Eggs of Floodwater Mosquitoes XII. Installment Hatching of *Aedes vexans* (Diptera: Culicidae)[1]

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ABSTRACT

Eggs of *Aedes vexans* (Meigen) obtained from mosquitoes reared and conditioned under controlled conditions will hatch in toto when subjected to a terminal hatching stimulus of reduced oxygen. However, eggs in the field, or when brought to the laboratory from the field, will not respond equally to this hatching stimulus. It was the objective of this investigation to demonstrate that sequential temperatures, location of the eggs, and circadian regimens of light during the antecedent generation could result in erratic or installment hatching.

Floodwater mosquitoes, particularly multivoltine ones such as *Aedes vexans* (Meigen), may appear as serial, partial broods from a population of eggs in natural sites. Breeland and Pickard (1967) proposed that hatching is partial at any 1 inundation, that serial inundations are required to induce some eggs to hatch, and that hatching is, therefore, by "installments." Because floodwater mosquitoes oviposit on soil prior to inundation, they may do so only when the soil is free of standing water. They do so 1 or more times during the season of adult activity when part or all of the soil at a site is exposed for oviposition. During any summer, therefore, eggs in any depression may represent ovipositing efforts any time from spring to fall. Variations, therefore, exist in parentage and age of the population of eggs present as well as in the duration and nature of environmental pressures to which the eggs have been exposed before submergence. Prior papers in this series have shown that temperature, humidity, inundation, and oxygen affect hatchability of eggs. This paper purports to show that light during rearing conditions, hatching temperatures, and location of the eggs with respect to the surface affect degree and time of hatching.

Although eggs of *A. vexans* are deposited at the soil surface they may become buried (Filsinger 1940). Eggs of *A. vexans* have been recovered from depths as great as 75 mm below the soil surface (Horsfall 1963, McDaniel and Horsfall 1963). However, little effort has been made to examine a population with respect to vertical displacement with advancing season or to examine the effects of burial on hatching.

Another factor which may influence hatching patterns in the field is the circadian rhythm of light experienced by the antecedent generation. Such influence has been demonstrated for *Aedes atropalpus* (Coquillett) (Anderson 1968). The effect of various daily patterns of alternating light and dark on the conditioning and hatching of embryos of floodwater mosquitoes has also been documented (Baker 1935, Kappus and Venard 1967, Love and Whelchel 1955, Vinogradova 1965). Field observations have been made that indicate that eggs of various floodwater mosquitoes resist hatching during late fall and winter (Dyar 1902, and others).

METHODS

Sampling for Eggs.—Eggs were removed from a field location to determine the vertical displacement. The site chosen for sampling is an embayment on the flood plain of the Sangamon River in Piatt County, Ill. Water inundates the area through a narrow cut in a roadway fill. The area is overgrown by a nearly pure stand of canary grass, *Phalaris.* The site produced a large brood in April. By late June oviposition was complete and no secondary flooding occurred. Several samples were taken at 6 different times between July 2 and Nov. 4. Circular cylinders of soil (10 cm diam and 5 cm deep) were removed by pressing a cutter with a piston inside into the soil to the desired depth. Samples were forced out of the cutter by pressure applied to the piston. Increments of 5-mm depth were shaved off and examined for eggs after screening and sedimentation had removed debris and soil (Horsfall 1956). Samples were taken repetitively throughout the season.

Burial of Eggs.—Eggs were buried beneath silt at known depths. Known numbers of eggs were subjected to a series of inundations. Prior to each inundation the soil was allowed to lose moisture (1) to a stage just prior to cracking and (2) to a state of extensive cracking. After an inundation, counts were made of free-swimming larvae that appeared in the water above the soil. These examinations revealed that a 2nd hatch occurred only when the soil was allowed to dry to a point of cracking.

