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CAN BLOOD-FED CULEX PIPIENS L. OVERWINTER?[1]

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Whether blood-fed *Culex spp.* mosquitoes are capable of surviving over-wintering conditions has long been a controversy. Such mosquitoes could serve as reservoirs of mosquito-borne viral encephalitis in temperate regions (Bennington et al., 1958). In the past, blood-fed mosquitoes have been subjected to various combinations of temperatures and humidities to test their "overwintering abilities" (Hall, 1967). Such tests, while providing interesting and valuable data, have not answered the intended question and have come upon the added complication of gonotrophic dissociation (Eldridge, 1966). Probably the time allowed for digestion and possibly oviposition after blood feeding and before the onset of overwintering conditions is a critical factor thus far overlooked in laboratory studies. Close observations of overwintering blood-fed mosquitoes in nature was investigated as an alternate approach to this question.

Obviously, throughout the summer and fall, some portion of any active *C. pipiens* population has blood-fed and oviposited. If, as suggested by Clements (1963), there is a late summer or fall "overwintering generation" which will not blood feed until the spring emergence from hibernation, its presence and increasing predominance should be detectable as an increase in the percentage of nulliparous females in the population. If mosquitoes which have oviposited are unable to overwinter, the percentage of uniparous and multiparous females should drop to zero by the spring emergence.

This report concerns observations of mosquitoes at Fort DuPont, Delaware City, Delaware, through the wintering of 1967-1968, with routine determinations of the numbers and parity of surviving females. The abandoned fort and its complement of four ammunition bunkers provided a series of ideal overwintering sites with sufficient mosquito populations for adequate statistical treatments of the data obtained.

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Materials and Methods

The study area consisted of six relatively isolated groups of hallways and rooms within the concrete fort and four brick-walled ammunition bunkers just to the northwest of the fort. One room in each of two of the complexes of the fort and one brick bunker were designated as control areas — to be preserved as natural study sites to determine overall population density changes. Maximum-minimum recording thermometers were placed in one concrete control and in one brick control.

Periodic observations were made in these three control rooms. Beginning on November 20 and continuing through April 25 temperature and relative humidity were determined in each of the three rooms each week with a sling psychrometer. The readings were taken between the hours of 3 and 4 p.m.

Both of the concrete control rooms included surfaces of limited areas which held by far the greatest concentrations of mosquitoes. All temperature and humidity readings were taken as close as possible to these compact aggregations without disturbing the resting mosquitoes. In addition, weekly flash photographs were taken of these two specific areas and of one specific area, originally arbitrarily selected, in the brick control. Later, the numbers of mosquitoes surviving in each successive week was determined from these photographs.

In addition, during this same period, specimens were collected weekly from other, similar concrete (fort) and brick (bunker) sites. It is important to note that these structures are not the same as those designated for control sites. Temperature and humidity data were recorded. While they were available (November 13 through March 7), 25 mosquitoes were removed weekly from each of the two types of test structures. Although some *Anopheles* spp. were present, only *Culex pipiens L.* was collected.

TEMPERATURE, RELATIVE HUMIDITY, AND ATTRITION DATA FOR OVERWINTERING SITES OF *CULEX PIPIENS* (L.), DELAWARE CITY, DELAWARE, 1967-1968.

Location	Temperature (F)			% Relative Humidity			% Attrition
	Mean	Variance	Range	Mean	Variance	Range	
Outdoors	43.4	124.8	52	72.8	382.7	64	-----
Concrete Room 1	45.4	54.3	26	74.3	290.7	56	93.2
Concrete Room 2	45.4	29.4	17	72.6	224.7	54	87.7
Brick Bunker	44.4	30.8	16	82.3	166.8	62	50.0

When the available population was reduced, the number in the collections was lowered from 25 to 7. Approximately 7 mosquitoes from each type structure were obtained from March 14 through April 25.

