PRELIMINARY STUDIES ON TWO BIOLOGICALLY DIFFERENT STRAINS OF *AEDES TRISERIATUS* IN NEW YORK[1]

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ABSTRACT. *Aedes triseriatus* mosquitoes collected from tires and treeholes in New York, and those from an established colony in Maryland (Gerberg) were reared in the laboratory. Eggs from each of these 3 colonies were conditioned at 80% RH, 26° C and flooded weekly. Females from the established colony and those from tires were found more aggressive and efficient in feeding than those from treeholes, and their eggs were deposited in clusters of more than 100 and hatched rapidly after 4-5 weeks of conditioning. In contrast, the treehole strain females deposited less than 30 eggs per cluster which hatched intermittently for over a 13-week period.

Aedes triseriatus (Say) has been well established as the main biological vector of La Crosse (LAC), a California group (CAL) virus causing human disease in north central United States (Gauld et al. 1974, Thompson and Evans 1965, 1972, Watts et al. 1972, 1973a). These investigations have also shown that LAC virus can be transmitted transovarially by *Ae. triseriatus* (Watts et al. 1973b). Further, overwintering of LAC virus in the eggs of *Ae. triseriatus* has been demonstrated through isolations from larvae collected before the seasonal emergence of adults and from larvae taken throughout the summer from treeholes which had been enclosed since the previous winter (Watts et al. 1974, Beaty and Thompson 1975).

In New York State, CAL complex viruses were first isolated from mosquitoes in 1965 and have since been shown to cause human disease there (Whitney et al. 1969, Vianna et al. 1971).

Although *Ae. triseriatus* has been shown to transmit CAL complex viruses elsewhere, the role of this species in natural transmission cycles in New York has not been determined. Thus, studies on the biology of the *Ae.triseriatus* complex of mosquitoes were initiated in 1976 as part of an ongoing cooperative project between the New York State Science Service and the New York State Department of Health (Division of Laboratories and Research) to investigate the ecology of California encephalitis in the Albany region of New York. The purpose of this paper is to report on some preliminary findings regarding the bionomics of what appear to be two biologically different forms of *Ae. triseriatus*.

METHODS

To establish colonies of *Ae. triseriatus* from both natural and man-made habitats, larvae were collected from treeholes in American beech, silver maple, red maple, white birch and eastern hemlock and from discarded tires (fig. 1) all within 30 miles of Albany. The bionomics of *triseriatus* collected from all species of trees, regardless of location, was similar. Therefore, to simplify a comparison with those collected from tires, data on *triseriatus* obtained from a single silver maple treehole (fig. 2) were used to illustrate this particular study.

Larvae were transported to the laboratory in 8 oz. polypropylene jars with snap caps. The contents of each jar were added to a white fiberglass rearing pan containing 1500 ml of distilled water which had been kept at room temperature (23–25° C) for a 24-hr period.

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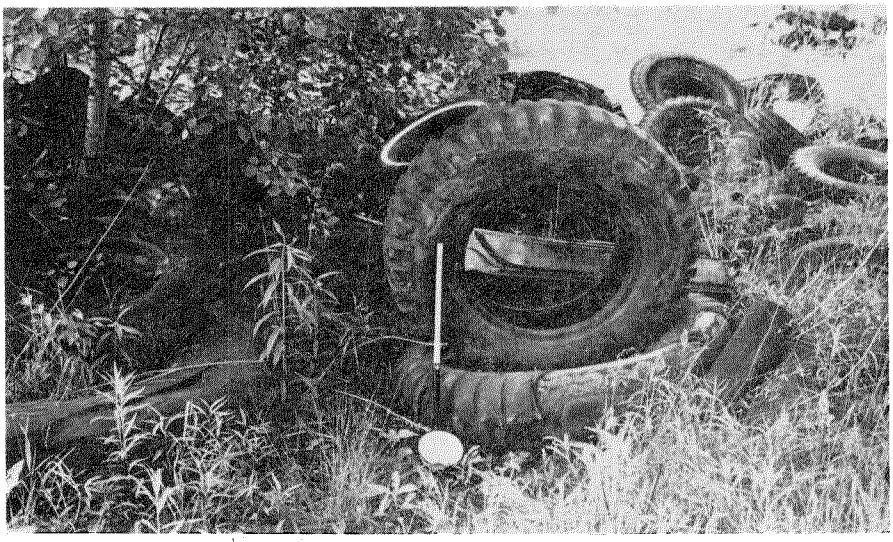

Fig. 1. Tires containing *Aedes triseriatus* larvae.

