# Biology of tree-hole mosquitoes: photoperiodic control of development in northern *Toxorhynchites rutilus* (Coq.)

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Carnivorous larvae of the tree-hole mosquito, *Toxorhynchites rutilus*, were collected from the northern portion of their range. Long days were found to promote rapid growth and metamorphosis from egg to adult; short days retard development during the second and third instars and evoke diapause in the fourth. All larvae exposed continuously to long days from embryos to the third or fourth instar developed without entering diapause. Diapause-averting long days experienced earlier in development could be reversed in at least some individuals by subsequent short days. Among laboratory-reared larvae or those caught early in the fall, the critical photoperiod for the maintenance of diapause is around 13 h of light per day. Among larvae caught in midwinter, diapause is not maintained in all larvae at any photoperiod and in 50% or less of the larvae at photophases shorter than 12.5 h. Winter conditions in the northern part of the range of *T. rutilus* appear to play a prominent role in the maintenance and termination of diapause.

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On a récolté des larves carnivores du moustique *Toxorhynchites rutilus* dans la portion nord de son aire. Les jours longs accélèrent la croissance et la métamorphose d'œuf en adulte; les jours courts retardent le développement durant le second et le troisième stade et déclenchent une diapause durant le quatrième. Toutes les larves soumises continuellement à des jours longs du stade embryonnaire au troisième ou quatrième stade se développent sans entrer en diapause. Le mécanisme est cependant réversible puisque l'effet des jours longs durant les premières phases du développement peut être inhibé par une exposition subséquente à des jours courts. Pour les larves élevées en laboratoire ou celles qui sont recueillies tôt à l'automne, la photopériode critique nécessaire pour maintenir la diapause est d'environ 13 h de lumière par jour. Dans le cas des larves recueillies au milieu de l'hiver, aucune photopériode ne réussit à maintenir la diapause chez toutes; des photophases plus courtes que 12.5 h maintiennent la diapause chez 50% ou moins de ces larves. Les conditions d'hiver dans la partie nord de l'aire de *T. rutilus* semblent jouer un rôle très important dans le maintien et la cessation de la diapause. [Traduit par le journal]

## Introduction

*Toxorhynchites* is a circumtropical genus of mosquitoes whose highly carnivorous larvae characteristically occupy container habitats. In North America, *Toxorhynchites* is represented by a single species, *T. rutilus*, which ranges from southern Florida and Texas north to about the 40th parallel (Jenkins and Carpenter 1946). In North Carolina, *T. rutilus* overwinter as fourth-instar larvae in the water of tree holes, where they undergo a seasonal diapause, the onset and termination of which is mediated by photoperiod and temperature (Jenner and McCrary 1964; McCrary 1965). In the present paper, we consider the effects of photoperiod on early larval development and diapause among *T. rutilus* from the northern part of their range.

## Collection and Maintenance

Larvae for experiments concerned with the initiation of diapause and development before diapause were derived from eggs collected in the field near Lahaska, Bucks County, Pennsylvania (40°20′ N latitude; 75°4′ W longitude; 180 m altitude). A single tree hole yielded 12–128 eggs per day. The eggs and subsequent larvae were exposed to long days (light:dark = 16:8) at 28 ± 1°C or short days (L:D = 8:16) at 23 ± 1°C. Constant-temperature facilities consisted of two underground cellars kept at these temperatures. Light was provided by a single 100-W tungsten lamp at a distance of 1–2 m. Light regimens were controlled by individual Intermatic time clocks. Temperatures were continuously monitored by a recording thermistor-probe thermometer. First- and second-instar *T. rutilus* were fed small larvae of the mosquito *Culex pipiens*; older *T. rutilus* were fed *Tubifex* obtained from a local pet supply store.

