SWARMING AND MATING OF UNIVOLTINE *AEDES* MOSQUITOES IN THE LABORATORY[1]

IVAN N. McDANIEL

Department of Entomology, University of Maine, Orono, ME 04469

ABSTRACT. Swarming is a requisite for mating in populations of *Aedes communis* and *Ae. stimulans* in Maine. Swarming can be induced in a large, walk-in cage in the laboratory by proper control of humidity, temperature and light. The act of mating appears to be controlled by the behavior of the females and is usually completed during flight. Receptive females may vary in age from 5 to 12 days. The physiological conditions that give rise to receptive females are unknown. Field studies suggest that numerous univoltine *Aedes* species swarm and mate under similar conditions.

INTRODUCTION

The ease with which a colony of mosquitoes may be maintained under laboratory conditions has been largely responsible for our current background of knowledge concerning the family Culicidae. That we know a great deal about *Aedes aegypti* (Linn.), the yellow fever mosquito, is more likely due to the fact that it is easy to rear than the discovery at the turn of the century that it can transmit the pathogen which causes yellow fever. A technique for inducing copulation artificially in the laboratory has made possible the maintenance of numerous, small colonies of various species over extended periods of time, but considerable skill, time and effort are required to produce sufficient eggs for the average research program (McDaniel and Horsfall 1957).

The vast majority of pest mosquitoes in the northern United States and Canada are single-generation (univoltine) species of *Aedes* of the subgenus *Ochlerotatus*. A single generation per year is sufficient to cause a high degree of annoyance to man since the females are long-lived and may persist as long as 5 or 6 months. We have laboratory records of individuals of *Aedes stimulans* (Walker) taking as many as 20 blood meals and some of *Aedes communis* (De Geer) taking as many as 9 from man. Since these species live a long time and may take blood on numerous occasions, it appears that they could have great potential as vectors of pathogens. Indeed, numerous positive isolations of viruses of the California encephalitis group have come from several species of univoltine *Aedes* (Sudia et al. 1971, Le Duc 1979).

It is the author's opinion that we have a long way to go before all of our vector problems in the field of medical entomology will be resolved. After recently losing my wife to cancer, it came to my attention that Maine, a state with no organized mosquito or biting fly control programs, has one of the highest cancer death rates in the nation (Anonymous 1983). Also, it has been well established that oncogenic viruses are responsible for many of the known neoplastic transformations in animals; and it also has been stated that most of the chicken tumors and the great majority of mouse tumors are of viral origin (Gross 1961). At the present time cancer in man is not well understood, but evidence is accumulating which demonstrates that viruses are involved (Poiesz et al. 1980, Gallo et al. 1983). It seems possible that biting insects could mechanically transmit the virus which causes AIDS, HTLV-III, since the virus is known to be transmitted by the use of blood, blood products and hypodermic needles. At the present time approximately 7% of the known cases do not follow the established patterns of transmission. In the case of HTLV-I, substantial data have been acquired which indicate that there are environmental factors in endemic areas that must be considered as important (Blattner et al. 1986). Improved methods for maintenance of colonies of various species of mosquitoes will lead to a better understanding of the relationships between them and various agents of disease.

Prior studies on swarming and mating of mosquitoes have led to conflicting reports as to the function of swarming. Many observers believe that swarming is in some way related to the mating act (Downes 1969). However, Nielsen and Greve (1950) and Nielsen and Haeger (1960) have conducted numerous studies and seem convinced that these acts are independent and swarming serves no known useful purpose. Perhaps the major reason for considering the two acts to be associated stems from early reports that mating occurred within swarms (Knab 1906, 1907).

MATERIALS AND METHODS

Swarming activity is initiated in our laboratory by simply opening a door between an

[1] Maine Agricultural Experiment Station publication No. 1133.

air-conditioned rearing room and our "colony room," which contains a large, walk-in cage 3.74 × 2.74 × 2.74 m located in a room equipped with a 2.74 × 1.80 m skylight. The relative humidity in the colony room is normally maintained between 50–70% at night and much of the day. During the afternoon we gradually reduce the humidity to about 25% by turning off the humidifier. The temperature is maintained at 20–21°C. Prior to a mating experiment, the rearing room temperature is reduced to about 15°C. The door between the two rooms is then opened about 20 cm, permitting cool, humid air to flow into the colony room. Theoretically, the cool, humid air stratifies into a layer near the floor. Swarming of the males begins immediately, and continues as long as there is sufficient light for observation, and probably into the dark. The swarming period is a minimum of 40 min and mating is observed as the illumination declines from 7 to 4 foot candles. In addition to the skylight, the colony room has windows equipped with venetian blinds along the south side. The illumination from the southern exposure was not critical for swarming since it made little difference whether the blinds were open or closed, so they were left open during the experiments. The skylight was also equipped with venetian blinds, but swarming took place only when the blinds were open.