Larvae were also reared from eggs obtained from an established laboratory strain in Maryland (Gerberg). These larvae were treated in the same manner as those collected from natural sites.

One hundred 3rd and 4th instar larvae were retained in each pan. They were fed approximately 5 ml yeast slurry, layered along the bottom of the pan in three places using a pasteur pipette. Yeast slurry was prepared fresh for each feeding by dissolving a one-fourth oz. package of dry baker's yeast in 100 ml warm water and letting it stand for 24 hr. Larvae were fed every other day and water added on alternate days to maintain 1500 ml in the pan.

Pupae were transferred daily to half-pint ice cream containers which were placed in cubic foot collapsible mosquito cages. The cages were kept at room temperature on shelves in the laboratory. Eighty to eighty-five% RH was maintained by means of a towel draped over each cage and immersed in a container of water directly behind each cage. A fresh cotton pledget saturated with 10% sucrose solution was placed in each cage daily for adult feedings.

Beginning 2 days after emergence, females were offered daily blood meals in subdued light. For the first feeding, 10 one-day old mice were placed in the cage for 1 hr; for successive feedings, a forearm was offered for 15-min periods. To evaluate relative aggressiveness and length of time required by each strain for engorgement, cages containing 10 females from each source, all 6 days old and unfed, were offered a blood meal (human hand) under identical subdued lighting conditions.

After the first feeding, oviposition containers were placed in each cage and examined daily for eggs. The containers were made from pint ice cream cartons painted with dull black enamel inside and out. The inside vertical surface of each container was covered with a piece of paper towel cut to fit. One hundred ml distilled water containing one ml yeast slurry was added to the container. This kept the paper towel moist, providing a suitable surface for egg deposition. A hole, one inch in diameter,

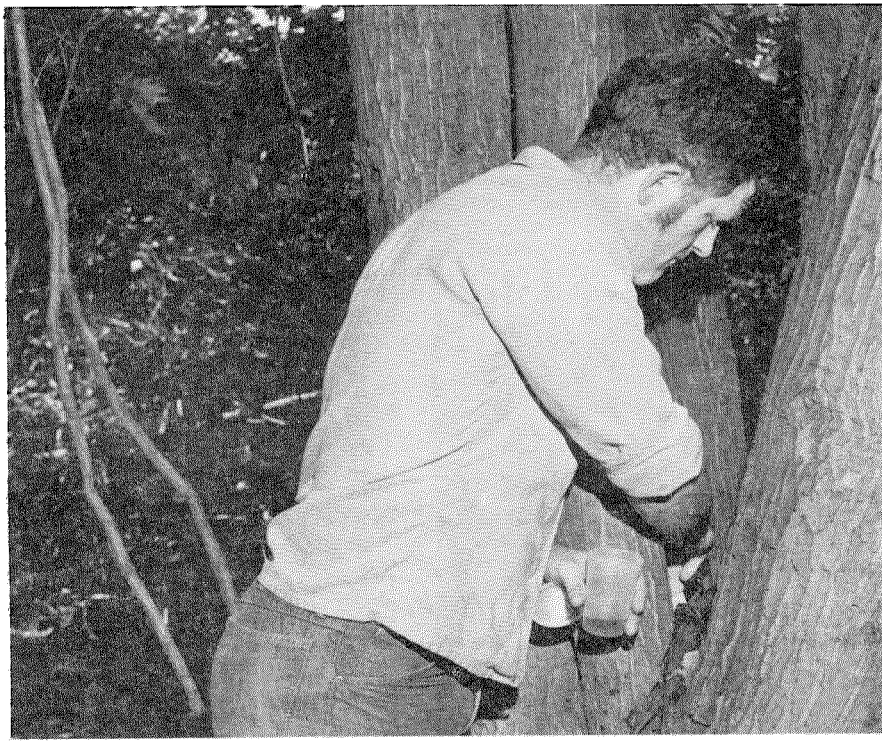

Fig. 2. Collecting *Aedes triseriatus* larvae from a treehole.

was cut in the cover which was also painted black, inside and out.