To establish a stock of diapausing larvae, eggs were collected from tree holes in late July and early August

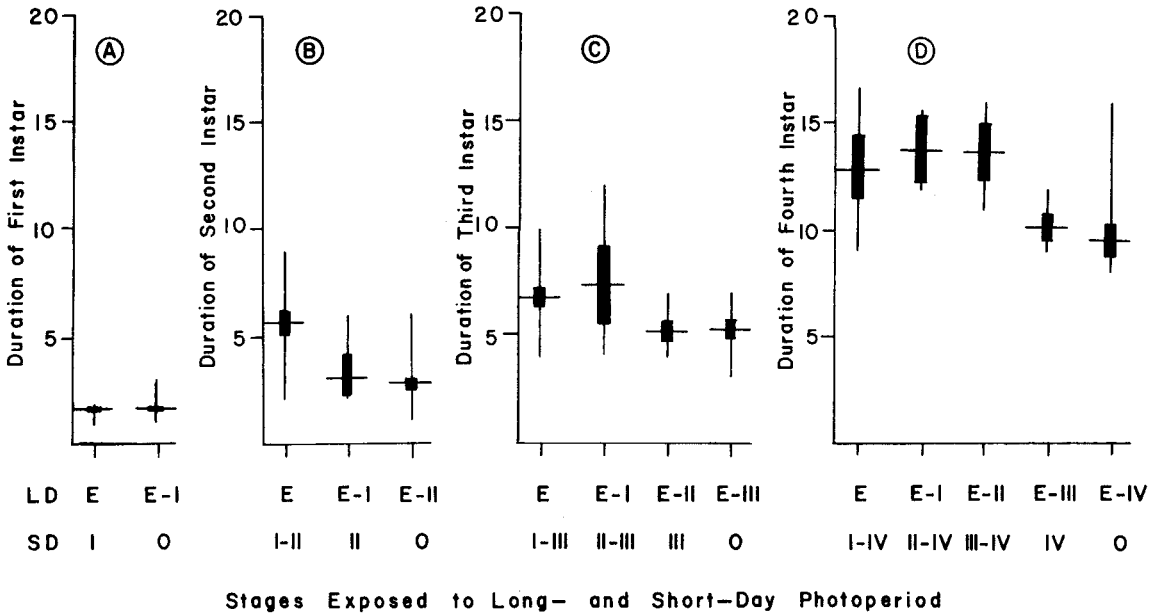


FIG. 1. Effect of photoperiod on the duration of larval instars. Eggs were exposed to long days and either maintained on long-day photoperiod at 28°C or switched to short days at 23°C after the egg stage or at the end of the first, second, third, or fourth instar. LD, long-day photoperiod, light:dark (L:D) = 16:8; SD, short-day photoperiod, L:D = 8:16; E, egg stage; I, II, III, IV, first, second, third, and fourth instar, respectively.

and the resulting larvae were reared at 23 ± 1°C with 8 h of daily illumination. Fourth-instar diapausing larvae were placed in individual compartments, packed in ice, and transported to Eugene, Oregon. In Eugene, they were kept at 4 ± 2°C in an ordinary refrigerator for up to 40 days before the initiation of experiments. In Eugene, experiments were carried out in photoperiod cabinets illuminated by 4-W cool-white fluorescent lamps at a distance of 10–50 cm from the larvae. These cabinets were kept in a constant-temperature room at 25 ± 0.5°C. Food consisted of third-instar larvae of *Drosophila melanogaster* and (or) white worms (*Enchytraeus* sp.).

## Experimental Results

*Initiation of Diapause*

To ascertain when diapause is determined, we exposed *T. rutilus* to long days (L:D = 16:8 at 28°C) during the egg stage ($n$ = 66), egg through the first instar ($n$ = 9), egg through the second instar ($n$ = 14), egg through the third instar ($n$ = 11), and egg through the fourth instar ($n$ = 24). The larvae were then transferred to a short-day regimen (L:D = 8:16 at 23°C) and observed until they had either pupated or had completed 20 days as fourth-instar larvae. The latter were scored as having entered diapause. Table 1 shows that exposure to long-day photoperiod through the third or fourth instar resulted in 100% development. Long days through the second and first instars or through the egg stage resulted in development of proportionally less of the sample, but not even the latter regimen prevented pupation in the entire population.

