HATCHING OF THE EGGS OF *AEDES TAENIORHYNCHUS* (WIEDEMANN) (DIPTERA:CULICIDAE) IN RESPONSE TO TEMPERATURE AND FLOODING [1]

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INTRODUCTION. *Aedes taeniorhynchus* (Wiedemann), a widely distributed American salt-marsh mosquito, is known to be a multivoltine species (Smith, 1904; Horsfall, 1955; Pratt, 1959). Presumably the eggs of this mosquito enter a state of facultative diapause in the more northern parts of its range. Travis (1953) studied the effects of repeated flooding and desiccation, and of various hatching media on egg viability. Elmore and Fay (1958) reported on variations in numbers of larvae hatching from sod samples exposed to different temperatures. However, not much work has been done on the response of *A. taeniorhynchus* eggs exposed to chilling and freezing temperatures. Mallack *et al.* (1964), liberally quoting Clements (1963), briefly reviewed the literature pertinent to diapause, quiescence, conditioning, and reactivation of aedine eggs.

The objectives of this investigation were as follows:

1. To determine the effects of different periods of freezing (14° F.), chilling (40° F.), and exposure to a summer temperature (75° F.) on hatching and viability of the eggs.
2. To compare the effects of continuous flooding with alternate flooding and drying.
3. To compare the response to alternate flooding and drying of eggs taken at random from a laboratory colony with the response of eggs from isolated females.
4. To determine any variation in the hatching response of chilled eggs from isolated females.

METHODS AND MATERIALS

LABORATORY COLONIZATION OF *Aedes taeniorhynchus*. Haeger (1958) and Davis (1958) independently reported the successful colonization of *A. taeniorhynchus* in the laboratory. To provide eggs for the present study, a laboratory colony was established. Eggs were initially obtained from a Florida strain of *A. taeniorhynchus* under colonization at the Department of Entomology, Walter Reed Army Institute of Research. They were reared in an insectary described by Murray and Bickley (1964) at a temperature of 80° F. and a relative humidity of about 80 percent. Eggs used for colony maintenance were flooded for 24 hours in a small enamel pan (12¼" x 7¾" x 2½") with approximately one gram of "Difco" nutrient broth (Difco Laboratories, Detroit Michigan) mixed in one liter of tap water. Two "Misco Pellets" (Misco Mills; Bozeman, Montana) were added as food for the newly hatched larvae. Larvae were then transferred to large enamel pans (16⅜" x 9¾" x 2½") containing about 2 liters of tap water, two "Misco Pellets" and a handful of straw. Six enamel pans with 300 larvae per pan yielded sufficient adults to maintain the colony. One week after hatching, 80–90 percent of the larvae had pupated. The pupae were strained from the large enamel pans and placed in the smaller enamel pans which could be easily placed in a 20-inch-square aluminum cage with an orthopedic stockinet sleeve. These pans were

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left in the cage from 3 to 5 days or until the majority of the adults had emerged.

Haeger (1958) attempted to intensify the stimulus for mating in *A. taeniorhynchus* by improving the opportunities for increased flight activity. He reported that changing light of low intensity and crowding increased the flight activity of both sexes of this mosquito. Following Haeger's suggestion, between 800 to 1,000 individuals of each new generation were concentrated in a 20 cubic-inch cage. This degree of crowding was sufficient to induce flight activity and mating. A 150-watt incandescent bulb was placed above the insectary and regulated so that the mosquitoes received 14 hours of light and 10 hours of darkness. This lighting seemed to have little effect on flight activity.

The adult mosquitoes were continually offered a 6 percent sucrose solution which was placed in a 25 ml. flask and made available by putting a sheet of rolled 9-cm. Whatman filter paper into the flask. A few days after emergence, the females were given their first blood meal. A rabbit in a wooden restraining device was placed on top of the mosquito cage. The adult females fed on the depilated abdomen of the rabbit through mosquito netting. Females were offered a blood meal twice weekly.

Initially, three oviposition sites were ofered to the females as follows: (1) moistened 9-cm. Whatman filter-paper, (2) moist *Sphagnum* wrapped in cheesecloth and (3) wet mud wrapped in linen sheet cloth. Although the females oviposited on all three sites, the most satisfactory site proved to be the wet mud. The mud was placed in a small enamel pan. The pan was filled with water for several hours to cause complete saturation of the mud. The excess water was poured off and several layers of cheesecloth placed on top of the wrapped mud. The females would oviposit quite readily on the moist cheesecloth. The cheesecloth with the eggs was removed periodically and new cheesecloth placed on the mud.

