

ARTICLES

PHYSIOLOGICAL FACTORS ASSOCIATED WITH MALE SWARMING OF THE MOSQUITO *CULEX TARSALIS* COQ.ROBERT F. HARWOOD¹

Colonization of the mosquito *Culex tarsalis* Coq. was accomplished initially by simulating as nearly as possible the daily fluctuations of light, temperature, and humidity encountered by the insect (Brennan and Harwood, 1953). This regimen was based on observations of these factors in nature during the crepuscular period when swarming of males could be observed. Mating was seen during the swarming period in nature, and was commonplace when swarming occurred in a cage. Since this mosquito can now be readily reared without meticulous control of environment, it cannot be said that the male swarm is a necessary adjunct to mating. Male swarming of nematoceros Diptera is an interesting phenomenon which has been investigated frequently. The relationship between such swarming and mating has been fully reviewed for mosquitoes in general (Nielsen and Haeger, 1960). The object of this study is to examine various physiological occurrences coinciding with the swarming of male mosquitoes, using *Culex tarsalis* as the experimental animal.

MATERIALS AND METHODS. Mosquitoes were observed in nature near Othello, Washington (Adams County), and in a laboratory colony derived from normal stock with little autogeny originating near Bakersfield, California (Bellamy and Kardos, 1958). All experimental work in the laboratory, namely observations on relationship of photoperiod to swarming and on glycogen levels, was conducted with

this strain. Field readings of light were obtained with a Model 756 Weston illumination meter and refer to maximum measurable light. Temperature and humidity were determined with a psychrometric device.

Conventional microtomy was used to determine the state of adaptation of the compound eye. Living male specimens were taken at the time desired and placed immediately in Bouin's fixative. They were subsequently imbedded in paraffin and sectioned at five microns. No stains were used because these would obscure other details of the ommatidia in the presence of naturally abundant pigment granules.

Glycogen levels were determined on a live weight basis by the method of Kemp and Kits van Heijningen (1954). The live specimens were ground in methanol; the rest of the procedure was carried out immediately to prevent loss of glycogen.

Temperature-controlled chambers were used to discern the effect of photoperiod on male swarming. These were BOD incubators equipped with a timer-controlled 32-watt circline fluorescent tube. Temperature was set at 20° C., holding within a degree except for periods the door was opened to observe swarming details.

RESULTS. The evening departure of male *Culex tarsalis* from daytime resting sites just before swarming coincides with the initiation of dark adaptation in the compound eyes. This was determined by sweeping males resting in a wheat field before any flight was observed, and at the first flight activity. This agrees with observations of Sato *et al.* (1957) that *Culex pipiens* becomes active in the evening when the compound eyes start dark

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adaptation. The degree of adaptation can be classified by dividing the stable corneal diameter of an ommatidium by the variable "iris" width (Sato *et al.*, *ibid.*) As the iris width increases in the dark adapted condition, the ratio will be a lower figure, and the narrow iris width of the light adapted ommatidium results in a higher figure.

To determine these ratios, eight seemingly undistorted ommatidia from the dorsal portion of the compound eyes of each specimen sectioned were measured. For example, in caged specimens placed in a typical shady resting site, the midafternoon light-adapted eye of three males had an average cornea/iris ratio (hereafter referred to as eye index) of 20.9, and the average index for two males well after dark was 4.1. Light- and dark-adapted condition of the eye in this mosquito is illustrated in Figure 1.

Swarming takes place when the eyes are changing from light to dark adaptation or vice versa. Only evening swarms were observed in nature. But in photoperiod chambers where lights were either fully on or off, swarming was readily observed in the presumptive morning period shortly after the lights came on. This further indicates that transition of the compound eyes is important for initiating swarming of laboratory-adapted strains of this species, and not the actual decline or increase of light.

The relationship between state of eye

adaptation and crepuscular activity is graphed in Figure 2. This is a composite representation from two evenings of observation eleven days apart, placement of eye index of swarming individuals being based on time of light intensity. When the first male was noted flying from its resting site, the eye was well along towards full dark adaptation, having an eye index of 7.1. With an eye index of about 5.0 there appears to be a slight

