potential for biologic transmission of pathogens among animals.

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SWARMING AND MATING BEHAVIOR IN *CULEX PIPIENS QUINQUEFASCIATUS* SAY[1]

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The sterile male technique is being studied as a means of controlling *Culex pipiens quinquefasciatus* Say (= *C. p. fatigans*) one of the principal vectors of *Wuchereria bancrofti*. In order to employ the sterile male technique, as with any control program, a thorough knowledge of the biology of the insect vector must be known. In a control program dependent upon breeding characteristics it is necessary to investigate the mating behavior of the species. Unfortunately, there is disagreement among researchers as to the interrelationships of the two behaviors in mosquitoes. The most common idea (Bates, 1949) is that copulation is almost invariably associated with the swarming of males and that the two are interrelated. On the other hand, Nielson and Haeger (1960) stated that swarming and mating are independent activities, even though they normally occur simultaneously. The purpose of this research was to determine what factors influence swarming and how this was related to mating in *C. p. quinquefasciatus*. A study was also made of the copulatory behavior within the swarm.

METHODS AND MATERIALS. Outdoor Cage Study—A cage, 12 m x 4.8 m and 3.6 m high, consisting of an aluminum frame covered by 20-mesh plastic screen was used for the outdoor studies. It was located in a wooded area near the Laboratory for Insects Affecting Man, Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Gainesville, Florida. Within the cage a wooden shed, 3.6 m x 2.4 m and 2.1 m high was constructed with the south side covered with 20-mesh screen. The northern end and northern half of the east side were covered with plywood and the remaining left open. A small plastic-lined "pond" (0.9 m x 0.9 m) surrounded by grass and flowering plants was located in the southern half of the shed.

Periodically 1500 *C. p. quinquefasciatus* pupae obtained from the laboratory colony were placed in the pond for emergence. All observations of adult behavior and light conditions were made from the east side of the pond and facing west. Light readings were made with a Luna-Pro® electronic system exposure meter.

Adult female mosquitoes were removed each day at random from the cage with a mechanical aspirator. Samples were taken of 2-, 3-, and 4-day-old mosquitoes for spermathecal examination. Females obtained from the cages were

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knocked down in a cold room and transferred in glass vials to the laboratory where they were immolated with ether. The three spermatheca from each female were removed and placed on a glass slide in a drop of Ringer's solution. A cover slip was added and pressed down firmly to crush the spermatheca allowing the sperm to flow into the solution. The slides were then examined with a phase contrast microscope for the presence of active sperm.

At the peak of the flight activity samples were taken from the swarms on two successive nights. A standard insect net was swept through the swarm several times. The adults were taken to a cold room, (340° F.) knocked down, counted used for most of the studies was cubical, prevelant.

INDOOR CAGE STUDY. The indoor cage used for most of the studies was cubical measuring 90 cm on a side. The bottom was plywood and the ends were covered with screen. The front was clear Plexiglas containing a lower center entrance with a cloth sleeve. The top and back were Plexiglas covered with opaque white plastic sheeting to diffuse the light (designed by James Haeger, Entomology Research Center, Vero Beach, Florida). It was located in a room with a maintained temperature and humidity of 78° F. and 75 percent respectively.

Cotton pads saturated with 2.5 percent sugar solution were placed in a waxed paper cup on the floor of the cage to serve as a food source. No other objects were inside the cage.

Controlled lighting was obtained with three series of fluorescent lamps operating on separate 12-hour on-off cycles. Each group was separated by 15-minute intervals. The first two groups of lights were located behind the cage and the third was the overhead lights in the room. Two 200-watt bulbs controlled by a rheostat were located behind the cage. These were used to vary the light intensities to enable the study of the swarms. The light intensity at the beginning of each experiment measured about 2 foot candles and was gradually reduced at 5-minute intervals to 0.065 foot candle.

Light intensity was measured by aiming the meter at the back of the cage from the center of the front panel. Observations of the mosquitoes' behavior and activity were made from the front of the cage.

Since the age of the adult has a direct bearing on its behavior, only adults between 8 and 12 hours old at the start of the test were used. These were observed for at least three consecutive nights. Samples of the females were checked for insemination.

To determine if females would also swarm, 100 two-day-old virgins were placed in the cage alone. These were observed under the same light conditions that induce swarming in males.

