Maryland, and to Dr. Dale R. Lindsay, National Institutes of Health, PHS, for assistance with the completion of this study.

References Cited

1. Bonne-Wepster, J. and Swellengrebel, N. H. 1953. The Anopheline Mosquitoes of the Indo-Australian Region. J. H. de Buzzy, Amsterdam, 504 pp.

2. Causey, O. R. 1937. Some anopheline and culicine mosquitoes of Siam with remarks on malaria control in Bangkok. Amer. Jour. Hyg. 25(2):400–420.

3. Doughty, Frank M. 1953. Annual Report, Union County (New Jersey) Mosquito Extermination Commission, 12 pp.

4. Fergurson, Frederick F. 1945. Biological Factors in the Transmission of Arthropod-Borne Virus Encephalitides. USPHS Monograph #23. Gov't. Print. Off., 37 pp.

5. Griffith, Melvin E. 1955. Personal Communication.

6. Horsfall, W. R. 1955. Mosquitoes—Their Bionomics and Relation to Disease. Ronald Press, New York, 723 pp.

7. Iyengar, M. O. T. 1953. Filariasis in Thailand. Bull. Wld. Hlth. Org., 9:731–766.

8. Mackie, Thomas T., Hunter, George W., and Worth, C. Brooke. 1954. A Manual of Tropical Medicine. W. B. Saunders Co., 907 pp.

9. McCrumb, F. R. Jr. 1955. Personal communication. McCrumb, F. R., Jr., Diercks, F. H., Pond, W. L., Keegan, H. L., and Barnett, H. C. in preparation (from Annual Report of the Institute for Medical Research, 1954).

10. Sambasivan, G., Bhatia, M. L., Pranich, U., and Notananda, V. 1953. Report of Thai Govt./WHO/UNICEF Malaria Control Demonstration in Thailand. WHO/SEA Mal. 1–53–48. (mimeo.), 42 pp.

11. Vejjasastra, Luang Bhayung. 1935. Files of Ministry of Public Health, Thailand. Personal communication.

OVIPOSITION SITE PREFERENCE OF *AEDES* MOSQUITOES (CULICIDAE) IN THE LABORATORY

W. E. BECKEL [1]

Introduction. The problem of what characteristics of the substrate influence the female mosquito when she is ready to oviposit is of practical importance in the field from the point of view of where to find the eggs, and in the laboratory, in the effort to obtain the most eggs from blood-fed females. Some work has been done on where the eggs are found in the field (1, 2, 3 and 5) but this only indicates where the eggs are when the sampling is done and although there is a good chance that this represents where they were laid, the possibility of translocation by rain to a site not chosen by the adult must be considered, as pointed out by Filsinger (3). Beckel and Barlow (1) showed that *Aedes communis* (De Geer) in the field did not lay randomly around the pools; also Bodman and Gannon found this to be true of *Aedes vexans* (Meigen) (2). In both cases only speculation could be made as to what caused the discrimination. Where exactly the eggs of *Aedes hexodontus* Dyar are found in the field has not been discovered since such work presents some difficulty owing to the vast areas of damp and marshy terrain available in the sub-Arctic and Arctic in which these mosquitoes might lay. However, experiments have been carried out to find some of the things which influence the adult female in the laboratory and from this the possible influence in the field may be inferred. No attempt was made to evaluate the variation in the chemical make-up of the water. This type of approach was made with *Anopheles quadrimaculatus* Say. (6) but the results were relatively inconclusive. In the experiment to be described below, only the effects of temperature, brightness of the substrate, and its physical consistency were evaluated.

[1] Defence Research Northern Laboratory, Fort Churchill, Manitoba.

MATERIALS AND METHODS. *Aedes* females were given a blood meal from a guinea pig and caged in open mesh cages in the laboratory at 70° F. for from five to seven days. At the end of this time they were just about to lay their eggs. They were then transferred to the experimental conditions where the choice of substrates was offered. Within 12 to 24 hours some of the adults would commence to lay eggs and laying would be fairly well finished within 48 hours. The oviposition sites were removed after 72 hours and the number of eggs on each counted.

