THE DISPERSAL OF *AEDES TAENIORHYNCHUS* V. A CONTROLLED SYNCHRONOUS EMERGENCE [1]

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INTRODUCTION. Dispersal studies of migratory mosquitoes pose one of the most challenging problems. In order to observe the movement of a reared and marked population, many variables must be brought under control. One such variable is the timing of the adult emergence, and another is the concentration of this emergence in time. Knowing in advance the day and hour of emergence is of great value in dispersal studies because the efforts of many persons in the deployment and operation of collecting devices must be anticipated and then coordinated.

In late May and early June of 1963 a preliminary experiment was conducted to test the feasibility of producing a synchronous emergence of the salt-marsh mosquito, *Aedes taeniorhynchus,* at a predetermined time. A further objective was to observe the behavior of mosquitoes reaching departure age subsequent to the normal crepuscular hour for a mass exodus, viz. 20 to 40 minutes after sunset. The earliest permissible age for spontaneous departure from the emergence site was assumed to be 6 to 8 hours.

Relevant to the timing of such a synchronous emergence is Nielsen's (1958) observation that the departure of these mosquitoes from their emergence site occurs only during the dark portion of the 24 hours. He also speculated that those individuals which had attained the proper age by sunset left in the twilight exodus while those which reached departure age after the twilight exodus might stay behind and leave, either a few at a time during the night or all together on a mass exodus at twilight of the next day.

The data of Nielsen and Evans (1960) on velocity of pupal development as regulated by temperature posed the possibility of controlling both the time and the synchrony of a brood's emergence. It was theorized that by harvesting pupae at intervals and retarding the development of each group by holding them at specified low temperatures, it would be possible to compress the emergence of all groups into a short time interval. With a temperature control of $\pm 1^\circ$ C. in both the concrete trough of the salt-marsh mosquito nursery (Pausch and Provost 1965) and the incubators in the laboratory, the stage was set for a trial.

Our basic assumption was that if temperature alone determined the rate of development and if the relationship did not vary with degree of completion of pupal development, then the total duration of the pupal stage, at any combination of temperatures, could be calculated on an incremental basis. We would use two temperatures, 28° in the marsh (concrete trough) and 20° in the laboratory incubators.[3] At these temperatures, pupae complete 2.58 percent and 1.19 percent respectively, of their total development in one hour. Our assumption was then, for example, that pupae would complete 90.2 percent of their development whether at 28° for 9.25 hours plus 20° for 55.75 hours, or at 28° for 33.25 hours plus 20° for 3.75 hours.

PROCEDURE. Before any work was attempted in the field, a time-sequence "flow-sheet" (Table 1) was composed, which scheduled all events and procedures in the experiment. Since we wished the mosquitoes to attain departure age, 6 to 8 hours old, subsequent to a sunset which would be at approximately 19^h 30′, their emergence would have to begin at 13^h 30′ or later. This was therefore the departure point for all other calculations. The flow-sheet further supposed a 40-hour interval of pupation, with pupal harvests every 4 hours. Finally, the plan was to bring the development of all pupae into synchrony when about 90 percent of their development was completed.

[1, 2] See footnote p. 8.

[3] All temperatures are in degrees Centigrade.

TABLE 1.—Schedule for the experiment to compress duration of emergence.

Date	Time	Occurrence
5/30	1800	Brood swale flooded and held until 2400 hrs.
5/31	0800	Start transfer of larvae from brood swale to concrete trough.
6/3	1000	Start of pupation
	1400	Pupae #1 moved to 20° C. retardation chamber.
	1800	Pupae #2 "
	2200	Pupae #3 "
6/4	0200	Pupae #4 "
	0600	Pupae #5 "
	1000	Pupae #6 "
	1400	Pupae #7 "
	1800	Pupae #8 "
	2200	Pupae #9 "
6/5	0200	Pupae #10 "
	0545	Pupae #10 returned to 28° C. concrete trough.
	0900	Pupae #9 "
	1230	Pupae #8 "
	1600	Pupae #7 "
	1915	Pupae #6 "
	2245	Pupae #5 "
6/6	0215	Pupae #4 "
	0545	Pupae #3 "
	0915	Pupae #2 "
	1100	Pupae #1 "
	1330	Start of emergence.

Data from Dr. P. T. M. Lum (personal communication) showed that larvae maintained at 28° under optimal feeding conditions would begin pupating four days and 12 hours after hatching. With 13^h 30′ of June 6 as the chosen time for start of emergence, simple calculations (Table 2) indicated that pupation should begin on June 3 at 10^h 00′. Calculating further backwards, hatching would then have to be at 22^h 00′ of May 30. However, since the newly hatched larvae would be in the exposed and uncontrolled environment of a brood swale for several hours and consequently exposed to night temperatures lower than the 28° of the rearing trough, larval development would be somewhat retarded. The exact amount of retardation was unknown but estimated at 4 hours. The time of hatching was therefore adjusted to 18^h 00′ of May 30.

