RESPONSE OF COLONIZED *CULISETA MELANURA* TO PHOTOPERIOD AND TEMPERATURE

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ABSTRACT. *Culiseta melanura* were tested for response to changes of photoperiod and temperature in efforts to induce larval diapause. Conditioning of larvae and the preceding maternal generation were attempted: photoperiods of 9 hours light and 15 hours dark (9:15) and 16 hours light/8 hours dark (16:8) were combined with temperatures of 28, 19, 10, and 4° C in a series of experiments to determine their effects on this mosquito. Conditioning of the maternal generation had no effect on progeny. 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 approximately 20–30% of the larvae became pupae within the test interval (17 weeks). Regardless of photoperiod conditioning, all larvae could be kept from pupating by low temperatures. One group of 4th instar larvae survived over 7 months at 4° C. Upon rewarming a substantial number pupated and emerged. All procedures were conducted with laboratory reared specimens.

INTRODUCTION

Culiseta melanura (Coquillett) is an unusual mosquito because of its peculiar bionomics. In addition, it is considered one of the most important species in the eastern United States because it is now recognized as the primary enzootic vector of eastern equine encephalomyelitis (EEE) in this region (King et al. 1960; Hayes 1961a; Howard and Wallis 1974). It is one of the few mosquitoes that overwinters in the larval stage; however the environmental factors regulating the ability of the larvae to enter and terminate their seasonal diapause remain poorly understood.

The life cycle of *C. melanura* has received considerable attention. While observing larval behavior, Wallis (1954) noted the onset of pupation on the 9th day post-oviposition when the larvae were reared at 75° F, and pupation was complete by the 16th day. Similar observations were reported by Siverly and Schoof (1962) and Burbutis and Lake (1966). Some investigators have reported that larvae of this species may require a longer period of development. Chamberlain et al. (1955) observed that larvae required over 5 weeks to pupate at 80° F, while Rutledge and Ward (1965) indicated that 1st instar larvae collected in August required up to 18 weeks to complete pupation. In field studies conducted in Maryland, Joseph and Bickley (1969) reported that development can be prolonged, requiring from 8 to 14 weeks for completion during the warmest part of the year.

C. melanura overwinter in the larval stage (Smith 1904), Burbutis and Lake 1956, Hayes 1961a; Wallis 1962, Joseph and Bickley 1969), and it is believed that the adults cannot survive winters at northern latitudes (Wallis et al. 1958, Joseph and Bickley 1969). The factors causing the cessation of larval development or the specific stadium at which growth is halted are not clear. Wallis (1962) reported an enriched diet of liver extract and calf meal was the only stimulus necessary to terminate diapause in 60 percent of dormant larvae. Subsequently, Hayes and Maxfield (1967) reported diapause termination by application of 20 hr of light per diel period and the same enriched diet used by Wallis. However, they stated that neither diet nor photoperiod alone terminated diapause. Burbutis and Lake (1956) questioned the significance of photoperiod when they reported that diapause does not seem to be completely linked to reduced photoperiod. Larvae brought indoors during the winter of

1954–55 failed to pupate until the following spring. Conflicting evidence appeared the following winter when the same investigators brought in another group of larvae which continued development and pupated in "a relatively short period of time." Wallis (1954) had previously observed in Connecticut that larvae obtained from a gravid female in November proceeded through to pupation within 2 weeks when they were warmed to room temperature.

Hayes (1961b) investigated the overwintering activity of *C. melanura* in the field by submerging pipe containers at the edge of a known breeding site and adding larvae to the water and mud within. Throughout the winter a number of the pipes were removed and examined for larval activity. It was noted that the larvae avoided freezing by burrowing into the mud. As the winter progressed, and became more severe, they moved deeper into the muck and could be found as much as 6 in. below its surface. Later, as the winter tempered, the larvae moved closer to the surface and by May they had left the mud and were found swimming in the water. This experiment helped to explain how *C. melanura* larvae escaped freezing in their normal habitat; unfortunately the underlying environmental stimulus involved in prolonging the larval stage still remained obscure. The purpose of this study was to examine the effects of photoperiod and temperature conditioning (the 2 most common inducers of insect diapause) to see if they are responsible for diapause in this species.

