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OVIPOSITION STUDIES WITH *AEDES VEXANS* IN THE LABORATORY

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INTRODUCTION. Females of *Aedes vexans* (Meigen) were engorged in the field to obtain fertile eggs for colonization attempts. This paper presents data on oviposition by these insects and results of laboratory experiments using several substrates to stimulate oviposition.

Mosquitoes were obtained while feeding on the collector in the field during the summers of 1961 and 1962 in Dane County, Wisconsin. Females were covered with vials only after blood became visible through the body wall. Feeding was rarely interrupted using this procedure. Mosquitoes were given no food other than this blood.

Oviposition vials were made by cutting pyrex glass tubing, 28 mm (OD), into 75 mm lengths. Cotton gauze was bound over the mouth of the vial with a rubber band. The base of the vial was filled with a plaster of paris layer approximately 6 mm thick. This plaster bottom was covered with an absorbent cotton disc as an oviposition substrate. Vials were placed in petri plates containing water. This procedure maintained the cotton disc at saturation and obviated the frequent addition of water.

DURATION OF THE PREOVIPOSITION PERIOD. Data are based on 107 females collected in each of the years 1961 and 1962. Oviposition occurred 2–8 days after engorgement in 1961 and 3–12 days afterward in 1962 (Table 1). Most of the ovi-

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TABLE 1.—The preoviposition period of 91 *A. vexans* females collected in each of the years 1961 and 1962

	Preoviposition period in days											
Year	1	2	3	4	5	6	7	8	9	10	11	12	Totals
1961	0	2	9	21	29	19	8	3	0	0	0	0	91
1962	0	0	5	33	21	12	11	6	1	0	1	1	91

positing females (93% in 1961 and 89% in 1962) laid eggs 3–7 days after feeding.

Other workers have also found that *A. vexans* most frequently oviposits within a few days after feeding. Mail (1934) found that 97 females oviposited 2.5–6 days after engorgement. Gjullin, Yates, and Stage (1950) reported that "Caged adults usually lay eggs from 5 to 10 days after they have been given a blood meal." Although the range of the preoviposition period was great for 30 mosquitoes (5–25 days), a "typical" female laid eggs on the sixth day after feeding (Breeland and Pickard, 1964). Variation in the length of the preoviposition period among females collected and fed together may be due to retention of developed eggs as well as to differences in the rate of egg development.

POSTOVIPOSITIONAL LONGEVITY. Although most females collected in 1962 died on the initial day of oviposition, 27% of the ovipositing females collected in 1961 survived oviposition by at least one day (Table 2).

TABLE 2.—The postovipositional longevity of 91 *A. vexans* females collected in each of the years 1961 and 1962.

	Postovipositional longevity in days					
Year	0	1	2	3	4	Totals
1961	62	25	2	2	0	91
1962	78	3	8	1	1	91

Figure 1 shows the relationship of the preoviposition period to postovipositional longevity. The majority of mosquitoes ovipositing on any given day after engorgement died that same day. For example in 1961, 20 females oviposited on day five and only 8 of these mosquitoes survived oviposition by one or more days. Several exceptions were apparent (i.e., groups ovipositing on days three and eight after feeding in 1961).

Aedes vexans died soon after oviposition, in this study, probably because females were not fed carbohydrates nor blood after the initial blood meal. There is considerable evidence that this species may feed and oviposit several times. Gjullin, Yates, and Stage (1950) found that females laid 1–4 lots of eggs when blood meals were given subsequent to the laying of each lot. Mosquitoes were provided with sugar continuously. Breeland and Pickard (1964) found that 30 *A. vexans* females took 1–8 blood meals (mean 2.4) in the laboratory before death. One to three blood meals were taken during the preoviposition period. Females were provided with sugar continuously and offered a blood meal daily. Of the 1,417 engorged females tested for multiple host feedings in Kansas, over 18% or 263 females were positive (Edman and Downe, 1964). These multiple blood meals may represent multiple gonotrophic cycles as well as interrupted feedings.

FECUNDITY. Ninety-one (85%) of the 107 females considered in both years of study laid eggs. The 107 specimens used in the 1962 study were dissected after death and eggs in the abdomen were counted. The numbers of apparently normal and malformed eggs deposited by each female were also recorded. Ninety-one females laid 4–203 eggs, not including the abnormal eggs. The mean number of normal eggs deposited was 67. Three females laid eggs on more than one day.

Figure 2 shows the frequency distribution for the numbers of eggs deposited

(A) and the total numbers of eggs produced (deposited plus retained) (B) by the 107 insects. The class value used in these distributions was 14 mosquitoes. The modal class of A was 0–13 eggs and contained 22 specimens. The modal class of B was 70–83 and contained 19 specimens. Sixteen of the 22 individuals of the modal class of A (those laying 0–13 eggs) deposited no eggs but only 5 of these mosquitoes failed to develop any eggs. Five of these 22 mosquitoes were redistributed into the modal class of B. Therefore consideration of the eggs in the ovaries normalized the frequency distribution of egg production.