With the time sequence determined, a

TABLE 2.—Number of hours involved in pupal development at the ten combinations of 28° (2.58% per hour) and 20° (1.19% per hour) exposures.

	28° C.*		20° C.**		28° C.*		
Group #	Hrs.	%	Hrs.	%	Hrs.	%	Total %
1	4	10.32	69.00	82.11	0	0	92.4
2	4	10.32	63.25	75.27	1.75	4.52	90.1
3	4	10.32	55.75	66.34	5.25	13.54	90.2
4	4	10.32	48.25	57.42	8.75	22.58	90.3
5	4	10.32	40.75	48.49	12.25	31.60	90.4
6	4	10.32	33.25	39.57	15.75	40.64	90.5
7	4	10.32	26.00	30.94	19.00	49.02	90.3
8	4	10.32	18.50	22.02	22.50	58.05	90.4
9	4	10.32	11.00	13.09	26.00	67.08	90.5
10	4	10.32	3.75	4.46	29.25	75.46	90.2

* Concrete trough. ** Incubator.

swale (Pausch and Provost, 1965) with an adequate egg deposit was selected and the larval collection trap installed. At 18h on May 30 the swale was flooded to its greatest depth, 46 cm, and the water maintained at that level until midnight, by which time all larvae were assumed to have hatched. Pumping was stopped and the water began to slowly recede. By 08h of May 31 the water had dropped to a level where the larvae were accumulating in the trap and the transfer of these first instars to the concrete trough began. When all larvae had been transferred, ample quantities of pelletized food[4] were scattered over the water. Larval development was watched closely and additional food was given daily.

On June 3 the larvae had reached the prepupal stage and were kept under almost continuous observation to detect the start of pupation. At approximately 10h, as predicted, the first pupae appeared, so the anticipated schedule of pupal harvest could be followed. Since pupae were to be harvested and removed to 20° retardation incubators every 4 hours, the first separation was set for 14h. We would continue to harvest pupae every 4 hours until they had all been transferred to the laboratory incubators.

Between separations, the larvae continued to pupate, so that at the end of each 4-hour period, the age of the pupae differed by as much as 4 hours. This necessarily placed a minimum time range of 4 hours over which the adults could emerge. Decreasing the separation interval could theoretically compress the emergence further, but a 4-hour interval was thought adequate. Furthermore, there was concern over the frequency of larval disturbances with numerous pupal separations.

The original plan to separate the new pupae from the larvae with the device developed by Fay and Morlan (1959) proved unsatisfactory because of clogging with debris and food particles. To circumvent this difficulty a cold-water technique was developed which was essentially as described by Weathersby (1963). All collections of pupae were held in 10″ x 15″ enameled pans, covered with a glass plate to reduce evaporation, and placed in incubators at 20°. They were kept at this temperature for the scheduled time interval given in Table 2. Since pupation was practically over in 40 hours, the ten expected groups of pupae were collected. By 11h of June 6, all pupae had passed through their cooling period and had been returned to the concrete trough to complete their development. At this time they were all at approximately the same stage of development, slightly over 90 percent complete, and about 3 hours and 45 minutes from the start of emergence at 28°.

After all the pupae had been removed from the rearing cell in the concrete trough and transferred to 20° incubators, an emergence cage was placed over the 6-foot section of trough to which the pupae would be later returned. This cage had a low front side to permit photographing the back side, and the top folded back to allow ready escape. All sides were lined off in a grid system to enable spot counting for estimating the number of adults resting on the screens, but only the back could be photographed in sufficient detail to make accurate counts from the prints. The figures given for emergence and movements of adults (Tables 3 and 4) are therefore relative, being merely for adults resting on the back of the cage.

Two behavior characteristics of mosquitoes must be recalled in order to appreciate the technique used for estimating their numbers and movements. They do not respond to stroboscopic light flashes (Nielsen, 1957) and can therefore be photographed repeatedly without being disturbed. Secondly, once the newly-emerged mosquito has found its place on the screen it will not move for several hours unless disturbed; in other words it will not shift positions. This is true at least until the spontaneous departure age is reached.

[4] "Gaines Meal," a dog food.

TABLE 3.—Emergence of adult *Aedes taeniorhynchus* on an hourly basis, June 6, 1963. The 3400 leaving between 19^h 30′ and 20^h 00′ are subtracted from the accumulative total (see text).