TABLE 1

Initiation of diapause after exposure of various stages to long (L) or short (S) photophases at 28 and 23°C, respectively

| Egg | I | II | III | IV | % pupation | Sample size |
|---|---|---|---|---|---|---|
| L | L | L | L | L | 100 | 24 |
| L | L | L | L | S | 100 | 11 |
| L | L | L | S | S | 86 | 14 |
| L | L | S | S | S | 44 | 9 |
| L | S | S | S | S | 17 | 66 |

To evaluate the quantitative effect of photoperiod on development, we recorded the duration of each larval stage under the regimens above (Fig. 1). The duration of the first instar was not significantly affected by photoperiod during that stage ($F$ = 2.38; $P > 0.05$). The second instar was significantly longer when both the first and second instars had experienced short-day photoperiod ($F$ = 167; $P < 0.01$; means significantly different at the 1% level after Duncan's multiple range test: $P_{DMRT} < 0.01$); the third instar was significantly longer when the

larvae had experienced short days during at least the second and third instars ($F = 17.6$; $P < 0.01$; $P_{DMRT} < 0.01$); the fourth instar was significantly longer when the larvae had experienced short days during at least the third and fourth instars ($F = 30.4$; $P < 0.01$; $P_{DMRT} < 0.01$). These results show that long days hasten and short days retard the completion of at least the second, third, and fourth instars.

*Termination of Diapause and Postdiapause Morphogenesis*

Two stages of development may be distinguished between the onset of diapause-terminating stimuli during the fourth instar and the larval–pupal molt: the termination of diapause per se and the completion of postdiapause morphogenesis (Bradshaw and Lounibos 1972).

To determine the time required to complete the termination of diapause and postdiapause morphogenesis combined, 16 laboratory-reared, diapausing larvae from Lahaska were exposed to continuous long days (8 each to L:D = 16:8 and 15:9) at 25 ± 0.5°C. The first pupae were observed after 10 days and all 16 larvae had pupated by the 15th day (Fig. 2). To determine the number of long days required for the termination of diapause, individual samples of six laboratory-reared, diapausing larvae from Lahaska were exposed to 0, 2, 4, 6, 8, 10, or 13 long days (L:D = 15:9) at 25 ± 0.5°C. They were then transferred to short days (L:D = 10.5:13.5) at the same temperature for a total experimental time of 20 days. At the end of this time, total percentage pupation was scored and the experiment terminated. Figure 2 shows that 8, 10, and 13 long days stimulated one, two, and five of six larvae, respectively, to terminate diapause. Most or all of the period from the onset of diapause-terminating stimuli until pupation is therefore spent actually terminating diapause and no more than a small portion for the completion of postdiapause morphogenesis.

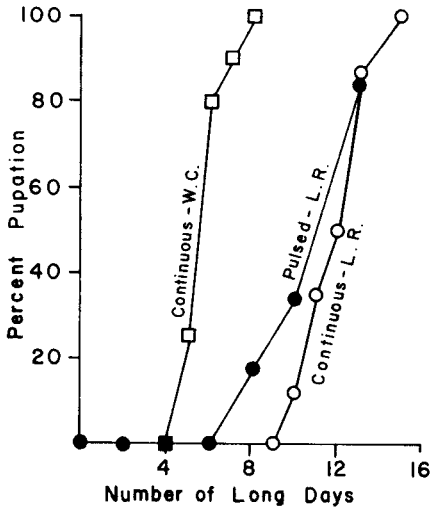


FIG. 2. Percentage of pupation in response to pulsed and continuous long days by larvae reared in the laboratory (L.R.) or collected in the winter (W.C). For continuous long days, larvae were exposed to L:D = 16:8 and 15:9; the figure plots cumulative percentage of pupation over a 16-day period; for pulsed long days, larvae were exposed to 0, 2, 4, 6, 8, 10, or 13 long days (L:D = 15:9) followed by 20, 18, 16, 14, 12, 10, and 7 short days (L:D = 10.5:13.5), respectively; the curve plots the total percentage of pupation after the entire 20-day period.