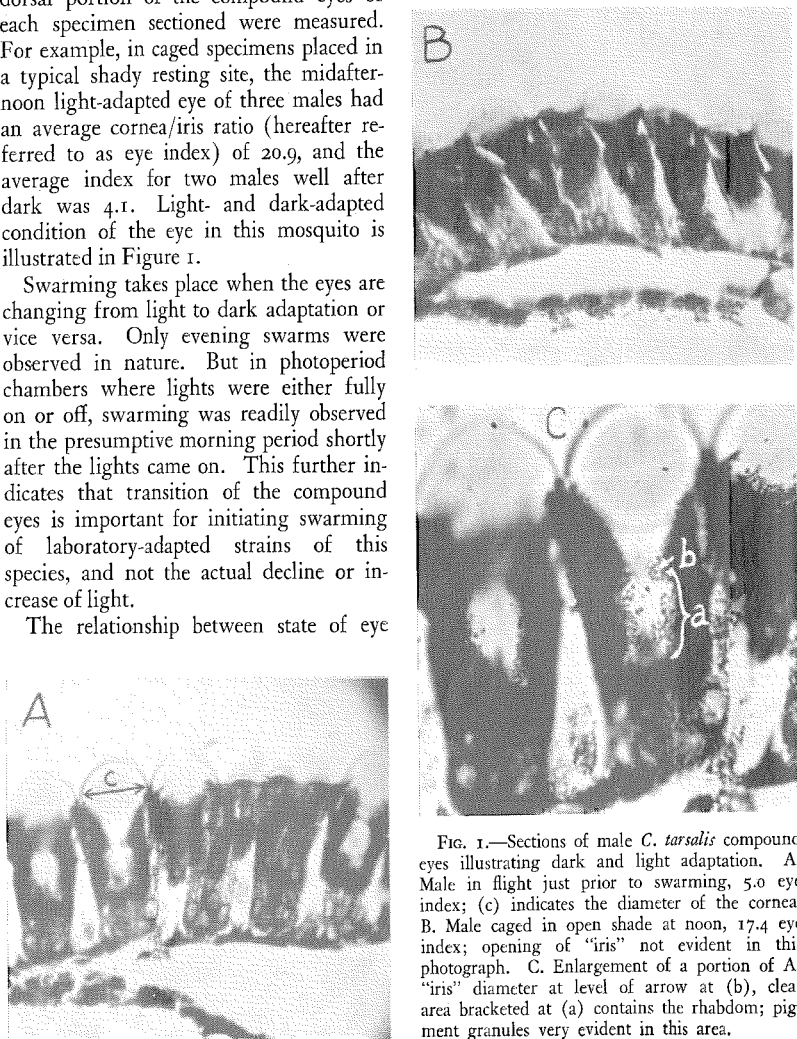


FIG. 1.—Sections of male *C. tarsalis* compound eyes illustrating dark and light adaptation. A. Male in flight just prior to swarming, 5.0 eye index; (c) indicates the diameter of the cornea. B. Male caged in open shade at noon, 17.4 eye index; opening of "iris" not evident in this photograph. C. Enlargement of a portion of A; "iris" diameter at level of arrow at (b), clear area bracketed at (a) contains the rhabdom; pigment granules very evident in this area.

plateau reached in the progression towards dark adaptation. In the natural setting studied, it was not possible to observe formation of the swarm, but on one occasion a very large swarm, likely in progress for at least ten minutes, was observed until it became lost in darkness. Males captured from this swarm provided the eye index of swarming individuals shown in Figure 2.

When this swarm was located the eye index averaged 5.0, but gradually declined in the next fifteen minutes to 3.4. At no time has an eye index smaller than this

terminal figure been observed. Temperature and humidity were often measured before and at the time male crepuscular flight activity began. These factors varied enough to indicate they were not of prime importance. In nature, the initiation of crepuscular flight was observed when maximum measurable light was near seventy-foot candles, and the compound eye was well along towards dark adaptation, with an eye index near 7 (Fig. 2).

Gradual increase or decrease of light seems unnecessary to cause swarming in the California-derived laboratory strain of