As eggs were found attached to the paper towel in each container, the latter was removed from the cage and replaced with another. Containers with eggs were stored in a chamber at 80% RH, 26° C until the water had evaporated (usually 3–4 days), after which the egg papers were removed and stored in covered petri plates under the same conditions.

After conditioning for 1 wk, papers containing approximately 500 eggs were submerged in deoxygenated water for 1 hr. Deoxygenated water was prepared by dissolving one 250 mg vitamin C tablet in 100 ml hot water and letting it stand for 24 hours.

Hatched eggs were counted, and papers which still contained unhatched eggs were returned to the environmental chamber. First instar larvae were transferred to bowls containing 100 ml distilled water with 5 ml yeast slurry added. The following day they were transferred to rearing pans containing 1500 ml water and fed yeast slurry layered along the bottom as described earlier.

PRELIMINARY OBSERVATIONS AND RESULTS

Each cage of 100 mosquitoes reared from original material contained 20–30% males, which emerged 12 to 24 hr earlier than the majority of females. Successful

mating under subdued lighting conditions was observed in all cages beginning 3 days after female emergence and continuing for 4 to 6 days. These observations were in agreement with the findings of Foster and Lea (1975).

Although some females took blood 3 days after emergence, most fed only after 5 or more days. Female *triseriatus* which emerged from larvae collected from tires and females from the laboratory colony (Gerberg) landed and fed on lab mice and a human arm much more readily than those emerging from larvae collected from treeholes. Also, they appeared more aggressive and persistent than females reared from treehole-collected larvae. In tests to determine time required for engorgement, females reared from tire-collected larvae and those from the laboratory colony all landed and began engorging within 15 sec. All were replete within 150 sec. Nine out of ten females of treehole-collected larvae landed gradually over a 3-min period beginning after 45 sec. The first female was replete 270 sec after first being offered the blood meal; all were replete after 540 sec (figure 3).

This same degree of aggressiveness and persistence was observed at the sites from which larvae were collected. In the vicinity of the tire dump, *triseriatus* females were always abundant and fed readily throughout the day in sun or shade. In the vicinity of treeholes, in a more sylvan habitat, there were usually few *triseriatus* females