*Critical Photoperiod*

In the above experiments, either long or short days were used; the experiments below concern the response to long, short, and intermediate day lengths. Fourth-instar, diapausing larvae were obtained from three sources in Lahaska: "laboratory-reared" in a cellar; wild-caught on August 26, 1973; and wild-caught on January 1, 1974.

Groups of 8–16 laboratory-reared larvae each were exposed to 11, 12, 13, 13.5, 14, 15, or 16 h of light per day at 25 ± 1°C. The larvae were fed and observed daily; those which failed to pupate after 50 days or more were scored as maintaining diapause. Figure 3A shows that day lengths of 12 h or less maintained diapause, while those of 13 h or more evoked pupation among all of the larvae within 22 days. A photophase of 13 h maintained diapause in 4 out of 14 larvae and retarded development in at least 4 more. These results indicate that the critical photoperiod of laboratory-reared larvae from Pennsylvania is about 13 h of light per day.

Of 32 fourth-instar larvae captured on August 26, 1973, 23 failed to pupate after 21 short days at 23 ± 1°C. These 23 larvae were presumed to be in diapause and transported to Eugene as explained above. To choose appropriate day lengths, they were not exposed to experimental photoperiods until results were forthcoming from the laboratory-reared larvae (Fig. 3A); consequently, they experienced 50 days of chilling before the initiation of experiments. The results in Fig. 3A also indicated that an experimental duration of 40 days was sufficient to dis-

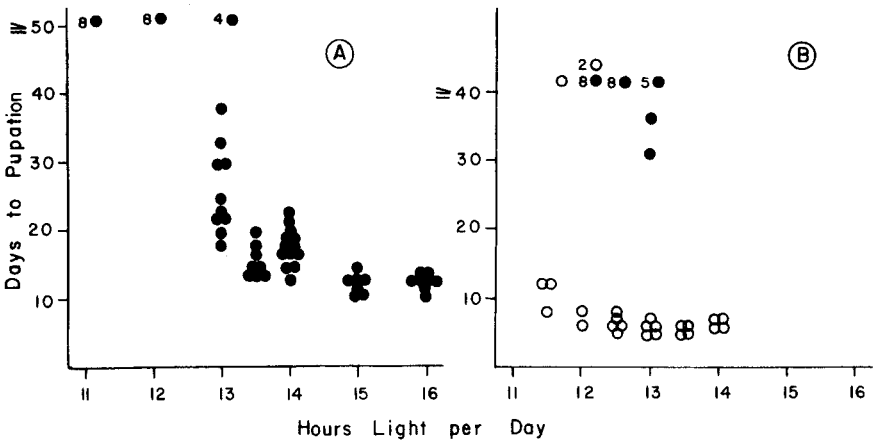


FIG. 3. Duration of the fourth instar in response to various photoperiods at 25°C. A, response of *T. rutilus* raised from eggs to the fourth instar on short days at 23°C in the laboratory; B, response of *T. rutilus* collected as fourth-instar larvae from nature either in late August (●) or in early January (○).

tinguish between diapausing and developing individuals. In the present experiment, larvae which had not pupated after 40 days or more were considered to be maintaining diapause. Figure 3B shows that photophases of 12 or 12.5 h maintained diapause, while a photophase of 13 h elicited development, albeit retarded, in two of seven larvae. These data show that the critical photoperiod of diapausing larvae caught in nature during the late summer was no shorter than that of larvae reared in the laboratory.