MATERIALS AND METHODS

C. melanura were obtained from the colony established by Wallis and Whitman (1969) in the Yale Arbovirus Research Unit (YARU) insectary. Material from this colony was used to start and maintain an experimental colony from which mosquitoes were taken for testing procedures. The YARU colony also served as a control for all tests. The parent colony was kept at temperatures between 27 and 29° C, and at a photoperiod of 16 hours light and 8 hours darkness (16:8). Since the origin of this colony, the larvae have routinely been reared in covered pans; they developed in darkness except when the covers were removed for daily maintenance.

As they became available, pupae from the YARU colony were removed and placed in the experimental colony which was maintained at the same temperature as the parent colony (28° C), but the photoperiod was reduced to 9 hours of light per day (9:15). In this manner adults emerged directly into a decreased photoperiod and were never exposed to more than 9 hours of light per 24 hours. When the adult females were ready to feed, a chicken was offered twice weekly as a source of blood. At other times 10 percent dextrose was available for nutrition. Pint cardboard containers filled with moist paper towels were included to raise the humidity; they also served as resting surfaces for the adults.

Egg rafts were removed from the oviposition pans daily and proportioned equally into large (41 x 25 x 6 cm.) porcelain rearing pans that were filled to a depth of approximately 4 cm. of distilled water. When the eggs hatched a standardized diet of BHI solution (calf brain heart infusion; Difco Labs) was added, a stock solution of this nutrient medium was made by dissolving 20 gm of BHI powder in 1 liter of distilled water. The water in the rearing pans was skimmed daily to remove any surface pellicle, and on alternating days 5 ml of the BHI solution were added. Efforts were made to maintain a constant number of 300 larvae per pan, to avoid overcrowing.

One-half of the eggs obtained from the experimental (short photoperiod) colony were maintained in the short (9:15) photoperiod room while the remainder were moved (the morning after oviposition) into another cubicle where they hatched

and developed through a long (16:8) illumination cycle. The temperature was maintained at 28° C in both cubicles. Eggs were then obtained from the parent colonly (16.8), and placed into the same 2 cubicles of 9:15 and 16:8 hours photoperiod respectively. All larvae were maintained under the indicated photoperiod until they pupated. Four different combinations were thus tested: 1) a short photoperiod on both adults and larvae; 2) a short photoperiod on the larvae; 3) a short photoperiod on the adults; and 4) a long photoperiod on both stages. Consequently any influence of photoperiod on *C. melanura* held at insectary temperatures was observed. Pupae were recorded as they appeared in the pans but were not returned to the colonies.

To test the interplay of temperature and photoperiod 2 cubicles were set at 19° C. In one a short photoperiod (9:15) was used, and in the other a long illumination cycle (16:8) was maintained. Eggs from the experimental colony were placed in these cubicles. By this method any relationship between reduced temperature and the 2 photoperiods was observed. Both adult colonies were still kept at 28° C.

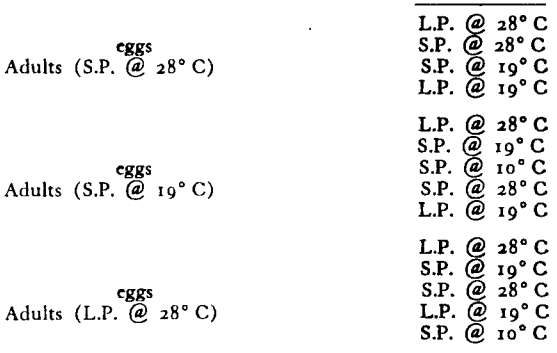
After completing this procedure, the temperature was dropped to 19° C in the room containing the experimental colony; these adults were thus exposed to both a short photoperiod (9:15) and lowered temperature. The adults were given 2 wk to adjust to the reduced temperature before the eggs they produced were utilized. Later, new adults emerged directly into the cooler environment, and their eggs were also used. Egg rafts were placed into the varied environments as previously described.

Finally, an environmental chamber was adjusted for a short light cycle (9:15), and a low (10° C) temperature. Larvae from the stock colony, and from the experimental colony (9:15 @ 19° C) were added, and to prevent excess mortality the temperature was lowered over a 5-day period. Control larvae in covered pans were also included.

The following flow diagram summarizes procedures.