Hour of emergence	# adults emerged	% of total population	# of adults on screen
1200–1300	300	2	300
1300–1400	475	4	775
1400–1500	475	4	1250
1500–1600	750	7	2000
1600–1700	700	6	2700
1700–1800	850	8	3550
1800–1900	2300	22	5850
1900–2000	3350	31	5800
2000–2100	500	4	6300
2100–2200	600	6	6900
2200–2300	525	5	7425
2300–2400	100	1	7525

TABLE 4.—Adult movement June 7, subsequent to completion of emergence.

Time	# adults photographed	# adults departed
2400	7525	...
0100	7300	225
0200	7200	100
0300	7100	100
0400	6600	500
0500	6300	300
0600	6100	200
0700	6000	100
0800	5800	200
0900	5600*	200
1000	5250*	350
1100	5100*	150
1200	4800*	300
1300	4600*	200
1400	4300*	300
1500	4000*	300
1600	3750*	250
1645	3600	150

* Visual estimate.

When the emergence cage had been put in place, a camera and stroboscopic light were positioned to photograph the emerging adults. Although a few adults emerged around noon on June 6, photography was started at 13^h 15′ and continued until 08^h 00′ of June 7. Photographs were made each hour until 17^h 30′, every 15 minutes from 18^h 00′ until 01^h 00′ on June 7, and every 30 minutes from then until 06^h 00′ when a one-hour interval was resumed. Starting at 08^h 00′, visual estimates were made hourly until 16^h 00′. A final photograph was made at 16^h 45′ to complete the series.

RESULTS AND DISCUSSION. Although this was a pilot experiment to test the feasibility of producing synchronous emergences, the results demonstrate the practicability of controlling, under field conditions, the development of broods of mosquitoes. In the study of mosquito ethology, the ramifications of this technique are many and far-reaching.

In regard to the synchronous emergence, it can be seen in Table 3 that the entire emergence took place in only 12 hours, 53 percent of the brood having emerged in 2 hours and 89 percent in eight hours. Since pupal development is controlled by temperature only, the emergence should have occurred over a 40-hour period, as did the pupation. The telescoping to 12 hours was therefore a reduction in time of 70 percent for the entire brood. The greatest reduction in time which could have been obtained was 90 percent, due to the 4-hour interval in separating pupae from larvae. With improved techniques the emergence may be compressed even more.

Between 19^h 30′ and 20^h 00′ on June 6, a twilight exodus was observed and also recorded by the camera. The photographs revealed a drop from 8750 to 5350 mosquitoes on the back panel of the cage, indicating an exodus of 3400. During this interval, and especially during the first 5 minutes, mosquitoes streamed out of the emergence cage and flew away. The peak 5-minute interval was 15 to 20 minutes after sunset; this is the peak exodus time for salt-marsh mosquitoes at this latitude, as previously demonstrated (Haeger, 1960 and Provost, 1960).

According to careful calculations, not over 1000 of the 3400 mosquitoes taking part in the exodus could have been over 6 hours old. The assumption of 6 to 8 hours as departure age may therefore be too high. At summer temperatures (mean of approximately 27°) departure age might be as early as 4 to 6 hours. Because of its

importance to migration research, this is a figure much in need of accurate determination.

Although the partial exodus withdrew a large number of mosquitoes, the population continued to increase as the emergence progressed. By 24^h 00′ of June 6 the emergence was complete and 7525 mosquitoes were resting on the back screen. Some of these flew away gradually during the balance of the night, as the camera revealed (Table 4). Others left the screen during the day but almost certainly for more comfortable situations close at hand and not on flights. And finally others left during the expected mass exodus at dusk of the second day.

The departure breakdown was then as follows: (1) 3400 or 31 percent left on the June 6 twilight exodus; since they were presumptively the oldest 31 percent, they had emerged between 12^h 00′ and 16^h 30′ and were thus 3 to 7½ hours old. (2) 1425 or 13 percent left between 00^h 00′ and 05^h 30′ of June 7; if they were the oldest left behind at the June 6 twilight exodus, they emerged between 16^h 30′ and 18^h 30′ on that day, were 1 to 3 hours old at exodus time and 11 to 13 hours old at dawn, and had to be somewhere between 6½ and 13 hours old when they flew away spontaneously between midnight and dawn. (3) 6100 or 56 percent left on the June 7 twilight exodus, including the 2500 which shifted positions away from the back screen during the day; assuming these were the youngest adults, they emerged between 18^h 30′ and 24^h 00′, were 5½ to 11 hours old at sunrise and 19½ to 25 hours old when they took off on the June 7 exodus.

Although the figures are clearly in need of further refinement, there are indications that departure age in *Aedes taeniorhynchus* at summer temperatures in Florida may depend on the time of night. Minimum age for departure after the crepuscular mass exodus period, i.e. beyond 40 minutes after sunset, may be the 6 to 8 hours assumed before this experiment. Minimum age for departure during the twilight exodus, however, may be as low as 4 hours. The possibility of an entrainment effect during the mass exodus, drawing very young adults into the migratory stream, is suggested by the findings of this experiment.