The mosquitoes used in mating experiments were collected locally as eggs, larvae or pupae. Criteria for insemination are based on dissection of spermathecae (10 females per experiment) in physiological saline and examination under sufficient magnification to observe the presence of sperm cells. All mating experiments were conducted in the evening at approximately the same time that swarming occurs in the field and the females were examined after 24 hr.

RESULTS AND DISCUSSION

By experimentation we found that males are ready to swarm within a day or two of emergence, but as with many species the act of mating is ultimately controlled by the females. At 21°C females are seldom ready to mate until they are 5 to 8 days old and some may require as long as 10 to 12 days. Coupling can be observed during swarming approximately 6 to 12 days after emergence. When the females are forced to fly by tapping on the plastic screen at the sides of the cage, males will grapple with them and some attempts at mating can be seen, but if the female is not receptive she will reject the male. These brief encounters are thought not to result in insemination. Pairing is often difficult to observe in the field but is readily seen in the cage when large numbers of mosquitoes are present. These species couple and usually complete the mating act during flight. On occasion a pair will terminate the act after coming to rest on the plastic screen at the sides of the cage (Fig. 1). More frequently the pairs separate when they fly against the screen.

The key to mating lies in the ability to provide conditions that initiate and maintain swarming activity in the laboratory. The important factors include temperature, humidity, lighting and a cage sufficiently large to permit copulation to be accomplished during flight. Field observations indicated that swarming activity is initiated by a rise in relative humidity near the ground as the air near it cools during the evening. The males are then stimulated to flight, and they maintain a swarm either at a favorable level with respect to temperature and humidity, or above a layer which is unfavorable with respect to these factors. Therefore, the height at which swarming occurs will vary depending on environmental conditions. Swarming activity typically commences just above ground vegetation in the field, and often the swarms move to greater heights. The quality of the overhead light at this time in the evening appears to be critical, since we have been unable to maintain swarming activity with

Fig. 1. Dorsal view of mating in *Aedes communis*. Coupling occurs within swarms, and the act is terminated in an end to end position. The female is at the top of the photograph (X3).

various types of artificial lighting. We have observed that the natural overhead light during swarming is strongly polarized in a single plane as determined by rotation of a polarizer, but other aspects of the quality of the light in addition to intensity could be important.

Table 1 deals with swarming activity, observed matings and percent insemination of *Ae. communis* and *Ae. stimulans* in the large cage over at least a 10-day period following approximately 94% emergence. The data indicate that swarming can be initiated shortly after emergence but mating does not take place until about the latter half of the experimental period. Table 2 presents data which demonstrate that a high degree of insemination of *Ae. communis* can occur with only 3 nights of swarming activity if the swarming trials are delayed until the females are at least 9 days old. During the present investigation several generations of *Ae. communis* and *Ae. stimulans* were maintained in our laboratory. Limited tests with small numbers of *Aedes provocans* (Walker) and *Aedes abserratus* (Felt and Young) have resulted in similar results. The experiments suggest that these species, and perhaps others, swarm and mate under similar conditions and may be likely prospects for colonization. It appears that females mate only once, since very little mating activity can be observed after 70–80% of the females have become inseminated. As yet, we have not been able to achieve more than 80% insemination in the laboratory, which suggests that we are not producing optimum conditions and some selection is taking place.

Table 1. Percent insemination of *Aedes communis*[1] and *Aedes stimulans*[2] females as related to evenings of male swarming activity and visual observations of matings.

	Swarming activity		No. of matings observed		Percent insemination	
Day[3]	c	s	c	s	c	s
1	weak	strong	0	0	0	0
2	strong	strong	0	0	0	0
3	strong	strong	0	0	0	0
4	strong	strong	0	0	0	0
5	strong	strong	0	4	10	10
6	strong	strong	3	2	20	0
7	strong	strong	5	5	40	20
8	strong	strong	3	4	40	50
9	strong	strong	4	7	60	70
10	strong	strong	1	0	60	70
11	strong	—	0	—	70	—
12	weak	—	1	—	70	—

[1] Population ca. 3000 mosquitoes.
[2] Population ca. 4000 mosquitoes.
[3] Trial started with emergence of ca. 94% of the population.
c = *Aedes communis* s = *Aedes stimulans*.

Table 2. Percent insemination of *Aedes communis*[1] females when swarming was delayed until the females were at least 9 days old[1]

Day[2]	Swarming activity	No. of matings observed	Percent insemination
1	strong	12	30
2	strong	9	50
3	strong	3	60

[1] Population approximately 2000 mosquitoes.
[2] Trial started 9 days after emergence of all females.