RESULTS

LARVAL DEVELOPMENT RELATED TO ENVIRONMENTAL MANIPULATION OF THE ADULTS. Larvae in 3 groups obtained from adults maintained at 19° C and a short photoperiod required 13, 15, and 18 days respectively to begin pupation when they were reared at 28° C and a long (16:8) photoperiod. Most of the larvae rapidly pupated or died, and pupation was



L.P. = long photoperiod (16:8)
S.P. = short photoperiod (9:15)

observed to be complete 28 days post-oviposition. Two groups of larvae obtained from adults that were maintained at 28° C in a long photoperiod required 9 and 14 days before pupae began to appear after being reared in the same light/temperature regime. Pupation was complete in these groups by the 24th day post-oviposition.

The pupation rates between groups of larvae, obtained from adults held at each environmental extreme (28° C @ 16:8 and 19° C @ 9:15) were compared. Both larval groups were maintained at 19° C and a long photoperiod (16:8) after removal from the adult cage. Pupation began on the 30th day among the larvae obtained from adults kept under a long photoperiod at 28° C, and on the 39th day for the other group. The rate at which pupae appeared was similar for the 2 groups.

None of the larvae maintained at 10° C developed into pupae regardless of the photoperiod conditioning of their parents. Development was retarded at this temperature, as evidenced by the lack of pupation after 12 weeks. There was no difference in development between groups, as the larvae in all pans were 2nd and 3rd instars.

LARVAL RESPONSES TO PHOTOPERIOD AND TEMPERATURE CONDITIONING. This experiment was designed to detect the effects of photoperiod upon larvae kept at elevated temperature. Eggs were obtained from each of the 3 adult colonies which had undergone previous environmental conditioning (see flow diagram). Each egg batch was split so that ½ was placed under a long (16:8) light regime and the other half reared under a short (9:15) photoperiod. All larvae were maintained at 28° C. Pupae were first observed on the 12th, 14th, and 15th days respectively in the pans containing the short photoperiod larvae, whereas pupae appeared on the 12th and 13th day post-oviposition for the extended illumination group. The rate at which the pupae appeared was the same in the 2 groups.

At 19° C larvae developed more slowly than at 28° C. Whereas the majority of *C. melanura* usually required 11–19 days to complete larval development at 28° C, at the lower temperature larvae required at least twice as long to reach the pupal stage. At 19° C the earliest that pupae appeared was 23 days, and 50 days commonly passed before the 1st pupae were seen (the longest period was 61 days). A number of control larvae (from the parent colony) were also tested to observe their response to reduced temperature. They exhibited the same developmental rate as the experimental larvae, as 34 and 41 days respectively were required before they started to pupate.

All larvae exposed to 19° C and a short photoperiod (regardless of parental conditioning) had a similar pattern. After a somewhat variable period (24–61 days) pupae began to appear and continued to do so until approximately 20–45 percent of the population had pupated. At this point pupation ceased; some of these larvae were still alive 12 weeks later when the experiment was terminated. Efforts were made to induce these larvae to pupate by exposing them to 28° C and the long photoperiod. A month of this did not induce a significant number to pupate. One group, however, demonstrated a response to an increase in photoperiod and temperature. These larvae were from adults reared at 28° C and a long photoperiod. During their development this group was exposed to the short illumination cycle at 19° C. Originally these larvae were similar to other larvae maintained on this regime (i.e. there was an initial wave of pupation but the majority remained as larvae). Six weeks after the last pupae appeared in this group they were removed to the cubicle maintained at a long photoperiod and 28° C. After 13 days pupae began to appear, and a strong wave of pupation followed until all surviving larvae had pupated. As this observation suggested a sensitivity to changing environment and termination of diapause, 4 additional groups of larvae

with the same histories were introduced to identical conditions. The results could not be duplicated. After transfer to the new environment no wave of pupation was observed in the additional groups.

Whereas short light exposure and lowered temperature (19° C) impeded pupation, a long photoperiod at the same temperature did not. The period of time before pupae appeared (40 days) in the long photoperiod group was similar to the short photoperiod larvae maintained at the same temperature, except pupation continued until all larvae had either pupated or died.

