ARTICLES

THE DAILY RESTING CYCLES OF SEVERAL SPECIES OF MOSQUITOES

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INTRODUCTION. The active and resting cycles of many species of mosquitoes are not well known. The time and length of the daily active cycle is of importance in adult-mosquito aerosol and spray control programs. Knowledge of this cycle in species that transmit disease may also be of value in planning other protective measures.

The microclimate and resting places of *Anopheles quadrimaculatus* Say have been investigated by Eyles and Bishop (1943). They found that this species left its resting places in buildings during the 20 minutes following sunset and began returning shortly after sunrise. Light intensity was the only microclimatic condition that could be correlated with these movements. During the exodus of these species, the light in the open decreased from approximately 48- to 2-foot candles.

Adaptation of the eyes to darkness and to light has been shown to occur in several species of insects. In the codling moth, *Carpocapsa pomonella* (L.), adaptation to bright light was shown by Collins (1934) to occur when the pigment granules surrounding the retinal element move outward to exclude much of the light. Adaptation to darkness occurs when the pigment retreats to an inward position so that more light can enter.

Sato has shown that in *Culex pipiens* var. *pallens* Coquillett, (1950) *Aedes japonicus* Theobald, (1953a) and *Anopheles* [*hyrcanus*] *sinensis* Wiedemann (1953b), adaptation of the eyes to light and to darkness is accomplished by movement of the rhabdom and rhabdom pigment to decrease or increase the amount of light that reaches the retina. Suszuki (1954) found that *Culex pipiens* var. *pallens* Coquillett that were confined in cages began to fly when the light fell below 250 lux in the evening or rose to 2 lux in the morning. He sectioned and examined the eyes of groups of specimens taken at regular intervals during the period when the insects were flying and when they were at rest. When he found that rhabdom movement in the eye occurred while the insects were in flight but not after they had returned to rest, he concluded that flight was stimulated mainly by light.

The time and extent of the daily resting periods of *Culex tarsalis* Coquillett, *C. peus* Speiser, *Anopheles freeborni* Aitken, *Culiseta inornata* (Williston), and *Culiseta incidens* (Thompson) have been investigated in the study reported here. Information on the effect of light and other environmental factors has also been recorded.

METHODS AND MATERIALS. The mosquito resting cycles were investigated in Fresno, Calif. in a red box shelter of the type described by Goodwin (1942). The numbers of mosquitoes present on the back of the box were recorded at intervals with a time-phase camera. The frame surrounding the lens of the camera was inserted in an opening of a box that was placed opposite the opening in the red shelter box. The camera box was twice the height of the shelter box. The lower portion of the camera box, which was below ground level, housed the timeclock and other equipment for operating the camera. An open, basement area in the

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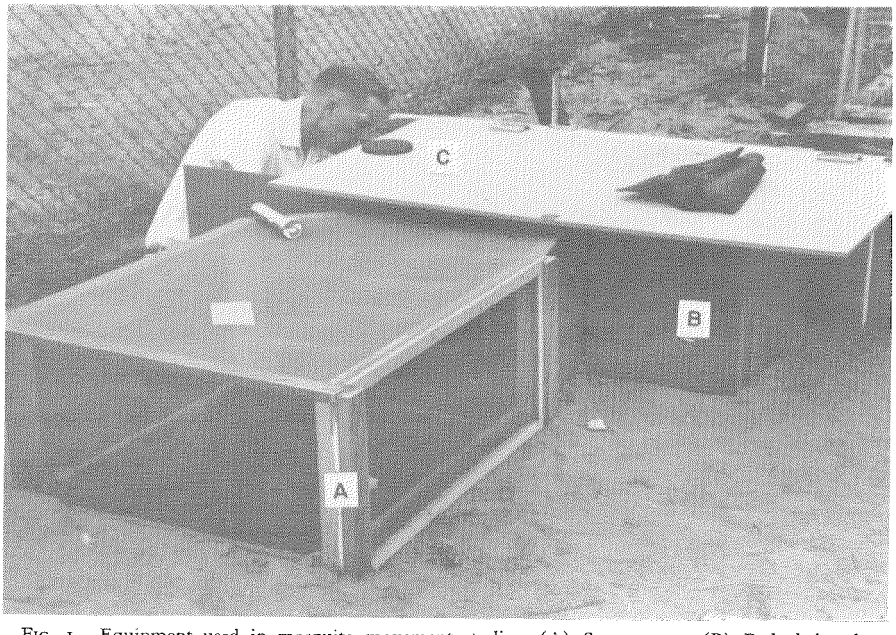