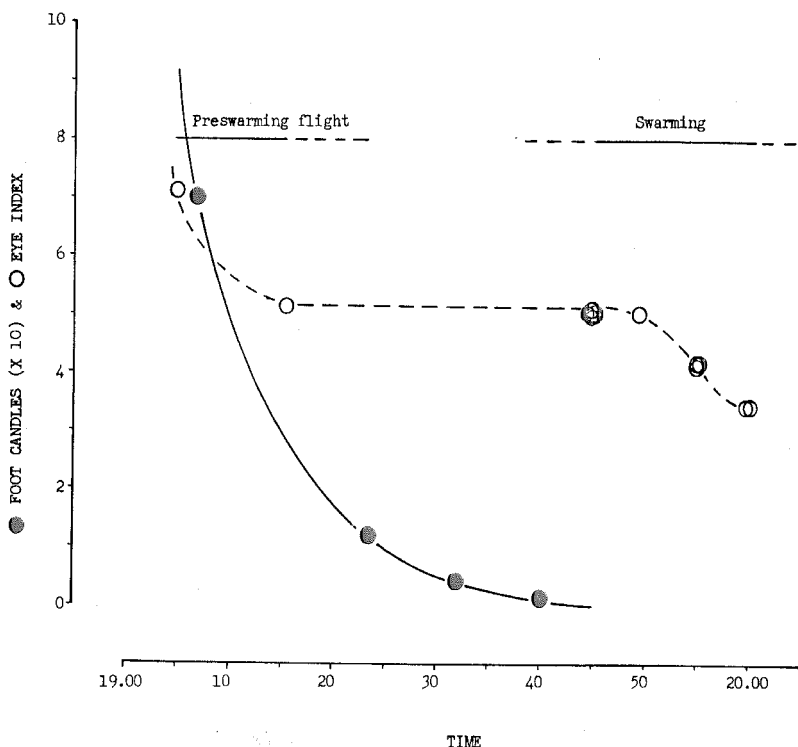


FIG. 2.—Relationship between natural decline of daylight and eye index of male *C. tarsalis* during preswarming flight and swarming. Swarming males captured eleven days after preswarming individuals, placement on graph determined by relationship of light decline to Pacific Standard Time. Upper interrupted horizontal line indicates the period of preswarming flight and swarming, the solid portion including known participation and the interrupted portion indicating the times not covered by capture or observation of individuals.

C. tarsalis kept on photoperiods wherein light was abruptly turned on or off. Analysis of proportion of males swarming immediately after the start of the photophase makes it clear that length of photophase (or scotophase) has a marked effect. This relationship is indicated in Table 1.

TABLE 1.—Photoperiod and swarming of male *Culex tarsalis*.

Hours of daily photoperiod		Maximum participation	
Larval	Adult	Days after Median emergence	% Participation
8	8	4	17
8	8	6	14
16	16	5	42
16	16	5	53
16	8	5	4
8	16	6	75
24	16	4	45
24	8	5	9

Observations were made on groups of males and females reared under varying larval and adult combinations of long (16 hour) and short (8 hour) daily photoperiod. Number of males in any given experiment was established by using one half the total number of pupae counted. From experimental groups, pupae were chosen over a six-day period to give an approximately even sex ratio as it is usual for males to pupate first. Numbers of males per experimental group varied between 350 and 1,000. Observations on swarming began two days after emergence, and were made daily thereafter for ten days. Maximum participation occurred six to nine days after first emergence, or four to seven after median emergence. Actual numbers of males participating in swarm flights were counted if small, or estimated if large. Estimation was likely within ten percent of the correct number through repeated close observation.

Actual numbers participating probably exceeded the numbers observed at any in-

stant because, as Nielsen and Nielsen (1962) found in *Culex pipiens*, it is improbable that individuals remain in flight throughout the swarm period, periodically resting on the side of the cage and rejoining the swarm later. It was observed that maximum participation took place from about 3 to 25 minutes after initiation of the photophase. Gradually diminishing numbers continued to swarm for about forty minutes. Disturbances, such as the close presence of the observer or breathing into the cage, would activate more males, causing them to fly and join the swarm briefly.

Long photoperiod enhanced male participation in swarming. This effect was noted whether larvae were reared on a similar photoperiod or on short photoperiod. It therefore appears that extent of male participation was most affected by photoperiod conditions in the adult stage. Photoperiod does not appear to affect eye index, as this did not significantly differ between long and short photoperiod males checked at identical times after the initiation of the photophase.

Exhaustion of energy reserves may be an important factor in terminating the swarming flight. Nielsen and Nielsen (1962) show that flight duration of swarming *Culex pipiens* is affected by rate of light decrease in crepuscular conditions. The actual termination of swarming must be related to factors other than increasing rate of light intensity under conditions of sudden maximum light in the photoperiod chambers used. One might suspect that energy reserve depletion is involved in termination, as Clements (1955) observed that glycogen was depleted in *Culex pipiens* flown to exhaustion. In a single test of glycogen level in connection with swarming, samples of two groups of ten long photoperiod males each contained 1.7 percent fresh weight of glycogen when the lights first came on, 0.8 percent and 1.0 percent after 45 minutes, and 1.5 percent and 1.3 percent 90 minutes

after lights first came on. Thus, glycogen dropped to nearly half of initial concentration after swarming, and was partially restored to initial level some 45 minutes after swarming ceased. The glycogen level of individuals actually engaged in swarming flight may have been lower than indicated, since no distinction was made between those that had flown and those that had not.