TREATMENT OF EGGS FROM THE COLONY. All eggs were stored on moist cheesecloth in polyethylene plastic bags. The plastic bags were placed in a Shiebler desiccator, the bottom of which contained an inch or more of water. Thus, the eggs were maintained in a saturated humidity. Eggs to be used for maintenance of the colony were stored at 75° F. Eggs exposed to summer temperature (75° F.) were also placed in the same desiccator for the designated period of time. Eggs subjected to chilling (40° F.) were first exposed to 75° F. for one week, to allow the embryos to develop, and then placed in a desiccator which was kept in a constant-temperature box at 40° F. Eggs subjected to 14° F. were also kept at 75° F. for one week before being placed in the freezer compartment of a refrigerator. As a desiccator would not fit in the freezer compartment, the plastic bags containing the eggs were put into the freezer. A hatching medium consisting of 0.1 percent "Difco" dehydrated nutrient broth in sea water and tap water (1:1) was found to provide a better hatching response than that resulting from several other mixtures of sea, tap, and distilled water.

Those eggs exposed to 40° F. and 14° F. must undergo a period of conditioning before they will hatch. An experiment was conducted to determine the period of time necessary to condition eggs exposed to 40° F. at 75° F. so that the 40° F. eggs would give a comparable hatch to that of eggs exposed only to 75° F. It was found that a minimum period of four days was required for proper conditioning.

After the eggs had been exposed for various periods to 75° F., 40° F. or 14° F. temperature and had been conditioned, they were removed from the plastic bags and brushed from the cheesecloth onto a strip of moist filter paper. Using a moistened water color brush, the eggs were separated into groups containing 100 eggs per group. The eggs were examined with a stereoscopic microscope, and those eggs which had collapsed or broken were discarded. Eggs uniform in size and turgidity were assumed to be viable.

With the aid of a wash bottle containing distilled water, the eggs were washed into small plastic baskets with 100-mesh bronze screen wire bottoms. The procedure for making these baskets was described by Mallack *et al.* (1964). The baskets were marked and then submerged in a glass bowl containing the hatching medium. Flooding consisted of one immersion for 7-days (continuous flooding) and four immersions for 2 days each with alternating 2-day drying periods (alternate flooding and drying). For each exposure period and temperature, one group of 300 eggs was continuously flooded while another group of 300 eggs was alternately flooded and dried. The hatching medium in all cases was changed each day. The baskets were checked daily, and all hatched larvae were removed and counted before the baskets were placed in fresh hatching media. Those baskets of eggs to be dried were stored in petri dishes on moist filter paper.

When flooding had been completed, all eggs which had not hatched were examined for viability. The determination of egg viability was accomplished by bleaching with a 5.25 percent solution of sodium-hypochlorite. This method was described by Mortenson (1950) who used it to study the embryos of *A. nigromaculis* (Ludlow). Christophers (1960) reported that eggs of *A. aegypti* which contained a fully developed larva ready to hatch, when bleached, would show three conspicuous dark spots towards the anterior pole. The two lateral spots are the pigmented ocelli and the dorsal spot is the egg-breaker. The appearance of a segmented embryo with pigmented ocelli and egg-breaker was, therefore, used to determine egg viability in this study. Submergence in the sodium-hypochlorite solution for a period of 10–15 minutes, revealed these characteristics in fully developed *A. taeniorhynchus* larvae.

TREATMENT OF EGGS FROM ISOLATED FEMALES. Females in the colony which had engorged to repletion on a rabbit were placed in a cage which lacked an oviposition site and were held for 4 days to insure digestion of the blood meal. These females were then placed individually into glass vials, 3 cm. (ID) x 8 cm., containing a 2 x 6 cm. strip of filter paper previously moistened by filling the vial with tap water to a depth of about 6 mm. The vials were plugged with cotton. Within 3 days most of the females had oviposited on the filter paper, but a few oviposited in the water. Eggs from each female that had oviposited were collected daily.

