Photoperiodic control of development in the pitcher-plant mosquito, *Wyeomyia smithii*

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Wyeomyia smithii diapause in the third larval instar. Long days avert or terminate and short days promote or maintain diapause. Diapause occurs early in the third instar and may be terminated by photoperiodic stimuli without the intervention of chilling or other factors. Fifty percent termination of diapause requires about 3 long days and another 6½ days are consumed in the third instar for postdiapause development. The critical daylength is identical for both the initiation and termination of diapause, 14.75 h of light per day. But, the photoperiodic clock monitoring diapause decisions is several times as accurate during initiation as in termination, reflecting the more drastic environmental consequences of development misdirection in the fall than in the spring. This accuracy is further enhanced by a prolongation of the second instar under short-day conditions. The doubling in the duration of the second instar exhibits the same critical daylength properties as diapause determination.

The third instar is divisible into four distinct developmental periods: prediapause, diapause, termination of diapause, and postdiapause. Methods for quantifying these periods are presented. Similar manipulations could be employed for other diapausing arthropods, regardless of the stage at which dormancy occurs or the cues used in its regulation.

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Les larves de *Wyeomyia smithii* subissent une diapause durant leur troisième stade. Des jours longs empêchent la diapause ou y mettent fin, alors que des jours courts la déclenchent ou la maintiennent. La diapause commence tôt au cours du troisième stade et des stimuli photopériodiques suffisent à y mettre fin, sans l'intervention parallèle d'un refroidissement ou de quelque autre facteur. Trois jours longs amènent la fin de la diapause dans 50% des cas; la croissance de la larve de troisième stade se poursuit encore pendant 6½ jours après la diapause. Qu'il s'agisse de déclenchement ou de cessation de la diapause, la longueur critique de jour est la même, 14.75 h de lumière par jour. Cependant, l'horloge photopériodique qui déclenche la diapause montre beaucoup plus de précision que celle qui la termine, ce qui reflète les conséquences de l'environnement plus graves en automne qu'au printemps d'une mauvaise orientation du développement. Des photopériodes courtes prolongent la durée du deuxième stade larvaire et de cette façon le déclenchement de la diapause se fait tellement plus précis. La longueur critique de jour qui double le deuxième stade larvaire est la même que celle qui déclenche la diapause.

On peut distinguer quatre périodes de développement durant le troisième stade: la prédiapause, la diapause, la cessation de la diapause et la postdiapause. On présente ici des méthodes de quantification de ces périodes. Des méthodes similaires pourraient s'appliquer à d'autres arthropodes devant subir une diapause, quel que soit le stade où se produit la diapause, et quels que soient les facteurs qui la contrôlent.

The bowl of the pitcher-plant, *Sarracenia purpurea*, provides an aquatic habitat for several endemic insects who subsist on organic debris trapped in the pitcher. Larvae of the midge, *Metriocnemus knabi*, and the mosquito, *Wyeomyia smithii*, frequently inhabit this pitcher-plant throughout Canada and northeastern United States. Both species overwinter as larvae in the pitcher. We have chosen to investigate the role of photoperiod in larval diapause and development of *W. smithii* in view of the mosquito's availability, distribution, and ease of rearing which make it particularly tractable for such studies.

Jenner (1951) undertook preliminary experiments whose results showed that the termination of diapause in *W. smithii* is regulated by daylength. Recently, Smith and Brust (1971) have presented more substantial evidence to this effect. Information from Spielman (1969, personal communication) indicated that both the initiation and the termination of larval diapause in *W. smithii* are mediated by daylength. We have confirmed Spielman's report and have given our results in a preliminary communication (Bradshaw 1971). The current paper expands upon certain of these findings and presents an ecological rationale for the various responses of *W. smithii* to photoperiod and for the ontogenetic timing of diapause.