The mosquitoes were collected by mechanical aspirator (Murphy, 1959) and were transferred to small, cylindrical screenwire cages. These collections were kept cool and were wrapped in wet toweling during transport back to the laboratory, where they were kept under conditions of 50° F. and constant light until five females from each type of site could be dissected (within a week). The condition of the ovaries, nulliparity, uniparity, or multiparity, was determined (Carpenter and Nielsen, 1965).

The remaining mosquitoes were transferred to 22-inch cubic rearing cages kept at room temperature and in nearly constant light. The cages were darkened only for blood feeding, the constant light being an oviposition stimulus (Eldridge, 1966). Larvae were obtained regularly from these females for positive identification to species.

The January 26 collection, however, produced an egg raft before blood feeding. From January 26 through March 7, four weeks were permitted between collection and the beginning of blood feeding to see if any more such egg boats might be found. All progeny derived from the non-blood-fed females were placed in a separate rearing cage in an attempt to demonstrate autogeny.

Results

The numbers of mosquitoes counted each week in each area photographed are represented in Figures 1 and 2, which also show the maximum and minimum temperatures for the week preceding the count. A logarithmic scale is employed because the data between the respective times of peak population and the times of presumed emergence from the overwintering sites closely conformed ($P<0.001$ in each case) to logarithmic functions. During this period, these functions for the concrete room 1, the concrete room 2, and the brick bunker, respectively, were as follows:

$$\log_{10}y = 2.9469 - 0.01134x,$$
$$\log_{10}y = 2.6193 - 0.007597x,$$
$$\log_{10}y = 1.6966 - 0.002793x,$$

(where "y" is the number of mosquitoes surviving and "x" is the number of days after the population peak in any one site).

These population data may, in turn, be compared with the weekly temperature and humidity data, which are expressed statistically in the table.

A total of 190 female *C. pipiens* was dissected from collections taken at the rate of 10 mosquitoes every week of the study excluding the collections of November 27, December 6, December 25, and April 4. In the