The filter paper was removed from each vial; all eggs from the vial were moved to the paper; and the paper was placed in a small plastic bag. The eggs were exposed to 75° F. for 7 days in a desiccator with a saturated humidity before being subjected to 40° F. for 20 days. After exposure to 40° F., the eggs were conditioned for 4 days at 75° F. The eggs from each female were washed into individual plastic baskets which were marked and placed in glass bowls for flooding. No attempt was made to remove abnormal eggs, which were presumed to be nonviable, from the baskets. All the eggs from isolated females were alternately flooded and dried in the manner previously described. The number of viable eggs remaining after the flooding was determined by bleaching with 5.25 percent sodium-hypochlorite.

RESULTS AND DISCUSSION

HATCHING RESPONSE OF EGGS FROM THE COLONY. In each experiment, three groups of eggs containing 100 eggs per group were subjected to the conditions shown in Tables 1 and 2. All results are based on the total number of viable eggs in each group of 300 eggs initially studied. These tests were planned with the idea that the response to alternate flooding and drying could be compared to the response to contінous flooding and with the idea that comparisons of the effects of different temperatures could be made.

Tables 1 and 2 show that eggs kept at 75° F. for 7 days hatched very readily after 1 or 2 days of flooding. During the second immersion after a 2-day drying period the increase in hatch was only 0.7 percent, and the third and fourth floodings did not induce additional hatching.

Eggs chilled for 20 days were slightly

TABLE 1.—Hatching and viability of eggs of *Aedes taeniorhynchus* after varying periods of exposure to three temperatures and alternating flooding and drying.

			Hatch after							
	Viable eggs		First 2-day flood		Second 2-day flood		Third 2-day flood		Fourth 2-day flood	
Exposure	No.	%[a]	No.	%	No.	%[b]	No.	%[b]	No.	%[b]
75° F.–7 days	293	97.7	289	98.6	3	99.3	0	99.3	0	99.3
40° F.–20 days	279	93.0	220	78.9	7	81.4	4	82.8	1	83.2
40° F.–45 days	285	95.0	188	65.9	30	76.5	5	78.2	7	80.7
14° F.–3 hrs.	297	99.0	289	97.3	5	98.9	0	98.9	0	98.9
14° F.–2 days	282	94.0	207	73.4	15	78.7	2	79.4	4	80.9
14° F.–14 days	279	93.0	92	32.9	10	36.6	5	38.4	5	40.1

[a] Percent viability based on 300 eggs; number of eggs selected at random from colony.
[b] Cumulative percent.

more sensitive to alternate flooding and drying. There was a very small but steady increase in hatch in response to second, third and fourth floodings. Eggs flooded continuously did not respond after the fourth day.

When eggs were chilled for 45 days the percent hatch was 65.9, 76.5, 78.2, and 80.7 in response to four flooding and drying periods. When eggs similarly chilled were flooded continuously there was no additional hatch after the fourth day at which time the hatch was 68.1 percent.

Comparison of alternate flooding and drying with continuous flooding of eggs exposed to 14° F. for 3 days shows that there was very little difference in response. In fact, this brief freezing period seemed to have little effect.

Eggs subjected to 14° F. for 2 days and alternately flooded and dried responded with an increased hatch of 5.3 percent following the second flooding and about 1 percent following each of the last two floodings. When these eggs were flooded continuously there was no increase in hatch after the fourth day.

Exposure to 14° F. for 14 days caused a marked reduction in total hatch. There was no great difference in response to alternate flooding and drying as compared to continuous flooding.

In general, the data reported in Table 1 show that eggs chilled for 20 or 45 days or exposed to freezing for 2 days responded similarly when alternately flooded and dried. These eggs were presumed to be in a state of facultative diapause before they were conditioned. A large majority of the eggs which hatched did so after the first 2-day flooding, but the percent hatch was lower than the hatch of eggs exposed only to a summer temperature. However, it should be noted that there was an appreciable number of eggs which hatched after the second flooding: 2.5 percent for those chilled 20 days, 10.6 percent for those chilled 45 days, and 5.3 percent for those exposed to 14° F. for 2 days.

The failure of some eggs to hatch until flooded the second time is probably due in part to the fact that all the eggs had not been fully conditioned before the first flooding. The additional two days on moist filter paper conditioned a number of eggs which did not hatch during the first flooding.

Experimental evidence presented in Table 1 also shows that a small number of *A. taeniorhynchus* eggs, having been exposed to cold temperature and alternately flooded and dried, were still hatching after the third and fourth floodings. An inherited response to hatching, as described by Gillett (1955), may account for this hatching in installments over a period of time. Those *A. taeniorhynchus* eggs exposed to 14° F. for 3 hours did not show this pattern. Because 99 percent of these eggs hatch after the first 2-day immersion (Table 1), it was thought

TABLE 2.—Hatching and viability of eggs of *Aedes taeniorhynchus* after varying periods of exposure to three temperatures and continuous flooding.