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Materials and Methods

Larvae of *W. smithii* were collected from a pitcher-plant bog near Boston, Massachusetts (42°34′ N; 71°30′ W). They thrived on a 3:1:1 mixture of pulverized guinea pig chow, daphnia, and mosquito larvae, the latter two obtained freeze-dried from Miracle Pet Products, Jersey City, N.J. The adults were fed on cotton soaked in a 1:1 honey–molasses mixture containing a few drops of mold inhibitor (*p*-methyl hydroxybenzoate).

Adults were bred at 25°C and 65–80% relative humidity in covered paper cups suspended over standing tap-water. For oviposition, females gained access to the water via a hole in the bottom of the cup. Eggs were collected every 2 to 3 days and the larvae reared at 21 ± 1½, 23 ± 1½, 25 ± ½, or 30 ± ½°C. The larvae grew fastest at 30°C, but maturation in the fourth instar at lower temperatures produced larger adults containing more eggs. We therefore routinely reared larvae at 25 or 30°C until the fourth instar and then transferred the fourth instar larvae to 21°C. Freshly pupated individuals were returned to 30°C until adult ecdysis.

These techniques have enabled us to rear 15 consecutive generations in the laboratory during the past 2 years. Price (1958) predicted a potential minimum laboratory life cycle of 1.5 months under optimal conditions. We have been able to reduce this to about 30 days but believe that an even shorter generation time might be possible at higher temperatures.

Various lighting sources were employed for photoperiod experiments. These included one 15 W incandescent, one 4 W cool white fluorescent, or two 48 W plus two 96 W cool white fluorescent lamps. The lamp–subject distance ranged from 5 to 50 cm for the former two lamps and from 1 to 3 m for the latter. Within a given experiment only one of these light sources was used. Both the experimental and control larvae were always exposed to the same source at similar distances.

Experimental Results

(1) Effects of Photoperiod on Growth and Molting

A collection of *W. smithii* on December 7, 1969 revealed only third instar larvae. We exposed two samples of these larvae to two different light regimens at 25 ± ½°C and observed changes in