FIG. 1.—Equipment used in mosquito movement studies: (A) Screen cage, (B) Red shelter box, (C) Camera box location under protective cover.

rear provided access to the back door of the box. The arrangement of these units is shown in Figure 1.

The shelter box was a 14-inch cube and the pictures covered a 12- x 12-inch area in the center of the back wall. The camera was 21¾ inches from the back wall. This arrangement left an opening of 7 inches between the camera box and the mouth of the shelter box through which the mosquitoes could enter and leave. The shelter box was insulated and a plywood shelter was erected over the top and three sides to provide additional shade and darkness. Cooling during afternoon periods of high temperatures was provided by ice water circulated through a radiator placed against the back wall of the box.

Laboratory-colonized mosquitoes were confined in a screened cage 34 x 30 x 15 inches high, which was narrowed at one end and attached to the sides of the opening between the camera box and the shelter box. Screen was also extended over the top and the opposite side of the opening between the two boxes so that the mosquitoes could move freely between the red box and the screen cage but could not escape. The portion of the screened enclosure adjacent to the red box was left bare, a central portion was planted with bermudagrass, and a small pond was excavated at the other end. The pond was lined with plastic and contained several inches of water during the tests.

The 5- x 5-inch pictures of the mosquitoes in the red box shelter were taken with a K-24 aerial camera modified to focus at 21¾ inches by moving the lens forward 3½ inches. The camera motor advanced the film when actuated by a timeclock that could be set for the desired intervals. The clock was operated by a

110- to 120-volt current. A transformer modified this current for use in the 28-volt camera motor. An electronic flash provided light for taking the pictures.

A small watch was inserted in the top corner of the back wall of the red box so that the time appeared on each frame of film. A Gossen Lunasix[3] light meter was inserted in the other corner with the sensory element pointed at the northern sky at an angle of 25° from the horizontal plane. The light-meter scale was exposed on the inside back wall of the box so that the position of the indicator needle would appear in the picture series. When adjusted for incident light, the meter gave closely approximate foot-candle readings. A relay activated by the timeclock closed the circuit through the light-meter battery to operate the light meter while the picture was taken. Two small themometers were taped to the back wall of the box. One was about 3 inches from the bottom and one 3 inches from the top of the box.

From 100 to 200 males and females of the species to be tested were released in the enclosure formed by the red box and the screen cage before the camera was started. Only a portion of these adults appeared in the pictures because many rested on the side walls of the box. Two or more populations of all species except *Culiseta inornata* were used in tests on different dates. A 10 percent sugar solution placed in the screen-cage section provided food during the tests.

Time-phase picture intervals are given in Pacific daylight time. Time of sunset and sunrise are also given in Pacific daylight time, as reported for Fresno in the *World Almanac* (1962). Pictures were taken at 10-minute intervals at sunset when mosquitoes were leaving the shelter, and at 15-minute intervals during the early portion of the morning flight. During other day and night periods, pictures were taken at 1- or 2-hour intervals. Electronic flashes at 10-minute intervals did not cause any visible disturbance of the resting mosquitoes.

Counts of the mosquitoes were made from the film with an 8X hand lens. It was possible to tell the males from the females and white leg markings could usually be seen at this magnification.