Exposure	Viable eggs		Hatch													
			Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
	No.	%[a]	No.	%	No.	%[b]	No.	%[b]	No.	%[b]	No.	%[b]	No.	%[b]	No.	%[b]
75° F.–7 days	296	98.7	291	98.3	3	99.3	0	99.3	0	99.3	0	99.3	0	99.3	0	99.3
40° F.–20 days	286	95.3	197	68.9	20	75.9	2	76.6	3	77.6	0	77.6	0	77.6	0	77.6
40° F.–45 days	288	96.0	162	57.3	23	64.2	9	67.4	2	68.1	0	68.1	0	68.1	0	68.1
14° F.–3 hrs.	296	98.7	284	95.9	7	98.9	0	98.9	0	98.9	0	98.9	0	98.9	0	98.9
14° F.–2 days	288	96.0	179	62.2	8	64.9	4	66.3	1	66.7	0	66.7	0	66.7	0	66.7
14° F.–14 days	279	93.0	83	29.7	1	30.1	1	30.5	2	31.2	0	31.2	0	31.2	2	31.9

[a] Percent viability based on 300 eggs; number of eggs selected at random from colony.
[b] Cumulative percent.

that this period of exposure was too short to instill a state of facultative diapause.

When eggs which had been subjected to chilling for 20 and 45 days were continuously flooded for 7 days, most of those which hatched did so on the first day (Table 2). The second day of flooding caused an additional hatch of 7.0 percent for eggs chilled 20 days, and 6.9 percent for those chilled 45 days. Only a few eggs hatched on the third and fourth days of flooding, and none hatched later. Eggs exposed to 14° F. for 2 and 14 days showed this same pattern, with a smaller proportion of the eggs hatching. Since many of the unhatched eggs were still viable, a period of drying after the seventh day might have conditioned more eggs which would have hatched on reflooding. The fact that a number of eggs subjected to freezing for 2 days hatched on the second day of flooding causes speculation that this was due to an inherited response in the eggs to hatch in installments. In those eggs exposed to 75° F. for 7 days and 14° F. for 3 hours, over 95 percent of the eggs hatched following the first day of flooding.

The data also indicate that *A. taeniorhynchus* eggs are very cold-hardy. The lowest viability obtained was 93 percent (Tables 1 and 2). This percent occurred in eggs exposed to 14° F. for 14 days and in eggs exposed to 40° F. for 20 days which were alternately flooded and dried.

Comparison of the percent hatch, after the final flooding, between eggs alternately flooded and dried and those continually flooded revealed the following points (Figure 1): (1) eggs continually flooded had a lower percent hatch than those alternately flooded and dried for each exposure period; (2) the longer the eggs were exposed to freezing temperatures, the lower the percent hatch, regardless of flooding procedure; (3) nearly all the eggs exposed to 75° F. for 7 days and to 14° F. for 3 hours hatched, regardless of the flooding procedure; and (4) in the egg groups exposed to chilling for 20 and 45 days or freezing for 2 and 14 days, at least 15 percent of the viable eggs did not hatch. On the whole, *A. taeniorhynchus* eggs responded to variations in temperatures, flooding, and drying in a manner very similar to the response of *Aedes sollicitans* as reported by Mallack *et al.* (1964).

HATCHING RESPONSE OF EGGS FROM ISOLATED FEMALES. In the experiment discussed here, eggs from each of 106 females were collected separately, chilled at 40° F. for 20 days, and alternately flooded and dried. The number of viable eggs from each female was determined after the fourth flooding, and the percent hatch was based on the number of viable eggs deposited by each individual.

The 106 females were selected from the colony at a time when there were approximately 850 individuals in the cage. The average number of eggs was 78.7 per female. The total number of viable eggs was found to be 6,950 as shown in Table 3. The cumulative percent hatch resulting from four floodings with 2-day drying periods between floodings is given in Table 3. The majority of the eggs from each of the 106 females hatched after the first 2-day flooding. Also shown in Table 3 are the results obtained when 279 viable eggs were taken at random from the colony and exposed to similar conditions.

Although the number of viable eggs studied from isolated females was large (6,950), and the sample taken from the colony was relatively small (279), the cumulative percent hatches were very similar (Table 3). A chi-square test (Snedecor, 1956) was carried out on the data obtained from these two experiments.