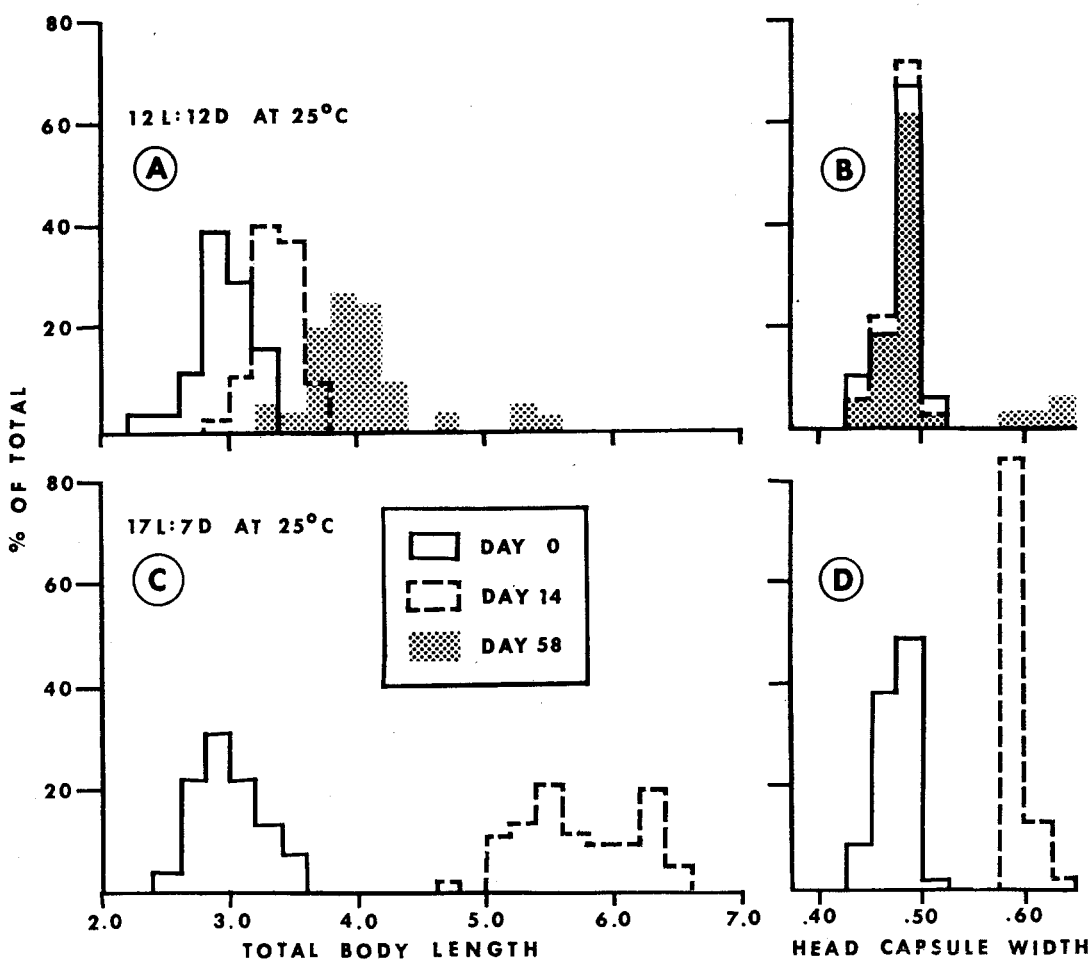

FIG. 1. Growth and development of overwintering third instar larvae in response to short- (A and B) and long-day (C and D) photoperiods. Total body length reflects the growth of soft parts within an instar; head capsules are rigid and their width indicates instar number (III or IV). Measurements are in millimeters.

total body length and in head capsule width. We found the former to be a quantitative measure of overall growth and the latter to be a reliable index of the larval instar. One group of 55 larvae received 12 h of daily illumination; the other group of 35 larvae received 17 h. Body length and head capsule width were measured with an ocular micrometer at the initiation of the experiment (day 0) and again on days 14 and 58.

Faster growth and higher frequency of molting were observed among larvae exposed to the longer daylength. By the 14th day, all these larvae had molted to the fourth instar (Fig. 1D) and had doubled their body length (Fig. 1C). By contrast, none of the larvae exposed to the shorter daylength had molted (Fig. 1B) and their body length had increased by only 15% (Fig. 1A). After 58 days, only 11% of the short-day larvae had molted; but, their body length increased by 50% (Fig. 1A). These results show that photoperiod controls development of overwintering *W. smithii*, but even developmentally arrested larvae may assimilate food and use it for limited growth.

(2) Effects of Photoperiod on the Onset of Dormancy

To examine the role of photoperiod in diapause initiation, we exposed cages of adults and their subsequent progeny at 25 ± ½°C to either 12 or 17 h of light per day and followed the course of egg hatching, larval molting, and adult ecdysis in these two groups.

As seen in Fig. 2, the duration of neither the egg stage nor the first instar was affected by daylength. Yet, larvae exposed to 12 h of daily illumination spend twice as long in the second instar as those exposed to 17 h of light per day. Furthermore, all the third instar larvae