ABNORMAL EGGS. Thirty-three *A. vexans* specimens from 1962 deposited 1–44

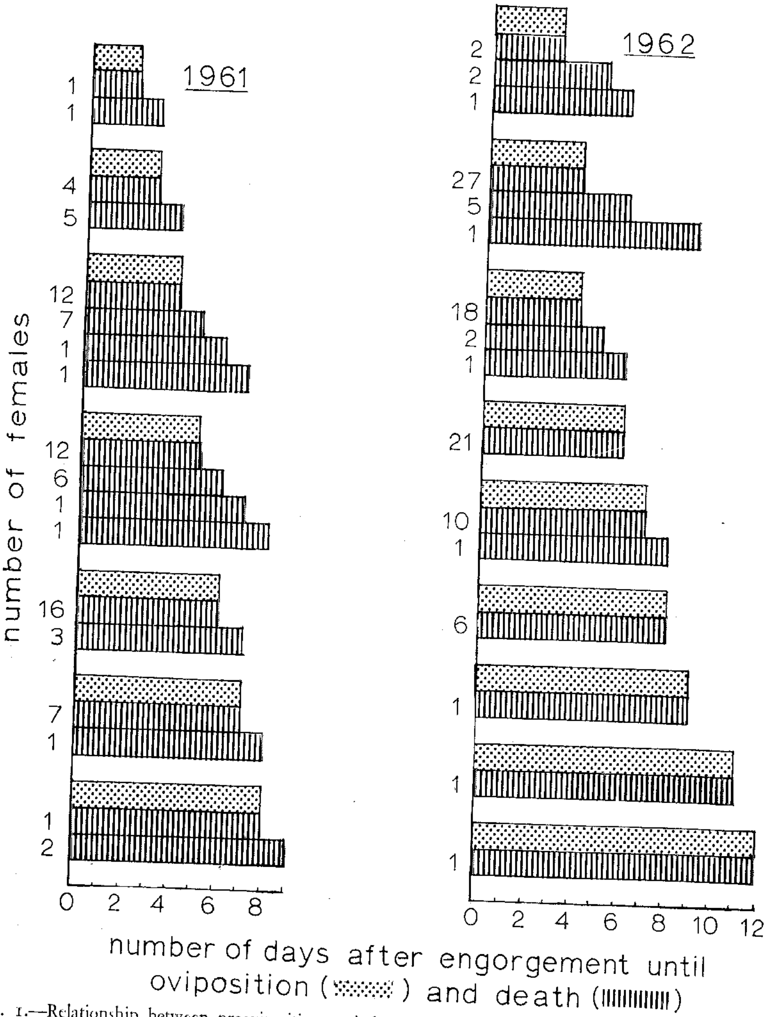

FIG. 1.—Relationship between preoviposition period and postovipositional longevity for 91 *A. vexans* females collected in each of the years 1961 and 1962.

