SEX DIFFERENCE IN PUPATION OF LARVAL *CULISETA MELANURA*

ROBERT C. WALLIS

Section of Medical Entomology and Yale Arbovirus Research Unit, Department of Epidemiology and Public Health, Yale University School of Medicine, 60 College Street, New Haven, CT 06510

Culiseta melanura (Coquillett) was colonized in this laboratory (Wallis and Whitman 1969) and has been in continuous rearing for over 400 generations. Until September 1986, it was maintained in the Yale Arbovirus Research Unit (YARU) insectary under controlled light and temperature conditions. Recently, when the insectary was closed for painting and renovation, the colony was moved to the Medical Entomology Laboratory. This provided the author an opportunity to observe the mosquitoes' reaction to variations in environmental conditions, and to determine if, after 18 years of continuous rearing in standard conditions without larval diapause, the *Cs. melanura* in the laboratory colony had changed and lost their inherent ability to overwinter as larvae. The results of experimentally manipulating the colonized *Cs. melanura* to induce and to terminate larval diapause are reported here.

Little attention was given to this mosquito until Chamberlain et al. (1951) isolated the virus of Eastern Equine Encephalomyelitis from it and understanding its bionomics became essential. A laboratory colony was required for study of virus-vector relationships, but for 15 years all attempts to rear this species were foiled by the onset of larval diapause. Therefore, considerable attention was given to breaking larval diapause—with somewhat conflicting results: Chamberlain et al. (1955), Barbutis and Lake (1956), Wallis (1962), Siverly and Schoof (1962), Rutledge and Ward (1965), Hayes and Maxfield (1967), Joseph and Bickley (1969). Wallis and Whitman (1969) finally reported establishing a colony of *Cs. melanura* that was uninterrupted by larval diapause by using an enriched larval medium, constant temperature of 27°C, and larval pans covered to obscure photoperiod variation.

Maloney and Wallis (1976) tested changes of photoperiod and temperature as stimuli for inducing larval diapause. At high temperatures (28°C) all larvae completed development within the same time interval regardless of photoperiod. At 19°C, pupation rates differed: nearly all of the larvae reared under the long (16:8) photoperiod pupated, whereas in the short photoperiod group, only 20–30% of the larvae became pupae within the test interval (17 weeks). This latter group was the only one that seemed to display an experimentally induced diapause, as indicated by the lack of pupation. When temperatures were elevated and photoperiods were extended, the diapausing larvae began to pupate after a delay. The experimental rearing described here was intended to again test if the combination of 9 hr light and cool temperature of 19°C would induce diapause and to determine if the long established laboratory colony had changed in this regard.

The *Cs. melanura* used here originated from the YARU colony established in 1969, that was reared thereafter under controlled temperature (27–29°C) and light conditions (16 hr light and 8 hr darkness) through at least 400 generations. Routine rearing for propagation of the colony under standard conditions of 16–18 hr of light and 23–25°C temperatures was begun and an adjacent laboratory cubicle with independent light and temperature controls was used to provide reduced light (9 hr) and temperature (18–19°C) conditions for experimental induction of diapause in test groups of larvae. For the standard laboratory colony (which served as a control) and experimental groups of larvae, routine rearing methods were used, as previously described by Wallis and Whitman (1969).

On December 23, 1986, two pans of larvae (approximately 400 first and second instars) were placed in the low light and low temperature conditions (9 hr light and 19°C) in a screened cage. They were examined every several days and compared with the stock colony material maintained in 18 hr of light and at 22–23°C. After six days it was apparent that larval development in the experimental group was quite retarded. By the 15th day, the stock colony (controls) began pupating and continued to completion on the 29th day, whereas no pupae appeared in the experimental group during this time. When another week passed without pupation, both experimental groups were taken from the low temperature cubicle and returned to the stock colony rearing laboratory at 23°C and 18 hr light. Pupae began to appear 12 days later, and within 14 days, 46% of the larvae pupated. As I harvested these 178 pupae individually with a hand aspirator tube, superficial examination indicated that they were all male pupae. This was confirmed by allowing these pupae to emerge in a separate cage. No further

development occurred for 16 more days—when another wave of pupation began and extended over a 10 day period, during which 209 pupae were harvested—all females. Dead larvae in the rearing pans were not counted, but were less than 4%.

This procedure was repeated with 200 larvae during a period from mid-March to the end of May, of 1987. It was repeated again between July and September, of 1987 and the results are shown in Table 1. They were similar with only minor variations in the number of days needed for larvae to recover from 5 weeks of dormancy at the low temperature. In the March–May experiment, 39% of the pupae comprised the first or all male wave of pupation that began 13 days after larvae were placed under warmer conditions. That male wave of pupation was completed 16 days later. This was followed by a 15 day delay before pupation of females began and extended over a 2 week period.

In the last replication of the experiment, after return to warm rearing conditions with a long photoperiod, the all male wave of pupation began after 11 days, included 42% of the larvae, and was completed within another 11 days, followed by a delay of 18 days, after which the remainder of the larvae, all female, pupated over a period of 13 days.

These results confirm that our long established colony of *Cs. melanura* still has the ability to respond to environmental changes in photoperiod and temperatures that induce larval diapause. They also confirm, in part, that the present YARU colony responds in the same way when exposed to the experimental conditions reported 10 years ago by Maloney and Wallis (1976), i.e., larvae reared at 19°C and short (9 hr) photoperiod. In the 1976 experiment, after an initial period of slow growth and dormancy, larvae returned to warm conditions exhibited two waves of pupation. The first extended from 23 to 61 days and accounted for 20–45% of the larvae. After the initial wave of pupation ceased, the rest of the diapausing larvae remained inactive for a variable number of weeks and then produced a second wave of pupation involving all of the remaining larvae.

Table 1. Sex difference in the onset and incidence of pupation in *Culiseta melanura* at 23°C and 18 hr light/24 hr following 5 weeks of larval development at 19°C and 9 hr light/24 hr.

Experimental group	Day pupation began		% pupation of larvae*	
	Males	Females	Males (1st wave)	Females (2nd wave)
1	12	42	46	52
2	13	44	39	57
3	11	40	39	59

* % varies due to larval mortality (2–4%).

Rutledge and Ward (1965) were the first to report that two discrete waves developed in *Cs. melanura* larval growth to adults—the first wave occurred between 6 and 11 weeks, with a second wave between 16 and 18 weeks. In both waves, adult males emerged first, but in each there was an overlapping in time of emergence of males and females.

In the present study, the first wave of pupation was composed entirely of male pupae, and a second wave was composed of all female pupae. The waves were separated by a prolonged delay with no overlap between the two. This indicates that either the two sexes respond differently to stimuli, or require different amounts of exposure to warmth and the longer photoperiod to induce resumption of development after a period of larval diapause. Another possibility is that male larvae do not undergo diapause but rather have development drastically slowed down by a few degrees of reduction in environmental temperatures. At the end of the 1976 studies, we concluded our discussion by suggesting—"that persistence of larvae throughout the winter can be the result of quiescence induced by low temperature rather than a short photoperiod." However tempting such an uncomplicated explanation may be, it is likely to be an oversimplication.

From our present work with *Cs. melanura* reared at a cool temperature and winter-time photoperiod it is concluded that further experimentation is warranted to explore the critical temperature and photoperiod threshold conditions that are important for induction of larval diapause, and for its termination. Particular attention should be given to the kind and degree of differences in responses of males and females to these conditions. Because our laboratory colony, even after 18 years of continuous rearing, still retains the capacity to diapause we have the opportunity for such study.

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