DISCUSSION. The observations made on natural and colonized groups of *Culex tarsalis* suggest factors involved in initiation, participation, and duration of male swarming. Results generally agree with the opinions of other investigators, though details vary since different species and different conditions were studied.

Initiation of the mating swarm is related to change in light intensity and coincides with transition of the compound eye from light to dark adaptation or vice versa. A graphic analysis of eye index for this mosquito is similar to the situation observed by Sato *et al.* for *Culex quinquefasciatus*.² One difference seen here is a seeming plateau in the eye index during dark adaptation. Since Sato *et al.* state eye transition occurs in both sexes, and coincides with crepuscular flight activity, this change is not exclusive with males. However, the coincident initiation of flight activity of both sexes would enhance opportunities for mating, and in males, eye transition can be the internal stimulus for initiation of the behavioral phenomenon called swarming.

Culex tarsalis differs from *Culex fatigans* (Nielsen and Nielsen, 1962) in that abrupt changes of light intensity result in male swarming in addition to the more gradual changes associated with sunrise and sunset. Nielsen and Nielsen note that *Aedes taeniorhynchus* will swarm in response to abrupt light changes. It therefore is evident that considerable variation

exists in the stimulus required to release swarming behavior, even between relatively closely related species, and comparative studies of initiating stimuli would appear profitable under controlled conditions.

Photoperiod is an important and relatively poorly observed facet of the swarming response. In the group of *C. tarsalis* studied it was apparent that a long photophase (or short scotophase) in the adult condition resulted in maximal participation in swarming. If this is a circadian rhythmic phenomenon it is obviously only important in adults since short larval photoperiod did not reduce adult response to long photoperiod. Shorter photoperiods were not tried in these experiments, but the observation that photoperiods shorter than five hours inhibited swarming of *Culex fatigans* (Nielsen and Nielsen) suggests a common relationship in male behavior of these two species.

Energy reserves may be a determining factor in swarm duration. Duration may be affected by rate of light change in some mosquitoes. However, male mosquitoes in general are not noted for prolonged flights, although females of the same species often are. The significant reduction of glycogen observed here during swarming of *Culex tarsalis* indicates that flight could not be continued much longer. The analytical method used only measured the glycogen level of the whole organism, and did not distinguish between the amount remaining in flight muscle or fat body. Histochemical analyses of this same mosquito by the PAS method show both locations to be rich in glycogen, and it seems likely that glycogen of the flight muscles would be depleted first during swarming. It is therefore possible that the glycogen still present just after swarming is in the fat body.

The findings related here suggest that gross observations of behavior in swarming male mosquitoes in natural and controlled settings can be supplemented profitably with histological, histochemical, and biochemical analyses.

² EDITOR'S NOTE: *Culex fatigans* of European authors is *Culex quinquefasciatus* of American authors.

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INSECT AND OTHER FAUNA ASSOCIATED WITH THE ROCK POOL MOSQUITO *Aedes atropalpus* (COQ.)

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INTRODUCTION. Although the rock pool mosquito, *Aedes atropalpus* (Coq.), is widely distributed in North America (Carpenter and LaCasse, 1955), it normally breeds only in rock pools along streams near rapids and waterfalls. It is a persistent biter near the breeding sites and is also a potential vector of pathogens of man and birds (Jakmaugh, 1940; Trembley, 1946). In Texas, Hedeem (1953) reported it from tree holes in hackberry, and Strom *et al.* (1960) stressed the importance of artificial containers, such as discarded aircraft tires, as a potential for disease transmission.

Though much has been published on its biology, little is known of its ecology, especially of relationships with other invertebrates in the normal breeding pools, except for observations made by Shaw and Maisey (1961). The present paper gives ecological data obtained in 1961 in investigation of an extensive breeding area at Cordova Mines, Ontario. Erosion of the precambrian bedrock by the Crowe River

at this point produced many rock pools that are well populated with mosquito larvae throughout the summer and autumn.

METHODS. Fifteen numbered pools from approximately 0.01 to 0.7 sq. meters in area were examined twice weekly from May 15 to November 15 for ten minutes or more each, to determine the presence of mosquito stages and those of other invertebrates. Specimens were collected for identification by means of a fine-meshed sieve 7.5 cm. in diameter or with a pipette. They were either preserved in 80 percent alcohol or reared to the adult stage in the laboratory.

The numbers of mosquitoes and associated invertebrates were investigated in ten additional pools of about the same sizes. Sampling was not feasible because of surface irregularities at the sides and bottom of the pools. Population data were obtained directly by taking a census: the pool was completely emptied, all macroscopic animals were collected for identifi-