RESULTS. All of the species that were studied in the red box shelter unit responded in the same way to light changes at sunset and sunrise. The times of the evening exodus and morning return of these species are show in Table 1. The initial movement of males and females from the shelter began at different times on different days. In the course of 27 days, the time of initial movement ranged from 17 minutes before to 31 minutes after sunset for this group of species. The time required for exodus ranged from 10 to 50 minutes. A few females and an occasional male remained in the shelter overnight. The numbers of females remaining overnight, as compared with the numbers present at 7 p.m., ranged from 0.32 percent for *A. freeborni* to 4.3 percent for *Culex tarsalis*. The adults began to return to the shelter during a period extending from 20 minutes before to 24 minutes after sunrise, but all did not return until some time after 8 a.m. Mosquitoes were still present in the screened, shaded opening leading to the shelter at that time.

Time of arrival and departure of the mosquitoes varied with the time of sunset and sunrise. *Culex tarsalis*, which had not begun to leave the shelter at 8 p.m. on August 1 when sunset was at 8:05, had all moved out by 7:40 on September 4 when sunset was at 7:23. Adults also returned to the shelter later in the morning when the time of sunrise was later.

The effect of longer periods of light at sunset was also tested on several species by replacing electric lights around the open areas at the mouth of the red box shelter. Four lights were placed so that they would light up the dark areas around the box without the light bulbs being visible to the resting mosquitoes. The lights were turned on at 5 p.m. and al-

[3] Mention of this proprietary product does not necessarily imply its endorsement by the U.S.D.A.

TABLE 1.—Movement of mosquitoes to and from a red box shelter.

Date	Evening			Morning	
	Time of sunset	Time of exodus		Time of sunrise [1]	Time of first arrivals
			Culex tarsalis		
Aug. 1	8:04 [2]	8:00	to 8:30	6:10	6:00
2	8:03	8:00	" 8:30	6:11	6:00
15	7:50	7:30+	" 8:00+	6:21	6:30
Sept. 4	7:23	7:20	to 7:40	6:33	6:30
5	7:22	7:20	" 7:40	6:34	6:15
6	7:20	8:00	" 9:00+ [3]	6:35	6:15
			Culex peus		
Aug. 28	7:34	7:40	to 8:30	6:28	6:15
31	7:30	7:20	" 7:50	6:30	6:15
Sept. 1	7:27	7:10	to 7:40	6:31	6:30
2	7:26	7:20	" 8:10	6:32	7:00
3	7:25	7:40	" 8:30	6:33	6:30
			Anopheles freeborni		
Aug. 17	7:48	7:40	to 8:30	6:19	6:15
18	7:47	7:40	" 8:20	6:22	6:30
21	7:43	7:40	" 8:10	..	..
Sept. 11	7:13	7:30	to 8:10	6:38	6:30
12	7:12	7:20	" 8:00	6:39	6:15
13	7:11	7:20	" 7:50	6:40	6:30
14	7:09	8:00	" 9:00 [3]	6:40	6:30
15	7:08	7:20	" 7:40	..	..
			Culiseta inornata		
Sept. 7	7:19	7:30	to 8:10	6:36	7:00
8	7:18	7:10	" 7:40	6:36	7:00
9	7:16	7:10	" 7:40	6:37	6:30
10	7:15	None	" 9:00+ [3]	6:37	7:00
			Culiseta incidens		
Aug. 23	7:40	7:40	to 8:30	6:24	6:15
24	7:39	7:40	" 8:00	6:24	6:15
25	7:38	7:40	" 7:50	6:25	6:30
26	7:36	8:00	" 8:30+ [3]	..	..
Sept. 16	7:05	7:20	to 8:00	6:43	7:00
17	7:04	7:10	" 7:20	6:44	7:00
18	7:03	8:00	" 9:00+ [4]	6:44	6:30
19	7:01	6:30+	" 7:10	6:45	7:00

[1] Sunrise is defined as "The time when the upper limb (edge) of the sun . . . appears above the sensible horizon. . . ." Sunset is defined as "the time when the upper limb . . . of the sun . . . disappears below the sensible horizon. . . ."