It is difficult to follow flight patterns of individual mosquitoes because of the intense activity taking place within the swarm. Numerous marker dyes were tried on the males but without success due to the dim light conditions. Even using an ultraviolet light to see the markers provided no help because it interrupted the swarming activities. It was therefore decided to photograph the swarming of the adults. A clear Plexiglas cage (22 cm x 16 cm x 27 cm) was constructed with a screen top and a cloth sleeve at one end for an entrance. To give a sharp contrast between the flying mosquitoes and the background the back, bottom and closed end were covered with white paper. Forty-eight-hour-old insects were placed in the cage to acclimate them. The cage was held under constant light for 24 hours to prevent the mosquitoes from swarming. It was then transferred to the environmental room and the lights gradually dimmed to an intensity of slightly less than 1 foot candle. Movies were taken with a high speed black and white film using a 16-millimeter camera at speeds of 16, 24, and 32 frames per second. Kodachrome II color transparencies of copulating adults were taken with a 35 mm SLR camera equipped with a F2.8 wide angle lens and 0.001 second duration

electronic flash. Observations were also made of isolated pairs of mosquitoes placed in the large cage.

To determine if males would continue to swarm after all the females were inseminated, 3-day-old virgin males were placed in the cage with 9-day-old inseminated females. Observations were made on these for two successive light cycles. Since males usually do not start to swarm actively in large numbers until they are about 72 hours old, tests were also outlined to determine if the swarming of older males would agitate younger males into swarming. Ten 58-hour-old males were placed in the cage with 25 twelve-hour-old males and observed.

RESULTS. Outdoor Cage Study—Most of the pupae placed in the pond had emerged within 24 hours. The adults were resting on the edges of the plastic and in the grass around the perimeter of the pond. When the light intensity had dropped to 1.5 foot candle, the mosquitoes became agitated and started to make short flights of less than one meter. As the light intensity decreased to 0.065 foot candle, approximately twenty mosquitoes started to swarm over the pond but no copulation was observed.

The following night, with most of the insects 2 days old, swarming had begun about 1 foot above the pond at a light reading of 1 foot candle and as the light diminished the number swarming increased. Copulation was first observed when the intensity had dropped to 0.75 foot candle. There was a corresponding increase of the number of copulations as the swarms increased. Other swarms, each consisting of several hundred individuals, were observed throughout the cage over objects such as ladders, racks and plants.

When the mosquitoes were 3 days old they started to swarm at approximately the same light intensity as the previous night and, as before, they did not copulate until the light intensity was much lower. The swarms on the third night were much tighter and copulations were more abundant than the previous night.

Females 2, 3 and 4 days of age removed from the cage daily and checked for insemination showed that an increase in percent insemination accompanies the increase in copulation (Table 1). These data therefore confirm the laboratory work of Sebastian and De Meillon (1967) on the optimum age for insemination for this mosquito in Burma.

Sweeps made through the center of the swarms confirmed our observations that they were composed primarily of males. Of the mosquitoes captured, 97 percent were males. These collections were made when the males were 48 and 72 hours old.

INDOOR CAGE STUDY. These indoor studies showed that the males while swarming flew in a formalized pattern within narrow spatial limits as described by Nielson and Haeger (1960). *C. p. quinquefasciatus* adults less than 12 hours old did not swarm at any light intensity. On the second night, one male exhibited swarming flight patterns at a light reading of 1.5 foot candles. As the light decreased, more males began to swarm in the front corners of the cage away from the light source. Only a few copulations were observed, with 30 percent of the collected females being inseminated.

When the mosquitoes were 72–76 hours old the size of the swarm was larger and the number of copulations was greater than the previous night. Of the females checked on the following day, 92 percent were inseminated.