The purpose of this analysis was to determine if any significant difference in response of eggs to similar stimuli existed in the large sample taken from isolated females and the smaller sample from the colony. The comparison based on those percentages is shown in Table 4. The value obtained for chi-square, with 4 degrees of freedom, was 2.065. This is non-significant at the 5 percent level (Snedecor, 1956). Therefore, the hatching response in the small sample was representative of that obtained in the larger sample of eggs.

The reason for studying eggs from isolated females was to attempt (a) to ascertain whether eggs in deep diapause are the products of a few females, all or nearly all of whose eggs require strong stimuli to induce hatching, or (b) whether each female produces a small proportion of such eggs. In Table 5 the 106 females are distributed by groups or classes according to the hatching response of their eggs. The same results are graphically shown in Figure 2. It will be seen that 50 females (or 47.2 percent) fell into the top 10 percent and that 92 females (or 86.8 percent) were in the upper 60 percent group following the third or fourth flooding. In the 10.01 percent to 60.00 percent range there was a decrease in number of females (from 17 to 14) attributable to second and third floodings. These data show that a rather large number of eggs from these 14 females failed to respond to four floodings.

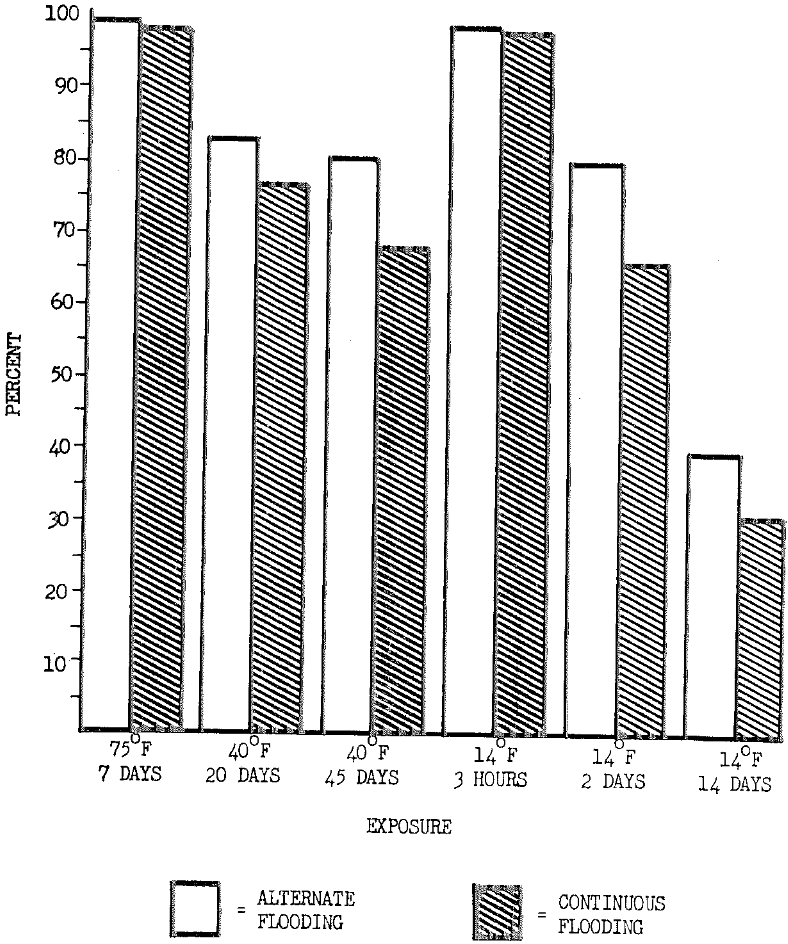


FIG. 1.—Comparison of percent hatch between eggs alternately flooded and dried and eggs continually flooded.

