taeniorhynchus* (Wiedemann) which is not resistant to organophosphorus insecticides were used for comparison. Rogers and Rathburn (1958) showed that these two species were about equal in susceptibility to malathion.

PROCEDURE. The susceptibility of females of both species was determined by exposure of adults (25 per cage) in a wind tunnel to contact sprays containing various concentrations of the insecticides in deodorized kerosene. The procedures were those described by Davis and Gahan (1961). Mortality was recorded after 24 hours. Duplicate cages were used in each test at each concentration. The tests with malathion were replicated 3 times while those with naled and fenthion were conducted only once because of limited numbers of adult female *A. sollicitans*.

RESULTS. The mortality data in Table 1 show that *A. sollicitans* was highly resistant to malathion; the concentrations required to kill 90 percent of the P_1 and F_1 adult females of *A. sol-*

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