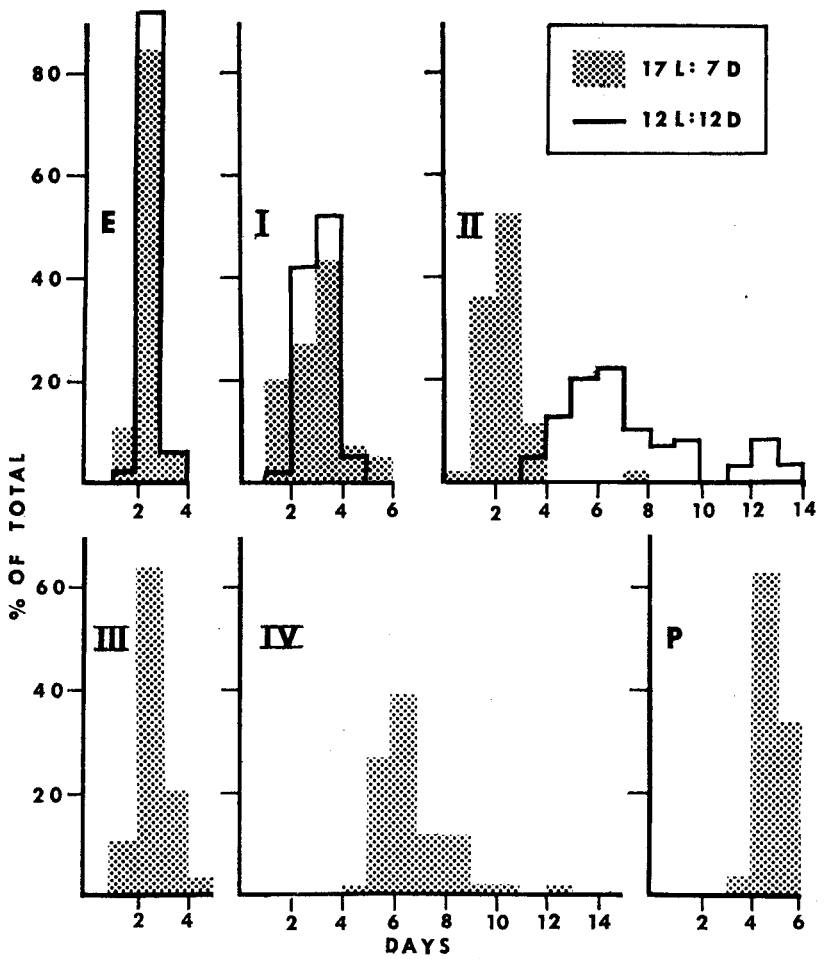

FIG. 2. Number of days consumed in various stages of development in response to long- and short-day regimens. E, egg stage; I–IV, instar number; P, pupal stage. Short days at 25°C prolong the third instar for several months.

exposed to the long day regimen molted to the fourth instar within 5 days, whereas larvae exposed to the short daylength remained in the third instar for 30 days and more without molting. It would therefore appear that the onset of dormancy as well as the duration of the second instar is mediated by environmental light.

(3) Developmental Response to Continuous Long Days

To determine whether diapause occurs early or late in the third instar, we performed the following manipulations. Larvae were reared at 23 ± 1½°C and 12 h of light per day up to the third instar. We then subdivided them into two groups: one group was transferred on day 0 of the third instar to 17 h of light per day at 23 ± 1½°C; the other group remained on the original short-day regimen as diapausing third instar larvae for two additional weeks and was then transferred to the longer photoperiod. Both groups were observed for 20 days after the transfer to long daylength. In each case the total sample size consisted of the number of larval survivors plus the number that had developed. Figure 3 (middle and right hand curves) shows that the response to continuous long-day photoperiod was similar for both groups of larvae, indicating that diapause is already established at the onset of the third instar.