No activity was observed in two consecutive nights at any light intensity with the 100 two-day-old virgin females placed in the cage. Therefore it would appear that the presence of actively swarming males or at least one male is essential to

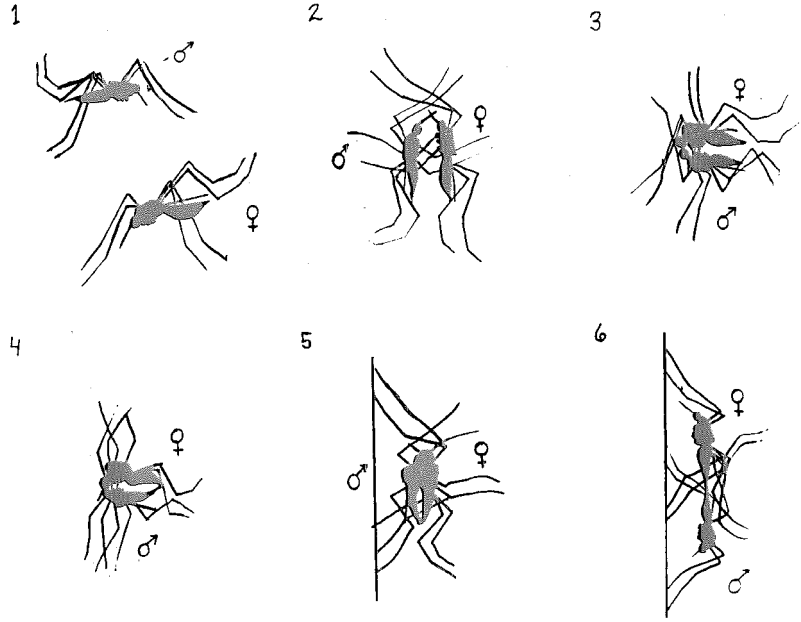
TABLE 1.—The relationship of age to insemination of *C. p. quinquefasciatus*.

Age in days	No. inseminated	No. checked	% inseminated
2	8	54	15
3	25	32	78
4	24	25	96

stimulate the females into flight and subsequent copulation. Observations of mating pairs indicate that as the male flies in the formalized pattern the female becomes agitated and starts to make flights across the cage to the opposite side. It is during this short flight period that she chances to meet the swarming males.

The act of copulation in this species occurs rapidly and lasts only a few seconds; however, with the aid of the photographs, movies and observations of isolated pairs the following information was obtained. Swarming males pay no attention to the females resting in the cage. There was no evidence that they search out and actively goad a female into flight. However, any female entering the area of a swarm may be attacked by one, two or even three of the males. Occasionally one male will attack another. Inseminated females if attacked by a male appear to ward him off and swiftly seek a resting site.

A virgin female, stimulated by the swarming males to take flight, eventually comes in close proximity to a male (Figure 1). When there is only one male present, the female may make several flights across the general area of swarming before she meets the male. The male attacks the female on one of her passes (Figure 2). The pair grapples with their legs in a ventral to ventral position. The female dominates the male and they assume a horizontal position (Figure 3). As soon as the male grasps the female with his claspers (Figure 4), the pair heads for a resting surface. The flight of the two is apparently dominated by the female as she is right side up. When she lands on the wall of the cage she pins the male under her with his dorsal surface against the wall (Figure 5). After a few seconds the male drops from under the female and hangs head down (still attached as in Figure 6). Within a very short period, 1–30 seconds, they separate and he flies back to the swarming area. The female remains quiescent apparently no longer stimulated by the swarming males. No matings were observed with



resting females as described by Tate and Vincent (1936).

Virgin males released in a cage with 9-day-old inseminated females would proceed to swarm as before but the females would not fly into the swarm unless disturbed by the observer. Once attacked by a male they quickly landed on the side of the cage, warding off any attempted copulations.

After 45 minutes of observation with decreasing light intensities, only 10 males were observed swarming, out of the 35 placed in the cage. The ten 58-hour-old males presumably did not influence the behavior of the younger 12-hour-old males which remained on the sides of the cage. On the following night when the youngest males were 34 hours old, more than 20 individuals were observed in flight 15 minutes after the initiation of swarming.

ABSTACT

Swarming in *Culex pipiens quinquefasciatus* Say is induced by a gradual change in light intensity to a certain threshold level—one foot candle. Only males take part in the swarming and are not affected by the presence or absence of other individuals. Swarming becomes more vigorous and prevalent as they grow older.

All observations indicated that copulation takes place within the swarm. The two sexes appear to meet by chance as there is no seeking of the opposite sex by either the males or females. Once they have encountered, the male appears to be the aggressor, although the female controls the flight of the coupled pair. The entire activity takes place in less than a minute. Following insemination the female remains quiescent. The number of copulations becomes more abundant with increasing age of the male and the percentage of inseminations subsequently rises. Thus, mating is dependent on male swarming but male swarming can occur independently of mating activity even in the presence of females.

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