Other eggs were placed in tubes of 6-mm bores containing sand or silt. Lots of 40 eggs were buried to depths of 1, 3, 5, and 10 mm under overburdens of sand or flood-plain soil. Selection of lots as to particle size was made by passing each through U.S. standard sieves. After burial the eggs were subjected to a hatching stimulus provided by deoxygenation in a solution of nutrient broth for a 24-hr period. The material was then examined for trapped larvae or eggs.

Exposure to Light.—Daily alternation of light and dark during ontogeny of the parents affects variability in hatching of F_1 eggs. Mosquitoes were reared

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under daily light:dark ratios (L:D regimens) of 6:18, 12:12, and 18:6 hr. All eggs were hatched at the start of the light phase and, thereafter, larvae, pupae, adults, and eggs were held at the starting sequence.

During all rearing the mosquitoes were maintained at a static 25.1±0.5°C. A light source consisting of two 7½-w incandescent bulbs was used. The intensity of the light at the water surface was 10.6 ft-c. By means of a 24-hr timer in conjunction with the light source it was possible to select any desired L:D regimen. Eggs were held for a minimum of 14 days at 25°C after oviposition to insure embryonation. Viability was assessed at the end of 14 days. Equal groups were then stored for 21 days at 30, 25, 21, 18, and 10°C. Eggs were stored in chambers permitting management of the light (Wilson 1970). After storage for 21 days the eggs were subjected to the hatching stimulus for 6 hr; the hatched larvae were counted, and the unhatched eggs were examined for viability. This examination was done by clearing the eggs in a solution of sodium chlorite and examining the intact embryo.

In an effort to determine in what stage or stages *A. vexans* was responsive to circadian L:D cycles, mosquitoes were reared under L:D 12:12 and transferred to L:D 18:6 at different times during development. All possible combinations from complete rearing at L:D 12:12 to complete rearing at L:D 18:6 were examined. The 2 L:D regimens were synchronized so that light periods began at the same time. All mosquitoes that were transferred from L:D 12:12 to L:D 18:6 were moved when the lights were on in both regimens. After the eggs were deposited, they were incubated throughout embryonation and beyond for a total of 14 days at 25°C, and then they were stored for 21 days at 18°C. At the completion of the combined interval, the eggs were subjected to the hatching stimulus for 6 hr, after which larvae were counted and unhatched eggs were examined for viability.

RESULTS

Installment hatching of *A. vexans* can be prevented, delayed, or made erratic by location of eggs in relation to depth of flood, degree of exposure above soil, and by rearing conditions of the parent generation. In the field any 1 site may be populated by eggs influenced by these factors in differing degrees.

Effect of Burial in the Field.—Eggs of *A. vexans* are deposited on the surface of moist soil and beneath any superficial layer of herbal detritus. They may not remain at the surface (Horsfall 1963). As the season progresses they may be displaced downward, a phenomenon that was demonstrated at a flood-plain site in a season of no secondary flooding. The downward progression of eggs in the field is shown in Table 1. As of July 2, 12% of the eggs were below the surface. These eggs were probably residual from 1968. By mid-July, 68% were still at the surface; less than 50% were at the surface by early August, and by November only 25% were at the surface. The mean depth in mid-July was 6 mm; it was more than 14 mm by November. The significant fact, though, is the shift in preponderant numbers from the surface to 6 or more mm deep by fall. Presumably the eggs below 6 mm were usually lost to the population because if any hatched, the larvae could not escape from the overburden.

The effect of burial in some cases is a failure to hatch in place while retaining viability. For example, buried eggs were recovered in the field after the completion of the vernal hatch. Seven vertical samples representing an area of 546 cm² yielded 178 eggs after the spring inundation had hatched the eggs at the surface. Of this number only 25 (14%) were within the top 25 mm. The remainder, 153 (86%), were found below 25 mm. The eggs were viable but presumably failed to hatch because of position.