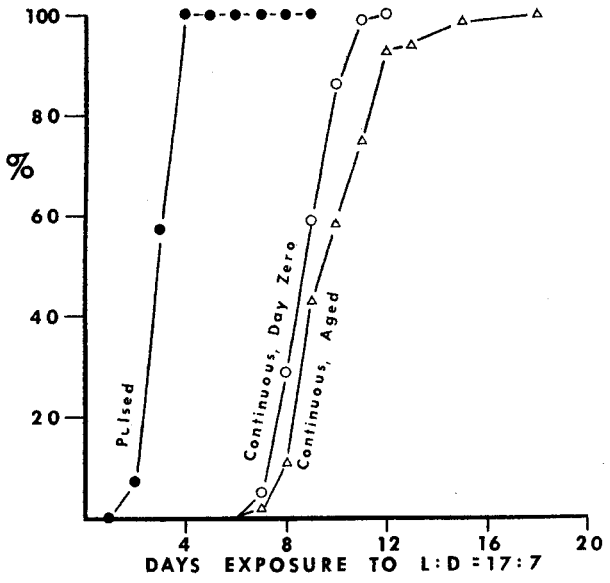

FIG. 3. Percent development of short-day reared larvae in response to long-day photoperiod in the third instar. Exposure to the continuous long-day regimen began either on the day of molting to the third instar (day 0) or after the diapausing third instar larvae had received 2 weeks of short-day photoperiod (aged). Pulsed larvae were reared and aged as above but exposure was restricted to 1, 2, . . . or 9 long days followed by short-day photoperiod.

(4) Development in Response to Pulsed Long Days

To determine how many long days are required to terminate diapause, we used a long day – short day pulse–chase experiment. Larvae were reared at 23 ± 1½°C on a 12 L : 12 D regimen until they had developed into diapausing third instar larvae. They were maintained on this daily schedule for 2 to 3 weeks before initiation of the experiment. On day 0 we placed nine groups of 15 larvae each on a 17 L : 7 D regimen at 23 ± 1½°C. One group was returned to the shorter daylength on day 1, another on day 2, and so on through day 9. All nine samples were observed until day 19 when development was scored and the experiment terminated. Figure 3 (left hand curve) graphs percent development as a function of days of exposure to the long-day photoperiod. All larvae required two or more long days to terminate diapause. Over half the sample terminated diapause after three long days, and 100% after four or more long days. It is therefore apparent that diapause is readily terminated in response to long-day photoperiod; moreover, the influence of the long days is not reversed by subsequent exposure to the short-day regimen.

(5) Precision of the Photoperiodic Clock

To determine the precision of the photoperiodic mechanism that controls the onset and termination of diapause, groups of first instar larvae, immediately after hatching, were placed at 21 ± 1½°C under photoperiods ranging from 12 to 17 h of light per day. Parallel experiments were carried out on diapausing third instar larvae. Termination of diapause was scored after 25 days by assessing the number of larvae having molted to the fourth instar; initiation of diapause was scored for each larva 15 days after the beginning of the third instar.

The results are summarized in Fig. 4A. The critical daylength (50% response) for both the initiation and termination of diapause is about 14.75 h of light per day. The threshold and saturating daylengths are not immediately apparent in Fig. 4A. First, 100% development is not always indicated by the end of the experiment; secondly, some larvae terminated diapause de-

spite the restraining influence of shorter days. Fortunately, more quantitative aspects of development in *W. smithii* permit a more accurate assessment of the threshold and saturating photoperiods.

Among the samples in which 50 or more percent of the population responded to the test daylength by terminating diapause, photoperiod had a marked influence on the time required for the median number of the sample to molt to the fourth instar. Hereafter this parameter will be abbreviated IV_{50}. As seen in Fig. 4B, IV_{50} was highest for the critical daylength of 14.75 h and declined with increasing photophase. A minimum IV_{50} is observed at 16 h of daily illumination; the saturating daylength thus lies between 15.5 and 16.0 h, or, by interpolation, is 15.75 h. If the precision of the photoperiodic mechanism controlling the termination of diapause may be assumed to be symmetric about the critical daylength, the above results would indicate that such a mechanism is accurate to approximately ± 1 h or about 4% of the daily cycle.

