USE OF A MODIFIED MARCHAND CAGE TO STUDY MATING AND SWARMING BEHAVIOR IN *CULEX TARSALIS*, WITH REFERENCE TO COLONIZATION[1]

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ABSTRACT. Selective pressures in laboratory rearing may account for the poor field mating of laboratory reared *Culex tarsalis* males. Previous studies of swarming behavior of field collected *Cx. tarsalis* had to be done in the field since such adults did not exhibit normal swarming in cages. Field collected individuals did not swarm normally nor mate effectively in cages. Normal swarming behavior by field collected mosquitoes, subsequent mating, and insemination were observed in a cage modified from a design by Marchand (1985). The use of such a cage could reduce one type of selective mating pressure involved in the colonization of mosquito species.

INTRODUCTION

Studies on the swarming and mating behavior of mosquitoes have been largely limited to those species which will mate in cages or to stenogamous populations of mosquito species which normally do not mate in cages. Mating behavior can differ substantially between stenogamous laboratory cage mating populations and "wild" type populations (Reisen et al. 1985). Studies of the mating behavior of mosquitoes which do not mate in cages have had to be done in the field or in field-like conditions. Mating and courtship comprise a set of behaviors which can require a large amount of space. In addition, lighting conditions required for mating in the natural environment may be difficult or impossible to duplicate in the laboratory. Without the necessary cues, normal swarming and mating may not occur.

Culex tarsalis Coquillett males form swarms in the field and mating occurs in association with these swarms. Swarming behavior is independent of the immediate presence of virgin females but not of the surroundings (Reisen et al. 1985). Males from the wild did not swarm when released into large outdoor cages. They flew up the walls of the cage, bouncing along the walls while ignoring the swarm markers and the females. A laboratory adapted population, in contrast, formed marker swarms over objects in such cages and mated with virgin females (Reisen et al. 1985).

The possibility that a horizon was needed as a cue for swarming mosquitoes was investigated by Marchand (1985). Marchand (1985) found that simulation of an evenly illuminated "sky," bounded by a dark "horizon" could release swarming in *Anopheles gambiae* Giles. A cage based on his design was used in this study.

MATERIALS AND METHODS

Our cage was modified from Marchand's design (1985) because of the availability of structural materials and to simplify construction. Our cage is diagramed in Fig. 1 and consisted of three parts. The lowest was a darkened area that served as a resting place. It was made from a 0.75 m^3 colony cage and had solid sides and a door for inserting mosquitoes and provisions. The intermediate observation level was made of dark screen stretched over a frame enabling an observer to see inside the cage through the screen. The screen was dark enough not to appear markedly different from the lower portion of the cage. Black muslin cloth was attached to the top edge of the observation level and draped over the screened observation area and the observer. The lower and middle portions of the cage were thus shielded from outside light.

The important differences between our design and that of Marchand (1985) were in the material for the upper portion of our cage and the arrangement and type of bulbs used for illumination. Stiff, white, translucent paper was used in the upper portion of the Marchand (1985) cage design. Marchand's (1985) illumination scheme used more, lower power incandescent bulbs for illumination than did our design. The upper portion (A in Fig. 1) of our cage was made of white sheets sewn together to form a cube 0.75 m on a side. The sheets were supported by a framework of polyvinylchloride (PVC) pipe. The corners of the white cloth cube were attached by nylon strings to the PVC supporting structure and were kept taut to avoid wrinkles. Light penetrating the white cloth was diffused and illumination was provided without an obvious point source. Flood lights were attached to the PVC pipe framework and arranged so