Gradual departure of new adults during the night, demonstrated in the 1952 dispersal test on Sanibel Island (Provost, 1957) and again here, must hereafter be considered an alternative to the far more frequent mass twilight exodus. The two types of departure must be studied independently in order to relate their respective contributions to the migration of a brood and to its ultimate dispersal. If only the mass exodus at twilight constitutes the initiation of a migratory flight, it might be possible to produce dispersals of any desired dimension by controlling the time of emergence, as was done in this experiment.

SUMMARY. In the spring of 1963 a controlled, synchronous emergence of the salt-marsh mosquito, *Aedes taeniorhynchus*, was produced at a predetermined time at Vero Beach, Florida.

The emergence of this brood, which should have lasted 40 hours, the period required for pupation, was reduced to 12 hours, with over 50 percent compressed into 2 hours. This was accomplished by separating the new pupae at 4-hour intervals and maintaining the 10 groups at a lower temperature for periods adjusted to bring them all into synchrony when 90 percent of pupal development was attained.

The emergence peaked at evening twilight, as planned. Adults 3 to 7½ hours old left on a mass twilight exodus on June 6, adults 6½ to 13 hours old left gradually between midnight and sunrise, and adults 5½ to 11 hours old at sunrise left on a mass twilight exodus on June 7 when 19½ to 25 hours old.

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References

FAY, R. W., and MORLAN, H. B. 1959. A mechanical device for separating the developmental stages, sexes and species of mosquitoes. Mosquito News 19:144–147.

HAEGER, JAMES S. 1960. Behavior preceding migration in the salt-marsh mosquito, *Aedes taeniorhynchus* (Wiedemann). Mosquito News 20:136–147.

NIELSEN, E. T. 1957. Use of the electronic flash to record the activity of small animals. Nature 179:1308.

———. 1958. The initial stage of migration in salt-marsh mosquitoes. Bull. Ent. Res. 49:305–313.

——— and EVANS, D. G. 1960. Duration of the pupal stage of *Aedes taeniorhynchus* with a discussion of the velocity of development as a function of temperature. Oikos 11:200–222.

PAUSCH, R. D., and PROVOST, M. W. 1965. The dispersal of *Aedes taeniorhynchus* IV. Controlled field production. Mosquito News 25:1–8.

PROVOST, MAURICE W. 1957. The dispersal of *Aedes taeniorhynchus*. II. The second experiment. Mosquito News 17:233–247.

———. 1960. The dispersal of *Aedes taeniorhynchus*. III. Study methods for migratory exodus. Mosquito News 20:148–161.

WEATHERSBY, A. B. 1963. Harvesting mosquito pupae with cold water. Mosquito News 23:249–251.

THE OVIPOSITION RESPONSES OF TWO SPECIES OF *CULEX* TO WATERS TREATED WITH VARIOUS CHEMICALS

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The larvae of *Culex tarsalis* Coquillett occur in a wide variety of relatively clean roadside ditches, pasture pools, log ponds, and other permanent and semipermanent bodies of water. The larvae of Culex *quinquefasciatus* Say are also present in many of these situations but are found in largest numbers in sewage waters, dairy drain pools, and street drains.

Evidence that mosquitoes are attracted to water containing various chemical compounds has been reported by a number of investigators. O'Gower (1963), in experiments with *Aedes aegypti* var. *queenslandensis* Theobald, found that egg depositions were influenced by olfactory, tactile, visual, chemotactile, and humidity stimuli. Chemoreceptor hairs that can detect appropriate concentrations of several sugars have been found by Owen (1963) on the tarsi, labella, and ligula of *Culiseta inornata* (Williston) and *Aedes dorsalis* (Meigen). Steward and Atwood (1963) have shown that several types of setae on the antennae of *Aedes aegypti* (Linnaeus) are the olfactory end organs that detect odors.

One hundred and fifty-one chemicals were tested in water samples against gravid *C. quinquefasciatus* females by Gjullin (1961) in a search for attractants and repellents that might be of value in control operations. Additional tests of a series of 296 chemicals against *C. quinquefasciatus* are reported here. Some of these chemicals were also tested against *C. tarsalis*.

METHODS AND MATERIALS. The tests were made in a 12 x 12 x 12-inch screen cage fitted with a cloth sleeve on one side. Twenty blood-fed females, not over 8 days old, were used in each test. Acetone or water solutions of the chemicals were added to 350 c.c. of distilled water in 400-c.c. beakers. The chemicals were tested at 5, 25, and 50 p.p.m. In the multiple-choice tests, three beakers containing different concentrations of the chemical and one containing distilled water were used. The beakers were placed in the four