Effect of Burial in Tubes.—Hatching and escape of larvae from overburdens of specific depth were observed in tubes of sifted soil. Eggs were unhatched in flood-plain soils irrespective of particle size when buried 5 mm or more. None emerged from a depth of 3 mm when the particle sizes were of 48 mesh or smaller, and only 10–30% could escape from 3 mm deep when particle sizes ranged between 28 and 48 mesh. One millimeter confined few or no larvae. Shore sand composed of round particles that yielded to larval movement permitted larvae to escape from

Table 1.—Vertical displacement of eggs of *A. vexans* in soil on a flood plain in Central Illinois throughout the summer and fall of 1969.

Depth below surface (mm)	2 July		15 July		2 Aug.		6 Sept.		22 Sept.		4 Nov.	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0–5	51	88	35	68	28	47	21	31	17	31	18	25
6–10	0	0	5	10	15	25	18	26	17	31	14	20
11–15	3[a]	5[a]	4	8	4	7	9	13	7	13	14	20
16–20	3[a]	5[a]	3	6	4	7	7	10	7	13	3	4
21–25	0	0	3	6	4	7	4	6	3	6	6	9
26–30	0	0	1	2	4	7	5	7	2	4	4	6
31–35	1[a]	2[a]	0	0	0	0	2	3	1	2	6	9
36–40	0	0	0	0	0	0	3	4	0	0	4	6
41–45	0	0	0	0	0	0	0	0	0	0	1	1
46–50	0	0	0	0	0	0	0	0	0	0	0	0

[a] Probably 1968 eggs.

Table 2. — Effect of daylength during ontogeny of P_1 and embryogeny of F_1 at 25°C for 14 days on hatching after exposure for an additional 21 days at 30, 25, 21, and 18°C.

Photoperiod L:D	Hatch after 14 days		Eggs hatched after an additional 21 day exposure at							
			30°C		25°C		21°C		18°C	
	No.	%	No.	%	No.	%	No.	%	No.	%
6:18	93	81	203	95	77	53	...	...	169	17
12:12	89	42	122	50	134	60	135	36	140	2
18:6	194	96	367	93	187	97	...	...	123	91

greater depths. Three-fourths of the larvae escaped from beneath 10 mm of sand (particle size 28–32 mesh) and even 10% escaped from beneath 10 mm of sand that passed through the 140-mesh sieve. No larvae were restrained by 5 mm of sand that was retained on a 32-mesh sieve, and almost 90% escaped from beneath sand retained on a 48-mesh sieve. Only 64% were restrained by an overburden of 3 mm even when the size of sand was that between 140 and 200 mesh.

Effect of Lighting During Parental Ontogeny.— Eggs from mosquitoes reared under L:D 18:6 continued to demonstrate a high percent hatch regardless of the storage temperature or duration of the storage period up to 3 weeks. Hatch ranged between 91 and 97% in all tests. Eggs from mosquitoes reared under L:D 6:18 and 12:12 showed a great deal of variation in hatchability after storage for 21 days at different temperatures. After 21 days of exposure, a progressive decrease occurred in the percent hatch with decrease in storage temperature (Table 2). At 18°C only 17% of the eggs from mosquitoes reared on L:D 6:18 and 2% of the eggs from mosquitoes reared on L:D 12:12 could be stimulated to hatch.

Eggs stored at 10°C demonstrated reduced hatchability regardless of the L:D regimen during rearing and storage. After 2 weeks exposure to 10°C, eggs from mosquitoes reared on L:D 6:18 showed a 26% hatch and those from mosquitoes reared under L:D 18:6 a 14% hatch.

Eggs deconditioned by storage at 10°C can be reconditioned by storage at 25°C. The ability of the eggs to recover from storage at 10°C appears to be related to the L:D of previous rearing. Examination of deconditioned eggs after 0, 2, 4, and 5 days at 25°C showed a more rapid and complete recovery of eggs from adults reared under L:D 18:6. After 5 days, 90% of those eggs from mosquitoes reared under a L:D 18:6 had hatched while 74% of those from mosquitoes reared under L:D 6:18 had hatched.