COLD HARDINESS OF LARVAE. When pans containing larvae that had been reared at 28° C were introduced directly into 4° C, very few (less than 10 percent) survived for 2 weeks. Larvae reared at 19° C were observed to be more cold resistant, as 60 percent of these larvae survived for 2 weeks after being placed directly into 4° C. All larval instars showed some capability of surviving at least 2 months at 4° C, provided they were cooled to this temperature gradually. One week of gradual temperature decrease gave all instars sufficient time to make the transition. After such a conditioning process, 1 pan of 4th instars withstood extended cold for over 7 months—50 percent of the larvae were still alive when the experiment was terminated. Upon re-warming, most of the survivors pupated within 3 wk. Healthy adults emerged from these pupae in 3–4 days. Photoperiod conditioning did not influence larval resistance to cold.

DISCUSSION

There are marked fluctuations in the rate of development among the larvae from the YARU colony. This character was strongly evident at 19° C and 10° C where the larval stage was prolonged. An obvious difference appeared in the time required for larvae to progress through their instars; this was evident even when they were reared under identical conditioning and had originated from eggs deposited the same night. When the first pupae began to appear, there was a marked diversity remaining in the larval population; 2nd and 3rd instars were also present (at 28° C larvae grew at a more uniform rate). If this happens in nature all stadia would be present in the fall as temperatures dropped, and therefore the population would be heterogeneous when cold stopped development. Since late instars seem better suited to survive winter cold a "stringing-out" of the population induced by cooler temperature would insure a certain number of them in the population at all times. Additional experiments should be conducted to determine if fast and slow temperature-sensitive strains of *C. melanura* exist in nature. The existence of these strains would help explain the diverse development periods reported for larvae collected from the field.

Eggs deposited at 28° C produced larvae that pupated more rapidly than larvae from eggs deposited at 19° C. This character was independent of photoperiod. Even when the rafts were moved to identical temperatures and photoperiod on the morning after ovipositon, the brief exposure to elevated temperature reduced the length of the larval stage. Most commonly, pupae appeared in the groups oviposited at 28° C about 4–5 days earlier.

All results were obtained utilizing *C. melanura* originated from a colony that was started 7 years ago, and therefore may not be representative of a natural population. The fact that larvae maintained at 19° C responded differently to the 2 photoperiod regimes may have been a bona fide light sensitivity, which has been modified or eliminated by colonization. In view of the fact that the "cut-off" character associated with rearing under the short photoperiod could not be ended and larvae induced to pupate, questions have arisen as to whether this was a valid example of diapause. If further work with wild caught mosquitoes produces results similar to those reported here, then it can be suggested that the length of photoperiod plays a limited role in causing the larvae

of this species to overwinter. Our results suggest that the ability of *C. melanura* larvae to survive the winter can be due to a quiescent period in development brought on primarily by low temperature. This would be consistent with many field observations, as this species is often found in underground crypts where sunlight could not penetrate.

CONCLUDING REMARKS

The environmental factors of photoperiod and temperature directed upon the maternal generation did not affect their offspring. Photoperiod did not affect larvae at 28° C, as all larvae pupated rapidly regardless of illumination cycle. At 19° C photoperiod and temperature may interact since larvae reared under a long photoperiod pupated over an extended period of time whereas 9 hours of light (9:15) induced some reduction of pupation. Regardless of photoperiod conditioning at 19° C, a certain portion of all populations pupated (at least 20 percent), and individuals reared under 16 hours light (16:8) approached 100 percent pupation among the survivors. However, in every experiment there remained a small number of larvae that would not pupate even when introduced to 28° C and a long photoperiod. Only 1 experimental group of larvae seemed to display an experimentally induced diapause. They were reared under 9 hr light at 19° C and exhibited the cut-off of pupation associated with this conditioning. Upon introduction to elevated temperature and long photoperiod, they pupated quickly. This could not be repeated using larvae with similar histories and conditioning.

C. melanura larvae developed a marked resistance to cold independent of photoperiod. All 4 instars were capable of surviving cold for at least 2 months, with no prior conditioning. Late instars survived cold better than 1st and 2nd instars, but all stages had a better survival rate if they were reduced to near freezing temperatures over a gradual period (at least 5 days). One group of 4th instar larvae was maintained at 4° C for over 7 months; more than one-half the original number were still alive when the experiment was terminated. Rewarming induced pupation and emergence in a substantial number of the survivors. It is suggested that persistence of larvae throughout the winter can be the result of quiescence induced by low temperature rather than a short photoperiod.

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