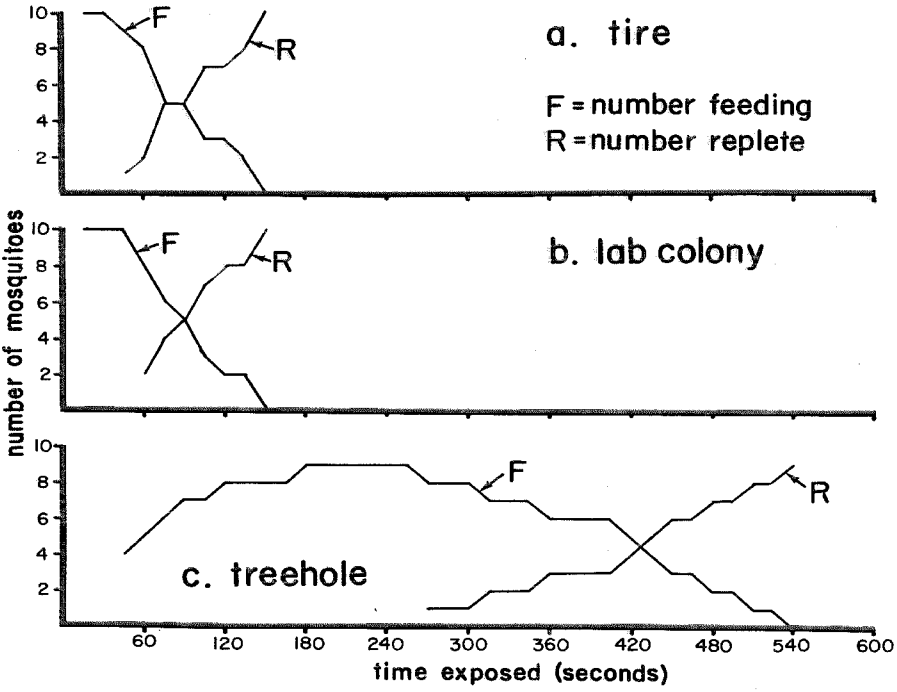

Fig. 3. Time required for 10 *Aedes triseriatus* females reared from larvae obtained from 3 sources to engorge in the laboratory.

present at any time; they were flighty, not very persistent in their feeding, and usually not present on sunny days.

Eggs were deposited by females from each of the 3 sources over a period of 8 to 16 days following the first blood meal. Although egg clusters deposited by some mosquitoes overlapped other clusters on the paper, they were usually in distinct locations and could always be distinguished. The mean number of eggs per batch obtained from the treehole colony was considerably smaller than those obtained from either the tire or laboratory colony: 40 batches of eggs with an average of 28 eggs per batch from the treehole colony; 39 batches with an average of 100 from the tire colony; 41 batches with an average of 115 from the laboratory colony (Table 1).

did not hatch. Of the remaining 72 percent of eggs from the treehole colony, 69% hatched gradually, approximately 8% with each weekly reflooding for 13 wk. The remaining 3% did not hatch (see fig. 4).

DISCUSSION

Although these are only preliminary observations made on a limited number of individuals reared from few sites, it appears that there may be 2 strains of *Ae. triseriatus* having somewhat different biological habits. Those breeding in the treeholes examined deposited fewer eggs than those breeding in tires, and the eggs hatched intermittently over a period of about 13 weeks after several successive floodings, which is typical of some flood-

Table 1. Eggs deposited in oviposition containers in cages containing 100 *Aedes triseriatus* mosquitoes

Number of days after first blood meal	Source of Larvae: Tires			Laboratory Colony			Maple treehole		
	N[1]	M[2]	R[3]	N	M	R	N	M	R
8	4	28	20–35	–	–	–	4	12	10–14
10	9	113	100–120	12	112	100–125	6	17	10–21
12	6	109	100–120	10	115	98–130	4	29	28–30
14	12	114	106–120	12	117	100–130	15	29	25–31
16	6	110	100–120	7	116	100–125	6	29	24–33
18	2	98	95–100	–	–	–	5	30	24–35
TOTALS	39	110	20–120	41	115	98–130	40	28	10–35

[1] N = number of batches of eggs.
[2] M = mean number of eggs per batch (rounded to nearest whole number).
[3] R = range in number of eggs per batch.

Approximately 4% of the eggs from each colony hatched each week when flooded after 2, 3 and 4 weeks of conditioning. After 5 weeks of conditioning, 72% of eggs from the tire colony, 4% from the laboratory colony and 8% from the treehole colony hatched. In the 6th week, all except 5% of the remaining eggs from the tire and laboratory colonies hatched, while just another 8% of the treehole colony hatched. The remaining 5% of the eggs from the tire and laboratory colonies

water mosquito species (Gjullin et al. 1950). In contrast, while a small percentage of eggs obtained from *triseriatus* mosquitoes breeding in tires hatched gradually during the first 4 weeks, the majority hatched abruptly after 5 to 6 weeks of conditioning.

Our observations also suggest that the females of *triseriatus* breeding in treeholes in a sylvan habitat are less aggressive and persistent in their feeding habits than those breeding in tires.