Ellis and Brust (1973) described a new species, *Aedes churchillensis* that is a sibling species of *Ae. communis*. It is autogenous for the first ovarian cycle and is stenogamous. This is undoubtedly the *Ae. communis* "form" that Beckel (1958) "mated" at Churchill, Manitoba. It is possible that some *Ae. churchillensis* could have been included in our mating tests, but this appears unlikely. As yet we have not observed autogeny or stenogamy in populations of *Ae. communis* in Maine. The populations with which we have worked in the laboratory bite man readily, while *Ae. churchillensis* is not known to take blood (Ellis and Brust 1973).

Our experience with swarming in univoltine species of *Aedes* suggests that females enter the swarms of males for the purpose of mating when they are receptive. This conclusion agrees well with an early published account of swarming in *Aedes punctor* (Kirby) in Alaska (Frohne and Frohne 1952). Also, from the many reports in the literature, it is apparent that flight or swarming activity is a requisite for copulation in many species of Culicidae and Diptera in general (Knab 1906, 1907; Downes 1969).

We have also used the large cage for the maintenance of colonies of *Aedes triseriatus* (Say) and *Aedes hendersoni* Cockerell which would not mate in small cages. However, in the case of these mosquitoes, temperature, humidity and the overhead illumination from the skylight did not seem to be important factors. By moving large groups of *Ae. triseriatus* first to a 60 × 60 × 60 cm cage for several generations and then to a 30 × 30 × 37 cm cage, we were able to select a strain that mated readily in the smaller cage. We also mixed *Aedes vexans* (Meigen) populations with both *Ae. stimulans* and *Ae. communis* but the number of inseminated females of *Ae. vexans* averaged about 10% and we did not succeed in starting a colony. It is possible that this technique could result in colonization of *Ae. vexans* if carried out over a period of time. The single large mating cage can be used to accommodate matings of various species during a given period of time, since it is necessary to maintain the mosquitoes there only for the time required for mating.

ACKNOWLEDGMENTS

This study was supported in part by research grant GM 13074 from the National Institute of General Medical Sciences, Public Health Service and the Maine Agricultural Station. Thanks are due Johnny L. McDaniel and Dale R. Webb who provided assistance with the work.

References Cited

Anonymous. 1983. State of Maine 1983 Cancer Registry Annual Report. Dept. of Human Services, Bureau of Health, Div. of Disease Control, State House Station 11, Augusta, Maine. 116 p.

Beckel, W. E. 1958. Observations on rearing of larvae, pupae and adults of some *Aedes* mosquitoes of northern Canada. Can. J. Zool. 36:797–808.

Blattner, W. A., A. Nomura, J. W. Clark, G. Y. F. Ho, Y. Nakao, R. Gallo and M. Robert-Guroff. 1986. Modes of transmission and evidence for viral latency from studies of human T-cell lymphotrophic virus type 1 in Japanese migrant populations in Hawaii. Proc. Natl. Acad. Sci. USA 83:4895–4898.

Downes, J. A. 1969. The swarming and mating flight of Diptera. Annu. Rev. Entomol. 14:271–298.

Ellis, R. A. and R. A. Brust. 1973. Sibling species delimitation in the *Aedes communis* (DeGeer) aggregate (Diptera: Culicidae). Can. J. Zool. 51:915–959.

Frohne, W. C. and R. C. Frohne. 1952. Mating swarms of males of the mosquito, *Aedes punctor* (Kirby), in Alaska. Mosq. News 12:248–251.

Gallo, R. C., P. S. Sarin, E. P. Gelmann, M. Robert-Guroff, E. Richardson, V. S. Kalyanaraman, D. Mann, G. D. Sidhu, R. E. Stahl, S. Zolla-Pazner, J. Leibowitch and M. Popovic. 1983. Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). Science 220:865–867.

Gross, L. 1961. Oncogenic viruses. Pergamon Press, NY. 393 p.

Knab, F. 1906. The swarming of *Culex pipiens*. Psyche 13:123–133.

Knab, F. 1907. The swarming of *Anopheles punctipennis* Say. Psyche 14:1–4.

LeDuc, J. W. 1979. The ecology of California group viruses. J. Med. Entomol. 16:1–17.

McDaniel, I. N. and W. R. Horsfall. 1957. Induced copulation of aedine mosquitoes. Science 125:745.

Nielsen, E. T. and H. Greve. 1950. Studies on the swarming habits of mosquitoes and other Nematocera. Bull. Entomol. Res. 41:227–258.

Nielsen, E. T. and H. S. Haeger. 1960. Swarming and mating in mosquitos. Misc. Publ. Entomol. Soc. Am. 1:71–95.

Poiesz, B. J., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna and R. C. Gallo. 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc. Natl. Acad. Sci. USA 77:7415–7419.

Sudia, W. D., V. F. Newhouse and C. H. Calisher. 1971. California group arboviruses: Isolations from mosquitoes in North America. Mosq. News 31:576–600.