Eggs from mosquitoes reared under L:D 6:18 or 12:12 can be variably deconditioned by storage at temperatures ranging between 30 and 18°C for 21 days (Table 2). After this same period, eggs from mosquitoes reared under a long day (L:D 18:6) maintain a high percent of hatch even though they were held under the same thermal regimens. However, eggs from mosquitoes reared on a long day (L:D 18:6) can be prevented from hatching if the storage period is continued for 28 days. After an additional 7 days storage, hatching of these eggs was reduced to 32%.

Efforts to determine the responsive stage in *A. vexans* revealed that only those mosquitoes in which the adults were exposed to a short day demonstrated a reduced hatch after storage for 3 weeks at 18°C (Table 3). All other combinations of rearing in which various instars were reared on L:D 12:12 and others on L:D 18:6 demonstrated a 90–100% hatch.

The ability to respond in this manner to seasonal L:D ratios and thermal changes may be of considerable survival value. With the approach of the cooler winter months, the accompanying short day may stimulate adult mosquitoes to deposit winter ova in the same manner as *Anopheles walkeri* Theobald (Hurlbut 1938). Ova from these mosquitoes may be responsive to cooling changes in temperature. At any given temperature, below some undetermined minimum, eggs will respond less to the hatching stimulus as the duration of the storage period is lengthened.

The sensitivity to temperature of embryos from imagines that developed in the fall can be demonstrated through an examination of 2 field locations. In 1955, and again in 1969, heavy rains in late September produced flooding conditions during the 1st week of October in areas known to contain large numbers of eggs. The 1955 flood resulted in a substantial hatch, whereas during the same period in 1969 no larvae could be found.

An examination of climatological data for these 2 periods provides an interesting contrast, and one

Table 3. — Effect of photoperiod during various developmental periods on the hatching of eggs stored for 21 days at 18°C.

Photoperiod during specific stages of development		Hatch after 21 days at 18°C	
L:D 12:12	L:D 18:6	No.	%
	1,2,3,4,P,A,E[a]	322	90
1	2,3,4,P,A,E	160	98
1,2	3,4,P,A,E	217	96
1,2,3	4,P,A,E	166	93
1,2,3,4	P,A,E	192	99
1,2,3,4,P	A,E	104	100
1,2,3,4,P,A	E	110	5
1,2,3,4,P,A,E		140	2
E	1,2,3,4,P,A	149	98

[a] Numerals indicate instars; P = pupa, A = adult, E = egg.

which, in light of the work presented in this paper, can be interpreted as having considerable ecological significance. In 1955 the mean weekly temperatures for the 3 weeks preceding the inundation (3rd and 4th weeks of September and the 1st week in October) were 24, 13, and 17°C, respectively. The temperatures for the same period in 1969 were 17, 16, and 19°C, respectively. It can be seen that the 1955 flood was preceded by 2 weeks of temperature in the 18°C range whereas the 1969 inundation was preceded by 3 weeks of temperature in the 18°C range. It would appear that the additional 7 days of temperature in the 18°C range in 1969 was a critical feature resulting in a zero hatch.

A point of some interest is that eggs from mosquitoes reared on L:D 18:6 can also be caused to enter the latent state. Laboratory examinations have indicated that those eggs will demonstrate a reduced hatch after 4 weeks at 18°C. Because eggs from mosquitoes reared on a long day may also resist hatching at cold temperatures, the question then arises as to what ecological advantage is provided those eggs from mosquitoes reared on a short day. Much more work is necessary before this question can be answered with any degree of certainty. However, it would appear that those eggs from mosquitoes reared on a short day are much more sensitive to graduated changes in temperatures than those from mosquitoes reared on a long day. The latter eggs respond more nearly in an all-or-none fashion. Eggs from mosquitoes reared during short days are much better adapted to respond to the fluctuating temperatures generally associated with fall periods. Another possible advantage of eggs from mosquitoes reared during short days is that their ability to pass the winter and hatch the following spring is enhanced.

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