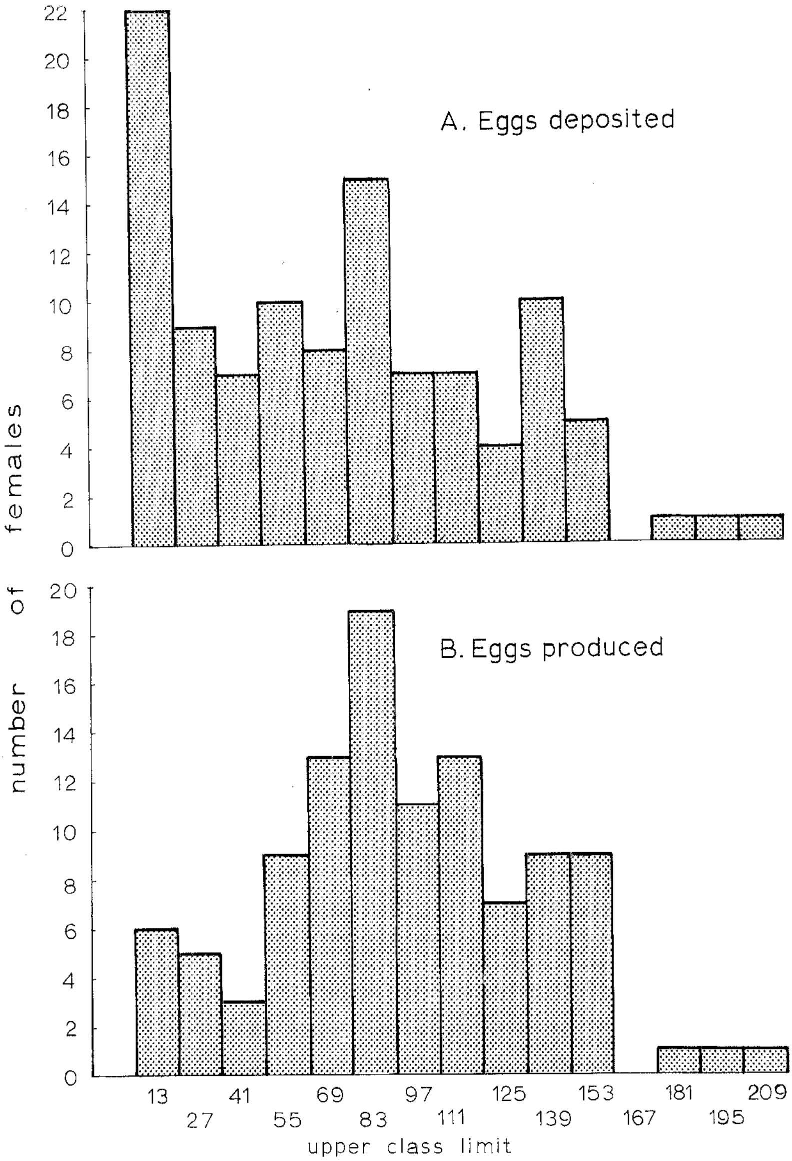

FIG. 2.—Number of eggs deposited (A) and total number of eggs produced (B) by 107 *A. vexans* females collected in Madison and the Mazomanie Wildlife Area, 1962.[a]

[a] Abnormal eggs are not included in these figures.

malformed eggs (mean, 7). The 16 specimens not laying eggs were dissected and produced no abnormal eggs. With 30 of the 33 specimens depositing abnormal eggs, less than 13% of the eggs were malformed. Although the numbers of eggs produced by these 30 females were varied, the percentage of abnormal eggs produced was consistently small (Fig. 3). Therefore there appeared to be little relationship between the total numbers of eggs produced and the numbers of malformed eggs produced.

On the other hand, the 3 mosquitoes laying more than 13% abnormal eggs produced 34, 40, and 48% malformed eggs respectively. These females were among the 7 specimens producing the fewest numbers of normal eggs. Barr (1958) reported similar results for this species. He said W. R. McKibben found that 2 of 22 ovipositing females laid abnormal eggs and that the same insects also laid the fewest normal eggs. Barr's suggestion that these females may not have engorged to repletion is very reasonable although all specimens fed in this study were distended with blood.

OVIPOSITION SUBSTRATE TEXTURE. An experiment was conducted to determine the relative potential of several common oviposition substrates to stimulate oviposition. Although this experiment would enable few inferences about the characteristics of natural substrates, it was intended to make possible more efficient oviposition in the laboratory.

Several oviposition substrates have been reported for *A. vexans:* cellucotton (Mail, 1934; Horsfall and Craig, 1958); cotton (Barr and Al-Azawi, 1958); gauze (Trembley, 1955; W. R. Horsfall, personal communication).

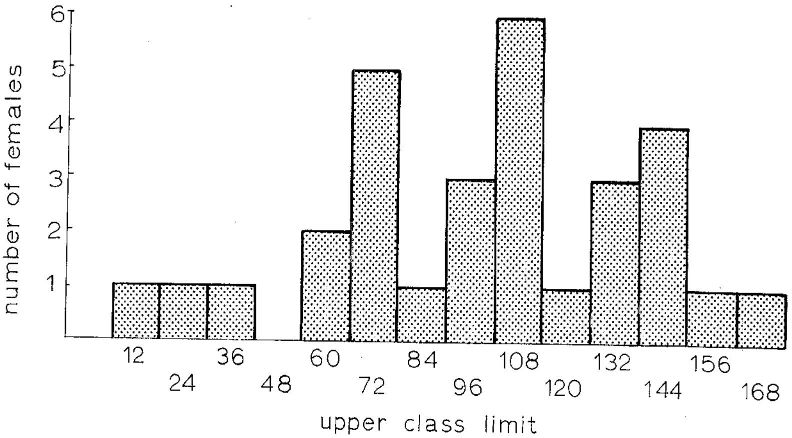

FIG. 3.—Total number of eggs produced by 30 *A. vexans* mosquitoes laying abnormal eggs.[a]

[a] These data include deposited, undeposited, and abnormal eggs.

Barr (1958) reported that *A. vexans* would not oviposit readily on light, coarse sand but Breeland and Pickard (1958) obtained large numbers of eggs using this medium. Several authors have reported poor oviposition with filter papers (Barr, 1958; Trembley, 1955).