Oviposition Site Preference. Wire mesh cages 12 by 12 by 24 inches were placed at 70° F. under fluorescent lights so that the lighting inside the cages would be uniform. Two round apertures had been cut in the bottoms of each cage so that the aperture centers would be equidistant from the walls of the cage and from each other. The cages were raised enough from the table on which they rested so that two petri dishes could be placed in the apertures, fitting tightly and with their surfaces flush with the floor of the cage. Each petri dish was filled with absorbent cotton and kept constantly soaked with distilled water. The experimental substrates were then placed on the absorbent cotton. The adults were removed from their storage cages with an aspirator and introduced into the experimental cages. Sugars were offered as a supplementary food.

The following substrates were offered. Trial 1: white filter paper and black filter paper (painted with India ink). Trial 2: white filter paper and white terrycloth towelling. Trial 3: white towelling and black towelling (painted with India ink). Trial 4: black towelling and black filter paper.

Temperature Preference. A temperature gradient was set up to enable adults just about to lay, to choose, in total darkness, the temperature at which they preferred to lay their eggs. The gradient consisted of a glass tube six and one-half centimeters in diameter and 72 cm. in length with one end of the glass tube surrounded by water kept constant at some temperature between 33° to 40° F. and the other end surrounded by water kept constant at some temperature between 98° and 115° F. A non-linear gradient was established with temperatures ranging from 99° F. at the warm end to 44° F. at the cold end. On a thin strip of wood, one-quarter of an inch wide and the length of the gradient, were mounted 24 thermocouples, two in the center of each six centimeter length along the strip. Absorbent cotton and white cotton cloth were wrapped about the strip of wood so that the bottom thermocouple at each station was covered by the substrate and the top was in the air above the substrate. The cotton and cloth were then soaked with distilled water. The gradient was insulated and made light-proof. An observation window was made available on the top. Fifty blood-fed female *Aedes hexodontus,* ready to lay, were then introduced, 25 from each end of the gradient, at or about the center. Upon observation they were noted to fly and walk about readily. The thermocouples were then read each 24 hours for the 72-hour duration of the experiment. The experiment was repeated three times on each of two successive years. At the completion of each trial the number of eggs in each six centimeter region was counted. The gradient was found to vary little in temperature once it was allowed to come to equilibrium and no difference between the temperature of the substrate and the air above was recorded. The set of thermocouples in the air above the substrate was discontinued in the second year. The thermocouple readings chosen for statistical treatment were those taken at the period when it was considered, on the basis of adult age and observation, that most of the eggs were being laid.

RESULTS AND DISCUSSION. *Aedes hexodontus* Dyar. The results of the oviposition site preference experiments are given in Table 1.

The choice of substrate may be made by the female either by sight or after she has made a tactile survey of the substrate. Also it may be neither of these but may

TABLE 1.—Preference for Brightness and Physical Consistency of Ovipositing *Aedes hexodontus* Females

(100 adults used in each trial)

Trial number	Type of substrate	Number of eggs 1953 Test 1	1954 Test 1	1954 Test 2
1	White filter paper	180	159	
	Black filter paper	275	216	
2	White filter paper	85	32	
	White towelling	887	925	
3	Black filter paper	574	11	69
	Black towelling	3807	842	896
4	White towelling	2202	140	192
	Black towelling	2717	904	1200

involve the awareness of a different humidity such as might exist over the towelling as a result of greater evaporating surface or an awareness of a difference in temperature. A complex interaction of all considerations may be the case. If the female must first touch the substrate before choosing, then she may lay some eggs on one substrate before being stimulated to move. Also the period between which the individual eggs mature and are ready for laying may be long enough that the female is stimulated to fly or walk from the original oviposition site and thus be forced to make a new choice of oviposition substrate each time she lays. Because of these considerations it was not possible to ascertain the number of females choosing one site or the other, therefore the absolute number of eggs laid on each substrate was used as indicative of preference. When this is done the preference for black over white as well as for towelling over filter paper is significant at the .005 level. Why there were fewer eggs laid when filter paper alone was offered as a substrate is not known.