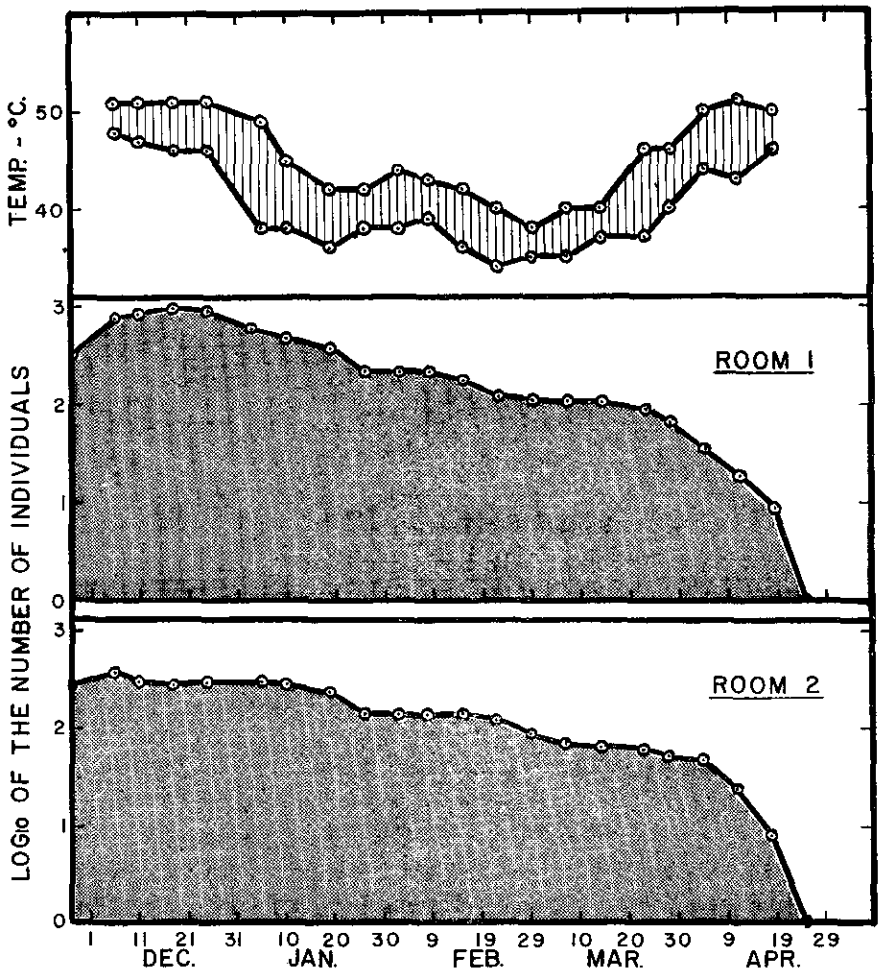

Fig. 1 — Weekly temperature maxima and minima and numbers of *Culex pipiens* at concrete control sites during the winter of 1967 - 1968.

November 27 collection, oviposition occurred before dissection could be performed, while in the other three collections, mortality occurred during transport and/or during holding in the refrigerated culture chamber to the extent that insufficient numbers remained for dissection.

The average percent of females having oviposited remained significantly constant ($P<0.05$) throughout the study. A mean of 36.3% (69 individuals) had oviposited before overwintering, and of these, four were biparous and one was triparous. In addition, of the 110 females collected

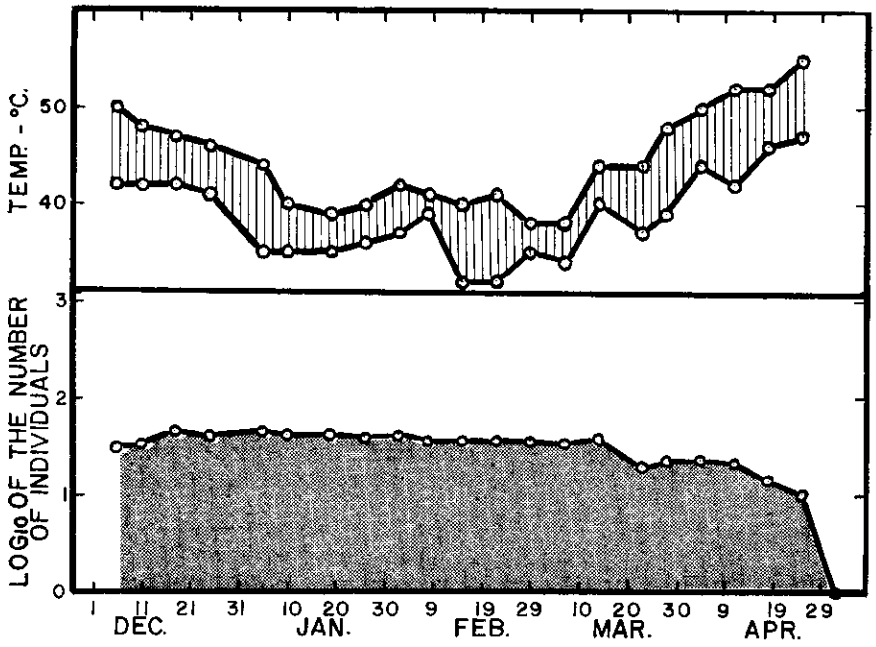

Fig. 2 — Weekly temperature maxima and minima and numbers of *Culex pipiens* at brick control sites during the winter of 1967 - 1968.

and examined for spermathecal contents during the last 11 weeks of the study, all were found to contain active spermatozoa.

Oöcyte development was not found in the dissections until the April 11 collection, but was found in all collections thereafter.

All females collected were found to be *C. pipiens* on the basis of identification of larvae developing from their eggs. A total of 3200 larvae was examined. During collection of egg rafts, 10 were obtained before females were blood fed in the laboratory and these egg boats yielded 530 larvae.