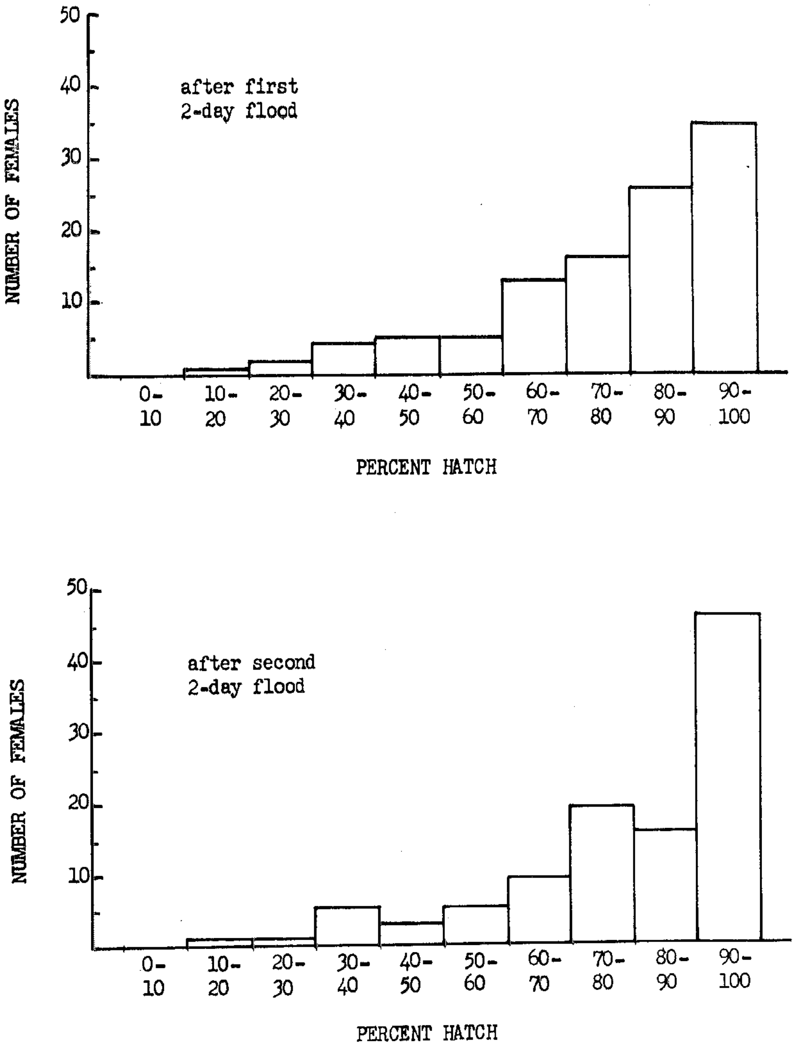
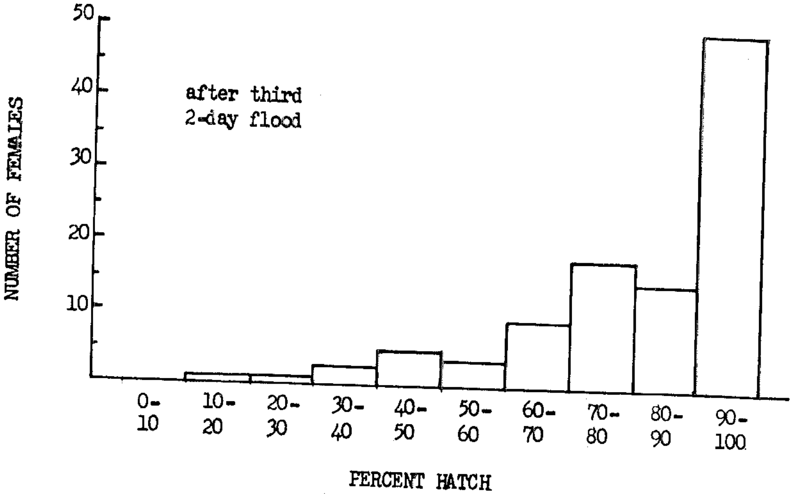


FIG. 2.—The distribution of 106 *A. taeniorhynchus* females by groups based on the percent hatch of the eggs.