[2] Chronological data are given in Pacific daylight time.

[3] Lights placed around opening of red box from 5 to 9 p.m.

[4] Lights placed around opening of red box from 5 p.m. to 8 a.m.

lowed to stay on until 9 p.m. in all except one run with *Culiseta incidens,* when the lights were left on all night. In all instances a considerably larger number of mosquitoes remained in the shelter until after 9 p.m. There was, however, a gradual reduction in the numbers remaining, and the light that was left on all night did not maintain a larger than normal number of adults after 10 p.m.

In some instances adults that were placed in the cage shelter unit remained for longer than normal periods in the shelter box on the first night. The reason for this behavior is not known, but it may have been due to previous conditioning resulting from the laboratory-colony environment.

Light-meter readings of incident light, recorded from the meter pointing north and upward at a 25° angle, were obtained for a portion of September. The results obtained with three species indicated that they began to leave the shelter on some days when there was still 3.1 foot-candles of light but remained on other days until the light fell to 0.9 of a foot-candle. Some mosquitoes began to move back into the shelter in the morning when the light had risen to 0.3 of a foot-candle. A 26-foot-candle reading obtained on one morning may indicate that adults had moved into the box but had not moved to the back wall where they could be recorded by the camera. The light-meter records for these runs are shown in Table 2.

The temperatures appearing on the two themometers taped to the back wall of the red box were recorded on the film. The upper half of the back wall was cooler than the bottom half in some runs at 8 a.m. and in others the two halves were equal in temperature. During the rest of the day, the bottom half was cooler by several degrees. A cooling unit was used to reduce the temperature of the lower half of the wall on hot days during a portion of these tests.

The numbers of females appearing on the top and bottom half of each frame of film were recorded. A majority of the *C. peus* population moved to the lower half when the temperatures on the top half changed from 79° to 87° F. Fifty-five percent of both *C. tarsalis* and *C. incidens* were on the lower half of the back wall when the temperature was 82.5° on the top half and 81° F on the lower half of the back wall. Sixty percent of the *C. inornata* had moved to the

TABLE 2.—Light intensity at the time of mosquito movement from and to a red box shelter.

	Foot-candles of light at time of initial movement in and out of red box shelter						
	Evening				Morning		
Date	7:00	7:10	7:20	7:30	6:15	6:30	6:45
			Culiseta inornata				
Sept.							
7	..	..	..	0.90	..	..	26
8	..	..	3:13	..	..	1.4	..
			Anopheles freeborni				
11	..	..	..	0.88	..	1.05	..
12	..	..	1.56	..	.021	..	..
13	..	..	1.28	..	.014	..	..
14	..	..	..	..	..	.88	8.4
			Culiseta incidens				
16	..	..	0.91	..	..	..	8.4
17	1.69	..	..	..	..	..	5.9
18	1.43	..	..	..	..	..	.30
19	1.21	..	..	..	..	..	.87

lower half when the temperature on the top half was 81.7°, and 76 percent of the *A. freeborni* had moved down when the temperature on the top half was 75° F. It is also possible that differences in humidity and light may have been sufficient in the top and bottom halves of the box to affect mosquito movement. Results of these tests are shown in Table 3.

Information on the rate of evaporation in the red box shelter and in the open was obtained by placing a dish of water in the mouth of the red box and a similar dish in direct sunlight. The dishes were refilled daily to the predetermined level. During the period from September 11 to 17, the rate of evaporation was 3.3 times greater in the open than in the mouth of the box. Temperatures in the open were also recorded. During the period from September 14 to 17, the temperature in direct sunlight ranged from 106° to 114° F.

Biting collections of *A. freeborni* and *C. tarsalis* on man were made approximately 40 miles west of Fresno on August 15 in an area where these species were present in considerable numbers. On this date, when sunset was at 7:50, the first *A. freeborni* was seen at 8:10, and *C. tarsalis* were collected a few minutes later.