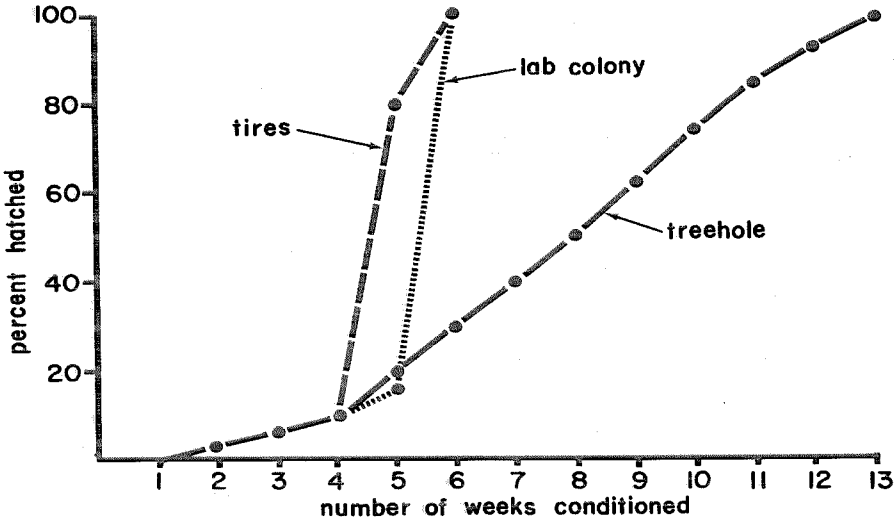

Fig. 4. Percentage of *Aedes triseriatus* eggs from 3 sources hatching after conditioning and reflooding for varying periods.

Further studies are needed to determine if these 2 populations constitute genetically different strains of *Ae. triseriatus* and if the existence in the Albany area of a biologically distinct sylvan strain breeding in treeholes and a semi-domestic strain breeding in tires is of potential epidemiologic significance in the transmission of CAL virus infections to man.

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MATING COMPETITIVENESS OF CHEMOSTERILIZED HYBRID MALES OF *AEDES AEGYPTI* (L.) IN FIELD TESTS

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ABSTRACT. Field tests were conducted to study the effects of heterosis on mating competitiveness (c) of chemosterilized males of *Aedes aegypti* (L.). When males of the normal strain were sterilized by exposing pupae to *P,P*-bis(l-aziridinyl)-*N*-methylphosphinothioic amide and released along with untreated males and virgin females of the same strain, c averaged 97%. When hybrid males replaced either the sterile or untreated normal males in releases designed to measure the effect of heterosis, no discernible effect was noted, whether the hybrids were sterile (c = 97%) or fertile (c = 109%). The results indicate that chemosterilized males may be suitable for genetic control of this species, but that hybrid vigor does not necessarily increase the competitiveness of the released males.

Research on the sterile male technique for mosquito control was stimulated by the report (White 1966) that thiotepa (tris(l-aziridinyl)phosphine sulfide) could be used to chemosterilize pupae and subsequently by the report of Lofgren et al. (1973) that some analogues of thiotepa were even better pupal sterilants. The use of these chemicals for sterilizing mosquito pupae is simpler than treatment of larvae or adults and also produces a fairly competitive insect apparently because of the low level of somatic damage. Thiotepa was used as the sterilant in an experiment that eliminated *Culex quinquefasciatus* Say from an island off the Gulf Coast of Florida (Patterson et al. 1970). One of the thiotepa analogues, *P,P* - bis(l - aziridinyl) - *N* methylphosphinothioic amide, was the sterilant used in the successful control of a population of *Anopheles albimanus* Wiedemann in the Lake Apastepeque area in El Salvador (Lofgren et al. 1974). Likewise, thiotepa was used to sterilize the mosquito species, *Culex fatigans*, in release experiments in India (Rao 1974). The results of the Indian experiments were not conclusive in terms of population reduction, but the best estimate of the performance of the sterilized males indicated good competitiveness. The same laboratory in India also worked extensively with thiotepa as a sterilant for *Aedes aegypti* (L.) (Grover and Sharma 1974), generally via a somewhat traditional approach including studies of sterilizing treatments, permanency of sterility in males, and mating competitiveness.

We have also been involved in studies on using chemosterilants for the control of *Ae. aegypti*, but our approach has been different. In our studies we have used one of the analogues of thiotepa and have made an attempt to increase the competitiveness of chemosterilized males by taking advantage of heterosis. In an earlier paper, we