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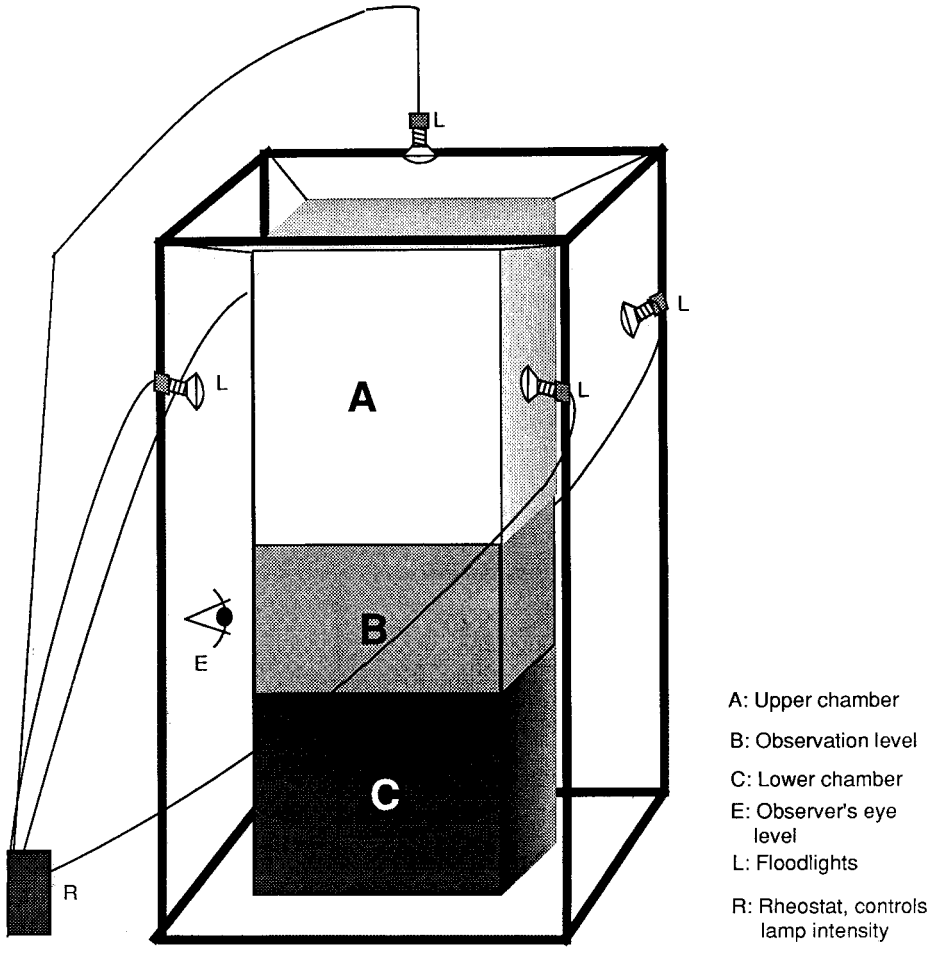

Fig. 1. Schematic drawing of the swarming observation cage modified from Marchand (1985).

that each lamp shone directly on the center of the sheets.

Illumination was controlled with a continuously variable rheostat by the observer sitting in the observation position. Light meter readings in the center of the observation level were correlated to the rheostat settings. The light levels at which various activities occurred could then be determined.

Adult mosquitoes from pupae collected at field sites near Bakersfield, CA were allowed to emerge in gallon size cages using standard laboratory rearing conditions (Reisen et al. 1985). After known numbers of virgin adults were released into the cage their behavior was observed at various light levels. Determination of insemination rates was performed before an experiment began and each morning once the experiment was in progress. On some occasions, samples for determination of insemination rates were also taken in the afternoon. Samples were obtained by aspirator from the lower part of the cage, after the mosquitoes resting on the cage floor were disturbed and had flown up to where they could be seen. Insemination was determined by dissection and search for sperm in the spermathecae or genital tract.

RESULTS

Table 1 presents insemination rates as determined in several experiments conducted in autumn of 1985. In the modified Marchand cage, described here, up to 80% of wild collected females were inseminated in one test.

Swarming behavior observed here was indistinguishable from that observed in the field. Previously, swarming behavior of wild *Cx. tarsalis*, if it occurred, in both outdoor cages and laboratory cages has been disorganized. When wild collected mosquitoes were released into the cage used in this study, they congregated at the bottom of the cage during simulated daylight periods when the cage illumination was bright.

Table 1. Insemination rates of wild-collected *Culex tarsalis* allowed to mate in a modified Marchand cage during 6 separate trials.