DISCUSSION. The close correlation between the arrival and departure of the five species tested in the red box shelter, with the time of sunrise and sunset suggests that these abrupt changes in light intensity are the cause of these movements. This behavior is similar to that observed by Suzuki (1954) for *C. pipiens* var. *pallens* and would suggest that in these species also flight activity is related to the rhabdom-movement in the eye, that in-

TABLE 3.—Numbers of females resting on the lower half of the red box back wall at various temperatures.

Date	Time	Temperatures (° F.) Upper half	Lower half	Mosquitoes resting on lower half (%)
		Culex pens		
Sept. 1–3 [1]	8 a.m.	68	69	39
	10 "	79	76	41
	12 m.	87	79	76
	4 p.m.	93	90	100
		Culex tarsalis		
5–6	10 a.m.	73.5	73.5	35
	12 m.	82.5	81	55
	4 p.m.	90	86.5	71
		Culiseta inornata		
7–10	8 a.m.	65.7	66.3	40
	10 "	73.3	73.7	61
	12 m.	81.7	78.3	66
	4 p.m.	88.7	85.7	83
		Anopheles freeborni		
12–15	8 a.m.	64.2	67.7	37
	10 "	75.2	73	76
	2 p.m.	85.2	81.5	83
	6 "	87	84.2	88
		Culiseta incidens		
16–20	10 a.m.	73.5	73.5	35
	12 m.	82.5	81	55
	4 p.m.	90	86.5	71

[1] Temperature averages are based on the number of days indicated by the inclusive dates.

creases or decreases the amount of light perceived by the insect. The failure of considerable numbers of mosquitoes to leave the shelter at sunset, when artificial lights were used to light up the entrance to the shelter box, is added evidence that these species are influenced by light.

The light-pattern response of the type observed in summer does not govern the winter populations of some of these species. *Anopheles freeborni* are active in the daytime in winter months. Unpublished information obtained by the California Bureau of Vector Control shows 15-minute landing counts in February ranging from 2 to 5 in the Redding-Cottonwood area to as many as 265 in the Colusa area. T. J. Raley (personal communication) stated that during the period when he was serving in the Malaria Control in War Areas group on the Pacific Coast, *A. freeborni* frequently attacked in large numbers on sunny as well as on cloudy days when the temperatures were favorable in January and February and occasionally in December. These observations were made in the Marysville-Yuba City area.

Protection from high temperatures and low humidities of summer in this area are the most obvious benefits obtained by this insect. This, and the daytime activity of *A. freeborni* during the more moderate climate in winter months, suggest that temperature and humidity may be involved in determining the time and the length of the resting period even though light is the triggering element.

Biting collections made near Fresno indicated that *A. freeborni* and *C. tarsalis* did not leave their natural shelters before sundown. Extensive biting collections of *C. tarsalis* made in Utah and Nebraska by Beadle (1950) also indicated that this species does not leave its shelter in summer and fall months until sundown.

Routine applications of aerosols and space sprays by ground or airplane equipment are believed to be relatively ineffective against adults in daytime shelters because they fail to penetrate into these protected areas. The results obtained in this study indicated that such applications should not be made before sunset or after sunrise during the summer months. The males and females that remain in shelters overnight further complicate effective control.

The daily resting period of *Culex tarsalis* has been shown to extend from 8 a.m. to sunset during the summer months in the Central Valley of California. This finding indicates that during equine-encephalitis outbreaks, transmission to humans by the bite of this species would rarely occur during this daily 8 a.m. to sunset period.

SUMMARY. Time-phase pictures of the daily resting periods of mosquito adults were made in a red box shelter attached to a screen cage. The screen-cage unit was placed in an outdoor situation and supplied with mosquito populations from laboratory-reared colonies.