Four oviposition substrates were used in the experiment: viz., cellucotton, cotton gauze (cheesecloth), filter paper, and blotter paper. All surfaces were white and not known to have been treated with any chemicals. Four sections of each substrate, 5 cm^2, were arranged in the form of a Latin Square. This array of replicates was placed on top of an 20-cm^2 section of the white blotter paper used as a test substrate. A nylon sponge base supporting the oviposition substrate arrangement was then placed in a 23-cm^2 aluminum baking pan. The sponge and substrates resting on it were maintained at saturation with distilled water. Over 300 females emerging from pupae collected in the field were held in a screened cage 30 cm^3 in size. The 30-cm^3 cage was placed in an insectary so that the oviposition surface was evenly illuminated during the 14-hr. period of artificial daylight. These mosquitoes were allowed to feed on the arm of a human host up to two weeks after emergence. Apple slices and water were provided continuously.

Five weeks after the first blood meal was taken, the baking pan was removed and eggs were counted. A 5-cm^2 section of acetate plastic was partitioned into 16 portions, 12.5 mm^2, by scoring it with a diamond marking pencil and crayon. This grid was placed over each 12.5-mm^2 portion of substrate and the eggs were counted within each division of the grid. All eggs were counted, including abnormal ones. Eggs laid on the sides of the sponge below the test surfaces were not counted.

B 369	G 246	F 120	C 1090
C 291	F 57	B 41	G 952
G 311	B 68	C 52	F 691
F 408	C 502	G 465	B 309

FIG. 4.—Arrangement of substrates in the Latin Square Design and total number of eggs laid within each replicate.[a]

[a] The letters "G," "C," "F," and "B" represent gauze, cellucotton, filter paper, and blotter paper, respectively.

The arrangement of substrates in the Latin Square and the total number of eggs deposited on each replicate are shown in Figure 4. These totals would indicate that the gauze and cellucotton materials were preferred over the blotter and filter papers. But the differences between cellucotton and gauze choices and blotter and filter paper choices were not great in the light of the following observations.

Although the experiment was designed so that a standard analysis (i.e., Latin Square) would produce meaningful results, two decided position effects in oviposition selection sites were noted. The lowest numbers of eggs were deposited on the four inside replicates. Three substrates were represented here and the blotter paper occurred twice. When the four replicates for each substrate were ranked in order of magnitude, the four inside replicates not only had the lowest numbers of eggs but also had many less eggs than any of those replicates on the outside (Table 3).

TABLE 3.—The number of eggs deposited on each replicate, tabulated in decreasing order from top to bottom.

	Substrate			
	G	C	F	B
Number of eggs per replicate	952	1090	691	369
	465	502	408	309
	311	291	120	(68)
	246	(52)[a]	(57)	(41)
Totals	1974	1935	1276	787

[a] The inside replicates in the Latin Square Design are in parentheses.

The second position effect occurred within those outer squares not found on the corners. In six of these sections very distinct gradients occurred from the inside margin to the outside margin (Fig. 5); in two squares (those having 291 and 246 eggs) the gradient was less pronounced.

The combination of these two effects produced a marked increase in egg deposition from the center of the oviposition sur-

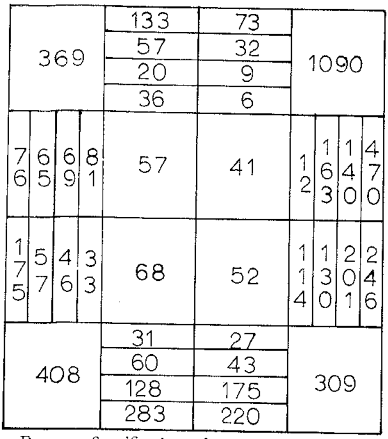

Fig. 5.—Stratification of eggs within several replicates.

face to the margins. This positioning effect obscured any differences attributable to types of substrate used. Possibly circular arrangements of the substrates would have avoided the bias shown in this experiment.

Summary 1. Of 214 engorged females, 182 (85%) laid eggs. The preoviposition period for 91 females collected in each of two years was 2 to 8 days (mode, 5 days) in 1961 and 3 to 12 days (mode, 4 days) in 1962.

2. Of 91 ovipositing females, 68% (1961) and 85% (1962) died on the day of oviposition. Brief life after egg deposition was attributed to lack of a carbohydrate food during retention in the laboratory.

3. Ninety-one females laid 4 to 203 eggs in 1962. Only 3 specimens oviposited on more than one day. The frequency of distribution of eggs produced was approximately normal.

4. Of 91 specimens ovipositing in 1962, 33 (36%) laid 1 to 44 abnormal eggs (mean, 7). Thirty of these specimens produced 13% malformed eggs. The 16 specimens not ovipositing apparently produced no abnormal eggs. No relationship was apparent between total number of eggs produced and the numbers of defective eggs.

5. *Aedes vexans* laid more eggs on gauze and cellucotton than on blotter and filter papers. Marked position effects occurred using a Latin Square Design.

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