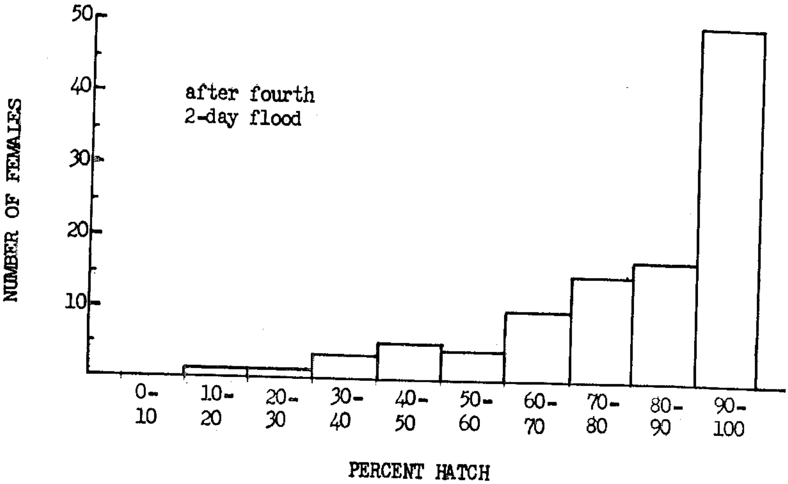


Fig. 2.—*Continued.*

TABLE 3.—Hatching response of eggs from isolated *Aedes taeniorhynchus* females and of eggs deposited by various females in the colony; eggs were exposed to 40° F. for 20 days and alternately flooded and dried.

	Viable eggs studied	Hatch after							
		First 2-day flood		Second 2-day flood		Third 2-day flood		Fourth 2-day flood	
	No.	No.	%	No.	%[a]	No.	%[a]	No.	%[a]
Isolated females	6,950	5,427	78.80	179	80.66	35	81.16	13	81.35
Colony	279	220	78.85	7	81.36	4	82.79	1	83.15

[a] Cumulative percent.

Presumably, they were in deep diapause. The single female in the 10.01 percent to 20.00 percent class has to be considered of importance in the maintenance of the population throughout periods of adverse environmental conditions because 90 percent of the eggs from this one individual were resistant to the hatching stimuli described. Egg behavior in this case might be categorized as "batch homogeneity" according to Gillett (1955).

Based on results reported here, it is suggested that most *A. taeniorhynchus* females produce heterogeneous egg batches in which very few eggs enter deep diapause, but a very small number of females (less than 10 percent) produce batches of eggs nearly all of which require a strong stimulus to break facultative diapause. In either case, there is no reason to doubt that variability in hatching behavior is an inherited characteristic. Installment hatching may be attributed to a few batches of eggs nearly all of which are "slow responders" as well as to a small number of "slow responders" in the vast majority of egg batches.

CONCLUSIONS

1. Eggs of *Aedes taeniorhynchus* exposed to 75° F. for 7 days or to 14° F. for 3 hours hatched readily; there was no evidence of installment hatching.

2. Exposure to 40° F. for 20 days or 45 days or to 14° F. for 2 days or 14 days induced facultative diapause; and in response to three subsequent 2-day drying and flooding periods small proportions of eggs (generally about 1 percent) hatched in installments.

3. Alternate flooding and drying induced a slightly higher percent hatch than continuous flooding.

4. Percent hatch was inversely proportional to duration of exposure to 14° F.

5. A small number of females (less than 10 percent) produced batches of eggs nearly all of which were in a state of deep diapause.

6. Installment hatching is due to the slow response of a few eggs in nearly all batches, but it is also due to the fact that a very small proportion of females lay eggs nearly all of which enter deep diapause.

TABLE 4.—Comparison of hatching response of eggs from isolated *Aedes taeniorhynchus* females with response of eggs taken at random from colony.

Percent	Isolated females	Colony	Chi-square value
Hatch after first 2-day flood	78.08	78.85	
Increase in hatch after 2nd 2-day flood	2.58	2.51	
Increase in hatch after 3rd 2-day flood	.50	1.43	2.0651 (NS)[a]
Increase in hatch after 4th 2-day flood	.19	.36	
Eggs which had not hatched after flooding	18.65	16.85	

[a] Nonsignificant at the 5% level.

TABLE 5.—The distribution of 106 *Aedes taeniorhynchus* females by groups based on the percent hatch of their eggs; eggs exposed to 40° F. for 20 days and alternately flooded and dried.

Groups by hatch	First 2-day flood	Number of females in each group after Second 2-day flood	Third 2-day flood	Fourth 2-day flood
90.01—100.00%	35	47	50	50
80.01—90.00%	26	16	15	17
70.01—80.00%	16	19	18	15
60.01—70.00%	12	9	9	10
Total	89	91	92	92
50.01—60.00%	5	5	4	4
40.01—50.00%	5	3	5	5
30.01—40.00%	4	5	3	3
20.01—30.00%	2	1	1	1
10.01—20.00%	1	1	1	1
Total	17	15	14	14
0—10.00%	..	..	..	..

It is reasonable to conclude that, as in *Aedes aegypti*, variability in hatching behavior is an inherited characteristic.

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