The time and extent of the daily resting period of *Culex tarsalis, C. peus, Anopheles freeborni, Culiseta inornata,* and *C. incidens* were determined. Both males and females of all of these species began to move out of the box shelter during a period ranging from 17 minutes before to 30 minutes after sunset. All had moved out in from 10 to 50 minutes, with the exception of a few females and males that remained in the shelter overnight. When artificial lights were placed around the opening of the shelter in the evenings, the adults stayed in the shelter for longer periods, but eventually moved out when the lights were still on. The mosquitoes began to return to the shelter during a period ranging from 20 minutes before to 24 minutes after sunrise, but all did not return until some time after 8 a.m.

Light-meter readings were taken at the time of arrival and departure of several species. These readings showed that the adults began to leave the shelter when the light ranged from 3.1 to 0.9 foot-candles in the evening and began to return to the shelter during the period when the light increased from 0.87 to 8.4 candles or more in the mornings.

Temperatures of the lower and upper halves of the back wall of the box were recorded on the time-phase film. All species began to move to the lower half of the box, which was cooler when the temperature of the upper half rose above approximately 80° F.

References Cited

BEADLE, LESLIE D. 1959. Field observations on the biting habits of *Culex tarsalis* at Mitchell, Nebraska, and Logan, Utah. Amer. Jour. of Tropical Med. and Hygiene 8(2):134–40.

COLLINS, D. L. 1934. Iris-pigment migration and behavior, codling moth. Jour. Exptl. Zool. 69:165–97.

EYLES, DON E., and BISHOP, L. K. 1943. The microclimate of the diurnal resting places of *Anopheles quadrimaculatus* Say in the vicinity of Reelfoot Lake. Reprint No. 2446 from Public Health Reports 58(6).

GOODWIN, MELVIN H. 1942. Studies of artificial resting places of *Anopheles quadrimaculatus* Say. National Malaria Society.

SATO, SHOZO. 1950. Compound eyes of *Culex pipiens* var. *pallens* Coquillett. (Morphological studies on the compound eye in the mosquito, No. 1.) Science Reports of the Tohoku University, 4th series (Biology), Vol. XIII, No. 3.

SATO, SHOZO. 1953(a). Structure and development of the compound eye of *Aedes (Finlaya) japonicus* Theobald. (Morphological studies on the compound eye in the mosquito, No. III.) Science Reports of the Tohoku University, 4th series (Biology), Vol. XX, No. 1.

SATO, SHOZO. 1953(b). Structure and development of the compound eye of *Anopheles hyrcanus sinensis* Wiedemann (Morphological studies of the compound eye in the mosquito, No. IV). Science Reports of the Tohoku University, 4th series (Biology), Vol. XX, No. 1.

SUZUKI, KENJI. 1954. Observations on the diurnal activity of a Japanese common mosquito, *Culex pipiens* var. *pallens* Coquillett. Jour. of the Faculty of Science, Hokkaido University, Series VI. Zoology, Vol. 12, No. 1–2:133–40.

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MASS PRODUCTION OF STERILIZED MALE *AEDES AEGYPTI*[1]

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The effectiveness of released sterilized male *Aedes aegypti* in reducing field populations of the species was studied at Pensacola, Florida, in 1960 and 1961 (Morlan *et al.*, 1962). Sterile males for these tests were reared at the Technical Development Laboratories, Savannah, Georgia, and air-shipped each week during the study periods.

Initial production methods used in 1960 were patterned closely after those used in 1955 to produce *Ae. aegypti* for previous biological activity studies (Morlan *et al.*, 1963). The present paper describes modifications both in the rearing methods and the irradiation procedure (McCray *et al.*, 1961).

Colony cages, as described by McCray (in press, 1963), were used to minimize the escape of adult mosquitoes during colony maintenance operations. Six colonies produced the eggs required. Each colony was stocked initially with 10,000 male and 10,000 female pupae and supplemented with 5,000 female and 1,000 male pupae per week for four weeks. A rabbit was used as a blood meal source for 3 hours every second day and a 1:1 mixture of 40 percent honey-water:raisin juice (from stewed raisins) was added twice weekly as supplemental liquid food.

A 3-inch-high stainless steel oviposition strip was covered on each side with a single layer of wet paper toweling from the top to within ½ inch of the bottom.

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