It is probable that the choice of black over white is made by the adult after a visual survey. Frost (4) found a definitely greater attractiveness with black light over white of equal foot candles. In the present experiment the intensity of reflected light from the black and white substrates was not measured but our results agree with those of Frost in showing the preference for black. What specific stimulus of the many which are combined in the towelling, causes the female to choose to lay eggs on towelling rather than on filter paper is not known. The depressions into which the eggs are inserted on towelling are darker than the surface of the filter paper when both substrates are white but this is not the case when both are black. The dampness may be important in that more moisture would be held in the depressions of the towel than would be found on the surface of the filter paper. Yet water alone is not sufficient stimulus since *Aedes hexodontus* when offered a choice of open water and a wet towel never lays on the water. When the female lays she makes a probing and stroking movement with her abdomen so it appears that the abdominal area carries the organs sensitive to at least a part of the factors influencing oviposition.

In the experiment to test temperature, three trials each year were carried out in two successive years, 1953 and 1954. The data, each year, for all three trials showed normal distributions which were approximately superimposed. On the basis of this it can be assumed that the temperature at the mean of the frequency distribution represents the preferred temperature. To compute the mean of the frequency distribution for each year, 29 classes were extracted from the pooled frequency curve of the three trials and the group mean calculated. A "t" test confirmed that there were no significant differences between the three trials. The variance for the pooled trials for 1953 was 76.09, the standard deviation was 8.78; the mean of the frequency distribution of all eggs appeared at 73.97° F. For 1954 the variance was 30.85, the standard deviation 5.55; the mean frequency distribution was at 74.18° F. The fiducial interval of the mean at the .01 level of significance was 73.97±0.422° F. for 1953 and 74.18±0.229° F. for 1954. It was also calculated at the 0.95 level of confidence, that 95 percent of the eggs of a similar experiment would be laid in a temperature range of 73.98±17.21° F. for 1953 and 74.18±10.88° F. for 1954. The confidence intervals for the mean show that close agreement exists in the preference of the sample of the populations from both years.

It was of interest that the mosquitoes laid a few eggs at both the gradient extremes of 99° F. and 44° F. If they react in the field as in the laboratory, a wide range of temperatures could be tolerated for oviposition.

Aedes aegypti Linnaeus. This species was used for oviposition site preference for comparison with the northern species. As shown in Table 2 the results again show

TABLE 2.—Oviposition Site Preference of *Aedes aegypti*

(100 Females used in each trial)

Trial number	Type of substrate	Number of eggs
1	White filter paper	65
	Black filter paper	2782
2	White filter paper	355
	White towelling	3525
3	Black filter paper	156
	Black towelling	1340
4	Black towelling	987
	White towelling	88

conclusively that black is preferred to white and rough to smooth.

Aedes campestris Dyar and Knab. An oviposition site preference experiment was also performed with *Aedes campestris*. The choice was between moist white filter paper and a dish of distilled water. The method used was similar to that used where the choice was white or black and rough or smooth. In the first trial with 75 adult females, 496 eggs were laid on white filter paper and 4,305 eggs on distilled water. In the second trial with 75 adults, 110 eggs were laid on white filter paper and 4,000 on distilled water.

The data show conclusively that *Aedes campestris* prefer to lay on open water rather than on a moist white substrate. It must be noted that no information was taken on the brightness of the surface of the water compared to that of the white filter paper but the dish of water was placed over a light background and on inspection gave light reflections.

Aedes communis De Geer. No controlled experiment was performed with *Aedes communis* to test oviposition site preference but during a large cage experiment, reported elsewhere, many eggs were found laid in damp dark corners of the cage. Only a few eggs were found on the dishes of white cloth covered absorbent cotton offered as oviposition sites.

SUMMARY AND CONCLUSIONS. 1. *Aedes hexodontus, Aedes aegypti* and *Aedes campestris* were tested for oviposition site preference. Black and white, and rough and smooth surfaces were offered to *hexodontus* and *aegypti*, open water and moist filter paper were offered *Aedes campestris*.

2. Black was conclusively chosen over white and rough over smooth for oviposition. *Aedes campestris* chose open water more often than moist filter paper.