The duration of the second instar (Fig. 2) is a continuous variable. It therefore offers a means of quantifying the precision of the mechanism which provides the go, no-go signal to a larva which must either initiate or avoid diapause in the next instar. For convenience, we determined the number of days from egg hatching to the day of molting to the third instar. Figure 4C shows that at 21 ± 1½°C the critical daylength for the duration of the first two instars is identical with that for the initiation of diapause (Fig. 4A). Moreover, the difference between the longest "short day," 14½ h, and the shortest "long day," 15 h, is only ½ h. The photoperiodic mechanism which mediates the onset of diapause is therefore accurate to approximately ± ¼ h or about 1% of the daily cycle.

Discussion

Wyeomyia smithii overwinters in the penultimate (third) larval instar. Although Smith and Brust (1971) also noted this phenomenon, they did not speculate on its significance. We propose that this ontogenetic timing, peculiar to *W. smithii*, relates to trophic considerations. Under long-day conditions, the first, second, and third larval instars are all of 3–4 days in duration; yet, the fourth instar is about twice as long as any of them, lasting about 7 days (Fig. 2). *Wyeomyia smithii* thus develops rapidly up to the fourth instar, then abates its rate of maturation. Presumably, since the mosquitoes are non-biting as adults, they use this instar to accumulate sufficient protein and lipid to insure adequate yolk in the future egg. Postponing the major trophic instar until the spring therefore makes excellent sense in terms of ecological energetics.

Wyeomyia smithii relies upon photoperiodic cues for controlling not only a developmental arrest in the third instar (Fig. 4A) but the duration of the second instar as well (Figs. 2, 4C).

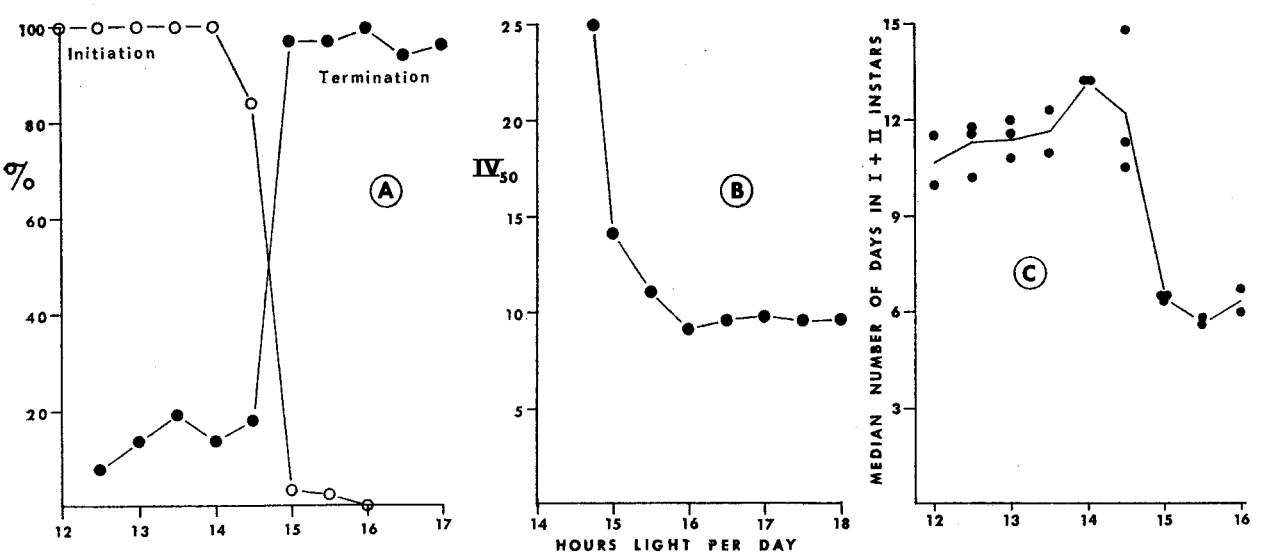

FIG. 4. A, percent initiation and termination of diapause as a function of photoperiod. B, number of days for 50% of the diapausing third instar larvae to molt to the fourth instar (IV_{50}) as a function of photoperiod. C, duration of the combined first two instars as a function of photoperiod.