Adults obtained from females that had not been blood fed in the laboratory were isolated in a separate cage and were not blood fed. Only one egg raft was obtained from this "colony." No viable larvae were obtained from the egg boat, but examination of the eggs revealed that the egg caps on nearly all of them had been detached. However, no larval remains could be found in the hatching medium. No problems were encountered in maintaining an autogenous colony.

In addition to *C. pipiens,* some *Anopheles quadrimaculatus* and *A. punctipennis* were found overwintering in both types of structure. Other

associated hibernating species were the moth, *Scoliopteryx libatrix (L.)* and the syrphid fly, *Eristalis tenax (L.)*. The latter species formed aggregations in crannies in the walls of both types of structure. Still other species found in the area of the hibernating mosquitoes were the spiders, *Tegenaria domestica* (Clerck), *Amaurobius ferox* (Walchenauer), and *Phalcus phalangoides* (Fuesslin), and a species of scutigerid centipede.

Discussion

Data presented in the table demonstrate the probable direct correlation between the degree of temperature and humidity variability and percent attrition. Although no statistical analyses of crowding (distribution) were performed, it was also observed that degree of crowding seemed directly correlated with percent attrition. The small sample size, taken from only three study sites, obviates the use of correlation determination.

Little correlation was seen of density with weekly temperature maxima and/or minima (c.f., Figures 1 and 2). The steadily logarithmic decline in population probably indicates some intrinsic determinant, such as fat body depletion, of attrition rate.

The photographs used to determine population levels were not tapped for their full value. It was observed that individual mosquitoes could be "tracked" from week to week in these photographs and that, during the logarithmic phase of the supervisorship curve, movement was negligible. As well as movement, crowding could be put in quantitative terms (e.g., mean distance from nearest neighbor). Even the loss to spider predation might be estimated from these photographs by counting the number of mosquitoes enmeshed in webs and/or "disappearing" near webs.

The problem as defined, though was to determine whether female mosquitoes which have oviposited are selected against in an overwintering environment. The data quite strongly indicate that no such selective death occurs; there was no statistically significant change in the percentage of nulliparous females in the population over the entire winter.

Uniparity alone is insufficient evidence of a previous blood meal. A segment of the population may be autogenous and may oviposit without blood feeding. It is thought, however, that a second group of eggs cannot develop without a blood meal (Clements, 1963). If this is true, then the multiparous females found in the study must have had previous blood meals and were thus potential overwintering reservoirs for viral encephalitis. In addition, if the 33.7% level of uniparity observed in this study actually representel an equivalent degree of autogeny (33.7% of the population), one would expect that this level of autogeny would have been apparent as laboratory egg production without blood feeding.

The apparent absence of *Culex salinarius* Coq. supports the findings

of Huffaker (1942) at the same site. It is not known why *C. salinarius* is not found overwintering in Fort DuPont, Delaware, while it is found in the Delaware Ordinance Depot in Salem County, New Jersey (Lomax, 1967).

Conclusions

Overwintering populations of *Culex* females at Fort DuPont, Delaware City, Delaware, in 1967-1968 were, as far as could be determined, entirely *C. pipiens*. Apparently, the most important external factors affecting survival rate at this site are variations in temperature and relative humidity.

The constant proportion of uniparity indicated within this population and the presence of some multiparity very strongly suggest that blood-fed *C. pipiens* can and do overwinter successfully in this area. This evidence is strong enough to justify further investigation. Particularly valuable in interpreting the present results would be some indication of the prevalence of autogeny in the local *C. pipiens* population and observations of seasonal parity in this population.

PRESIDENT HUGHSON: The next paper is on "Salt-marsh Water Management in Delaware Accomplished under Senate Bill #75, 1965-68" by Chester J. Stachecki, Jr. and A. Warren Wheatley, Mosquito Control Division, State Highway Department, Milford, Delaware. This paper will be given by Mr. Stachecki.