3. *Aedes communis* in the laboratory cages was noted to choose damp dark areas in which to lay in preference to moist white cloth.

4. *Aedes hexodontus* showed a temperature preference for oviposition at 73.97±0.422° F. one year and 74.18±0.229° F. another.

5. It appears that *Aedes hexodontus* could lay over a wide range of temperatures in the field as evidenced by the wide range of temperatures over which the eggs were laid in the laboratory but a preference might be shown for the temperatures mentioned in (4) above.

6. The data indicate that *Aedes hexodontus* and *Aedes aegypti* may prefer rough dark substrates to light colored smooth ones in the field. *Aedes campestris* may prefer open water to merely moist substrates.

ACKNOWLEDGEMENTS. The author wishes to acknowledge the valuable assistance of Mr. C. E. Law of the Operational Research Section of DRNL for carrying

out the statistical analysis, and to Mr. T. P. Copps for his usual high caliber technical assistance.

References

BECKEL, W. E., and BARLOW, C. A. 1954. Studies of the biology of the *Aedes* of northern Canada (Culicidae). III. Field oviposition of *Aedes communis* (De Geer) with a method of separating the eggs from the substrate. Defence Research Northern Laboratory, Technical Paper Number 8, Defence Research Board, Ottawa.

BODMAN, M. T., and GANNON, NORMAN. 1950. Some habitats of eggs of *Aedes vexans*. Jour. Econ. Ent. 43(4):547–548.

FILSINGER, CARL. 1940. Distribution of *Aedes vexans* eggs. Proc. 28th Ann. Meet. New Jersey Mosq. Exterm. Assoc. 28:12–19.

FROST, S. W. 1954. Response of insects to black and white light. Jour. Econ. Ent. 47(2): 275–278.

HUSBANDS, R. C. 1952. Some techniques used in a study of *Aedes* eggs in irrigated pastures in California. *Mosquito News* 12:3.

LUND, H. O. 1942. Studies on the choice of medium for oviposition by *Anopheles quadrimaculatus* Say. J. Nat. Mal. Soc. 1:101–111.

PREPARATION OF THE CHORION OF EGGS OF AEDINE MOSQUITOES FOR MICROSCOPY [1, 2]

GEORGE B. CRAIG, JR. [3]

Entomology Branch, Chemical Corps Medical Laboratories, Army Chemical Center, Md.

During recent years, there has been an increased interest in the bionomics of eggs of the *Aedes* group of mosquitoes. Mosquitoes of *Aedes, Psorophora* and related genera spend most of their lives in the egg stage. Survey procedures for locating eggs are being standardized, prehatching treatments for destroying eggs are being devised and physiological and embryological studies are being made. Workers have been hindered in these activities by inability to recognize the eggs that have been obtained in the field.

In connection with the general problem of identifying eggs of aedine mosquitoes, a method that permits rapid and accurate examination of egg shells has now been developed. This depends on the fact that the layers that comprise the shell of the egg of the mosquito bear distinctive surface features which are specific in many instances. Descriptions of eggs given by Goeldi (1905) and Mitchell (1907) called attention to some of these features. Later work on *Anopheles* showed differences even at subspecific levels (cf. Horsfall, 1955 for ref.). Howard *et al.* (1912) and James (1922) made further observations on the surface structures seen on whole eggs of aedine mosquitoes. Marshall (1938) noted that the eggs of *Aedes* could be determined by gross shape but did not study surface features. Horsfall *et al.* (1952) found that eggs of *Psorophora* bear distinctive markings that are visible for the most part by reflected light and are very distinct when fragments of chorion are examined by phase microscopy.

Former methods for preparing fragments of chorion for mounting on microscope slides have met with certain difficulties that have now been overcome to a large extent. The natural color of eggs of many species of *Aedes* and *Psorophora* is so dark that light can not pass through the shell. The untreated chorion is hard and brittle, shattering readily when pressed by the coverslip. Crushing the whole egg

[1] The author is indebted to Dr. William R. Horsfall and Dr. Dietrich Bodenstein for valuable suggestions in connection with this work.

[2] Portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Entomology at the University of Illinois.

[3] 2nd Lt., Medical Service Corps., U. S. Army.