Long days promote rapid development in the second instar and the aversion of diapause; short days prolong development in potentially diapausing larvae. The reason for this prolongation may reflect a changing larval habitat. The overwintering larvae live in a very different environment from that experienced during the rest of the year. They must endure several months encapsulation in a block of ice. It is therefore no surprise that the prediapausing larvae spend extra time in the second instar when they presumably make physiological adjustments before experiencing such extreme conditions.

The extended second instar of prediapausing *W. smithii* has a significant ecological as well as physiological rationale: confirmation of the seasonal cues offered by changing environmental photoperiod. While various geophysical phenomena may occlude an otherwise long day, none is capable of prolonging a short day. Cloud cover at daylengths close to the critical daylength could cause potential misdirection of development but the lengthened second instar in response to short-day photoperiod, whether real or apparent, permits a truer assessment of the environment. In addition, the prolonged second instar may permit the photoperiodic mechanism to ascertain whether daily illumination is increasing or decreasing. While such a mechanism may occur in other insects (Tauber and Tauber 1970), no investigation has yet been made of its existence in *W. smithii.*

Long days appear more important than short days in determining development in the moths *Antheraea pernyi* (Tanaka 1950*a*, *b*) and *Acronycta rumicis* (Danilevskii 1965, p. 73), in the aphid *Megoura viciae* (Lees 1959), and in the fly, *Sarcophaga argyrostoma* (Saunders 1971), although the reverse is apparently true for the butterfly, *Pieris brassicae* (Danilevskii 1965, p. 74). That *W. smithii* relies heavily on long days, at least for the termination of diapause, is evident in Fig. 3 (left hand curve) where only a few long days are required to commit the larvae to development, regardless of subsequent photoperiod.

The greater accuracy in the photoperiodic mechanism mediating the initiation of diapause (Figs. 4B, 4C) further enhances the reliability of the autumnal decision to enter diapause. The urgency of correctly interpreting environmental cues is proportional to the environmental risk confronting an organism. In the fall, a continuation of development beyond the third instar commits *W. smithii* to complete one extra generation before the next opportunity to diapause. In the spring, the precocious termination of diapause still leaves an individual with two larval and one pupal stages before it must eventually expose itself to the potentially hostile aerial environment. The consequences of misinterpreting environmental cues are therefore more severe in the fall than in the spring.

The findings summarized in Fig. 3 illustrate more than the position and depth of diapause in the third instar. The actual timing of other events surrounding diapause can also be calculated from these data. The third instar in *W. smithii* may be divided into four distinct periods: prediapause, diapause, termination of diapause, and postdiapause. Since there is no appreciable difference in the median response times of larvae exposed to long-day photoperiod immediately upon molting to the third instar (Fig. 3, middle curve) and of those "aged" in diapause for 2 weeks (Fig. 3, right hand curve), the prediapause period is virtually absent and little, if any, development takes place after the II–III molt. About three long days are required for the median number of larvae to terminate diapause (Fig. 3, left hand curve) although they do not molt to the fourth instar for another $6\frac{1}{2}$ days. This latter value constitutes a measurement of the postdiapause period. The postdiapause period is thus about twice as long as the entire third instar of larvae reared continuously on a long-day regimen (Fig. 2). It is unlikely that this substantial difference is due (1) to the 2°C change in experimental temperature or (2) to a necessity for postdiapause feeding since the larvae readily eat and assimilate food during diapause (Fig. 1). It is more likely that this long postdiapause period reflects physiological accommodation to resumed development in a climate unlike that which the overwintering larvae normally experience.

The form of analysis used to quantify the duration of the prediapause, diapause, termination of diapause, and postdiapause periods is not restricted to *W. smithii.* These same periods and basic methods of defining them are applicable to any other arthropod, regardless of the stage at which dormancy occurs or the cues involved in its regulation.

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