No. females/ males	Date checked	Time checked (hr)	Insemi- nated/ total checked	Per- cent insemi- nated
75/75	Aug. 9	1430	0/5	0
	Aug. 10	0930	0/5	0
	Aug. 12	0830	0/5	0
75/50	Aug. 12	1230	0/5	0
	Aug. 13	0830	2/5	40
100/120	Aug. 19	1200	0/10	0
	Aug. 20	0830	2/5	40
	Aug. 21	0730	1/5	20
	Aug. 21	1430	3/5	60
	Aug. 22	0900	7/10	70
	Aug. 23	1110	8/10	80
125/125	Sept. 3	1200	0/10	0
	Sept. 4	0900	2/10	20
	Sept. 5	0900	0/10*	0*
	Sept. 6	0900	4/10	40
125/125	Sept. 17	1200	0/10	0
	Sept. 20	0800	18/25	72
150/150	Sept. 23	1200	0/10	0
	Sept. 27	0900	16/25	64
Pooled final results			48/80	60

* A curtain blocking exterior light fell during the night and allowed exterior light to partially illuminate the cage.

During the simulated evening, when swarming and mating activities occur, male patrolling flight began at 2.5 lux. Patrolling flight across the cage at the level of the observation screen consisted of back and forth movements of males, very similar to the patrolling flights observed in the field by Reisen et al. (1985). Marker swarming (Reisen et al. 1983) began at 0.66 lux and peaked when light levels were reduced to 0.15 lux. The marker swarm was centered at or slightly above (5–10 cm), the area at which the patrolling flight was performed. The males did not approach the sheets of the upper cage closer than 5 cm during their swarming flight and did not land on the walls of the cage during the swarming period. Females however, would land on the cage walls after flying through the swarming area. Swarming ceased when the light intensity was below 0.075 lux.

Females began to fly upwards through the swarm at 0.15 lux. When females flew through the swarm, the males grappled with them. Successful copulation was observed frequently, at a rate of several times a minute during at least one observation period extending for several minutes. Often several males would attempt to seize the same female. This resulted in a cluster of grasping males surrounding the female. Successful matings were not seen to result from the multiple attacks by males. Grasping and capture of the females took place at or about the interface of the dark and the light areas of the cage. Copulation took place immediately after the successful capture of a female and occurred in flight. Copulating pairs left the swarm and landed on the upper cage walls as noted in Reisen et al. (1985). There, the females invariably landed with their heads up with the males hanging downward by their abdomens while coupled with the females.

DISCUSSION

Efforts to use sterile male techniques in the control of *Cx. tarsalis* have been delayed because males reared in the laboratory, when released in the field, did not compete successfully with the wild males for females (Reisen et al. 1981, 1982). Apparently, some factor in the colonization process or laboratory rearing altered the ability of colony males to mate with wild females.

One cause of this mating incompetence may be the lack of some dietary component which is required for successful swarming and mating behavior (Asman et al. 1985). The diet of larval mosquitoes in the field is different from a diet of laboratory chow and yeast. There could possibly be a deficiency of essential fatty acids in the laboratory diet which could contribute to poor flight and pursuit ability in the males and thus to mating incompetence.

An alternative explanation proposed by Reisen et al. (1985) and Reisen (1985) suggested that selection in the laboratory for a particular mating type may be a consequence of colonization. Laboratory cages which are illuminated without regard to artificial horizons or to the lighting conditions found in the field may lack cues to stimulate swarming. There may be many mating behavior types within the field population and one of the rarer types might be preadapted to laboratory conditions. Such a strain could thus successfully mate in the laboratory. Through selection, this mating type would then become increasingly adapted to a laboratory environment. Laboratory colonies would then be derived from minority mating types.

A higher insemination rate with field-collected mosquitoes would be expected in cages which did not select for a minority mating behavior type than in cages which selected for a minority mating type. Insemination rates observed in our cage were higher than usually observed in standard laboratory cages. A rate of at most 55% insemination is expected for field-collected *Culex tarsalis* brought into the laboratory and allowed to mate in standard screened cages for up to 4 nights (McDonald 1979, McDonald et al. 1979). In the modified Mar-

chand cage described here, up to 80% of wild collected females were inseminated in one test.

Although the cause of decreased mating competence in colonized *Cx. tarsalis* remains uncertain, the use of a cage as described above should reduce selective pressures against mosquito males which require certain visual stimuli found in the field but not in typical laboratory cages.

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