On January 1, 1974, we captured 32 fourth-instar larvae at Lahaska; of these, 26 survived the 73 days required for storage and transportation to Eugene. We exposed four or five larvae to each of six regimens ranging from 11.5 to 14 h of light per day at 25 ± 0.5°C. The larvae were fed and observed for 40 days, after which development was scored and the experiment terminated. Figure 3B shows that all of the fourth-instar larvae experiencing a photophase of 12.5 h or more pupated within 9 days. Larvae experiencing shorter photophases either developed within 12 days or maintained diapause. In Fig. 2 the response of these wild-caught larvae to continuous long days (12.5–14 h) is compared with the response of laboratory-reared larvae described earlier. These results show that the time required for the termination of diapause plus the completion of postdiapause morphogenesis among larvae caught in early January is about half the time required for individuals reared in the laboratory.

## Discussion

McCrary (1965) found that the initiation of diapause among *T. rutilus* from North Carolina was affected by both temperature and photoperiod. At low temperatures (19°C), the effect of long days on the first and second instar could be completely reversed by exposure to short days during the third and fourth instars. At higher temperatures (25 or 27°C), continual exposure to short-day photoperiod during the entire larval life failed to prevent development in 10% of the sample. However, even diapause-averting long days at 25 or 27° experienced during the first three instars were reversed by exposure to short-day photoperiods during the fourth instar. The data in Table 1 indicate that embryonic as well as larval photoperiods may be important in the determination of diapause among *T. rutilus* from Pennsylvania. In addition, larvae subjected to long days at 28° through the second instar were irreversibly committed to development, even if both the third and fourth instars subsequently experienced short days at 23°C.

Among *T. rutilus* from Pennsylvania, short days were found to prolong the duration of the second and third instars as well as the fourth (Fig. 1), but only if the previous stage had also experienced short days. By marked contrast, for the determination of diapause, short-day reversal of prior long-day stimulation requires that the fourth instar and the previous two stages encounter short days (Table 1). The mechanisms determining instar duration therefore appear to be distinct from and may be independent of those determining diapause.

Jenner and McCrary (1964) found that the critical photoperiod of *T. rutilus* in North Carolina was between 12 and 13 h of light per day. Diapause among laboratory-reared larvae

from Pennsylvania is firmly maintained by 11 or 12 h of light per day and rapidly terminated by day lengths of 13.5–16 h (Fig. 3A). A photophase of 13 h maintained diapause in some larvae and retarded or promoted rapid development in others. Among diapausing larvae caught in late August (Fig. 3B), photophases of 12 or 12.5 h maintained diapause, while a photophase of 13 h retarded but did not halt development in two out of seven larvae. These observations suggest that the critical photoperiod for the initiation and maintenance of diapause in *T. rutilus* is about 13 h of light per day in the northern part of its range.

The response of larvae collected during the winter contrasts sharply with those caught in the late summer (Fig. 3B). In the former, even day lengths as short as 11 h failed to maintain diapause in all of the larvae. Photophases of 12.5 h or longer evoked rapid development in each larva (Figs. 2, 3B). Chilling and (or) environmental photoperiods have apparently either effected the termination of diapause in certain individuals or depressed the critical photoperiod below 11.5 h. Evidence for the former interpretation stems from the observation that there are no strictly intermediate responses as seen in Fig. 3A; evidence for the latter may be derived from the observation that at 11.5 h of light per day, some larvae exhibited somewhat slower development. The lack of a larger sample size and shorter experimental photophases leaves these alternatives unresolved. McCrary (1965) found that *T. rutilus* from North Carolina, while entering diapause during late August and September, showed progressively rapid development to long-day photoperiods during the fall. However, larvae which were (1) captured and exposed to long days in late November, (2) captured in late November, exposed to short days until March and then to long days, and (3) captured and exposed to long days in March all exhibited mean time to pupation of around 12 days from the onset of long-day photoperiod. Apparently, winter conditions play a more prominent role in diapause termination among *T. rutilus* in the northern part of their range than in North Carolina.

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