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Photoperiodism and the Photic Environment of the Pitcher-Plant Mosquito, *Wyeomyia smithii*

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Summary. *Wyeomyia smithii* Coq. (Diptera: Culicidae) overwinters as a larva in a state of diapause which is initiated, maintained, and terminated by photoperiod. Both in the dawn and in the dusk, diapausing larvae are photoperiodically most sensitive to blue light (390–450 nm) with a shoulder in response in the blue-green and green (480–540 nm) region of the spectrum. The saturation curves for response to blue light in the dusk has a steeper slope than for response to blue-green and green light in the dusk, suggesting two distinct pigments or pigment complexes underly photoperiodic response in *W. smithii*.

The photic environment of *W. smithii* during twilight is rich in yellow-green light but sufficient light is available at 390–540 nm to trigger photoperiodic response early during morning civil twilight and to sustain response until late in evening civil twilight. Comparison of action spectra with spectra of available light indicates that the zenith angles of the sun which would result in 50% response are 95°48′ and 94°52′ in the dawn and dusk, respectively. Using these zenith angles to approximate daylength in nature provides a reasonable prediction of development in the field.

The flux density of photons necessary to elicit 50% development a 454 nm is about 9×10^7 photons/cm^2 s in the dawn and 3×10^8 photons/cm^2 s in the dusk. This high degree of sensitivity enables *W. smithii* to cue to the rapidly changing light intensity which occurs around the nautical-civil twilight transition. At the same time, the chromophore is not likely to be stimulated by the light of the full moon.

Despite the abundance of literature on photoperiodism among insects, the astronomic units which define a photoperiodic day in nature remain elusive. One method, that of exposing insects in the laboratory to an instantaneous dawn or dusk followed daily by exposure in nature to dusk or dawn twilight, respectively, has been used by Beach and Craig (1979). A second method could define a photoperiodic day by comparing the action spectra for photoperiodic response with the spectra of light available in the organism's natural habitat. To make this comparison, one needs two action spectra: one for the dark-light (dawn), the other for the light-dark (dusk) transition (Bradshaw, 1974). Many of the existing action spectra have not taken intensity into account (Kogure, 1933; Dickson, 1949; Geysptiz, 1957; Bünning and Joerrens, 1960; Williams et al., 1965; Claret, 1972, 1973a, b), have exposed insects to different wavelengths of light at only one intensity (Müller, 1964; Harris et al., 1969; Mangum et al., 1968; Pittendrigh et al., 1970; de Wilde and Bonga, 1958), have been concerned with light breaks during the dark period (Lees, 1966, 1971; Barker, 1964), or have considered but the dusk-terminal end of day (Hayes, 1971; Norris et al., 1969). Only two studies have considered energy or flux-density compensated action spectra for both the dawn and dusk transition (Bradshaw, 1972, 1974; Saunders, 1975). While both of the latter investigators speculated on the astronomic units which might define a day, neither actually measured light intensities in an environment normally inhabited by the subject organism. Indeed, defining the "typical" photic environment of a shallow pond midge (Bradshaw, 1972, 1974) or an adult parasitic wasp (Saunders, 1975) might well prove difficult. A better organism is the mosquito, *Wyeomyia smithii* Coq., which confines its pre-adult development to the container leaves of the purple pitcher-plant, *Sarracenia purpurea*. In the northern part of its range, *W. smithii* overwinters as a diapausing third instar. The initiation, maintenance, and termination of this diapause are mediated by photoperiod (Smith and Brust, 1971; Bradshaw and Lounibos, 1972; Evans and Brust, 1972), *W. smithii* being a typical long-day insect (Beck, 1968). Because of the strong influence of photoperiod on larval development and the specific, captive nature of its habitat, *W. smithii* is particularly tractable for a comparison between the action spectra for photoperiodic response and its photic environment.

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Materials and Methods

Larvae of *Wyeomyia smithii* used for the determination of action spectra originated from Moores Meadows, New Jersey (39°48′N latitude, 74°35′W longitude). They were maintained in the laboratory and diapause initiated by short days at 21° C (Bradshaw and Lounibos, 1972). Accurate phenological observations are available for a population of the same physiological race (Bradshaw and Lounibos, 1977) from near Forge Village, Massachusetts (42°34′N, 71°30′W) (Lounibos and Bradshaw, 1975). Larvae from the latter locality were used to determine critical photoperiods for the initiation and termination of diapause. Larvae from Forge Village were captured as diapausing third instars on April 1, 1974, and transported by air to Eugene, Oregon, within 48 h of capture.

Critical Daylength

To determine the critical daylength for the termination of diapause, aliquots of 15–20 larvae were exposed to a variety of daylengths at 16.5, 21, and 25° C. They were fed and observed daily

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for 20 days at 25° C, 25 days at 21° C, or 40 days at 16.5° C. After this time, development was scored by counting the number of fourth instars and the experiments terminated. To determine the critical photoperiod for the initiation of diapause, the F_1 generation resulting from development in the above experiment were reared at 21 and 25° C. Twenty to thirty freshly hatched larvae were placed on 12–15 h of light per day in half hour increments. They were fed and observed daily until each individual had either molted to the fourth instar (non-diapause) or had spent 20 days at 25° C or 25 days at 21° C as a third instar (diapause). The critical photoperiod was calculated as the number of hours of light per day required to evoke 50% development from the third to the fourth instar and initiate or maintain 50% diapause. Since exactly 50% development seldom resulted at any photoperiod, most critical photoperiods represent interpolated values.

Action Spectra

Action spectra were determined using the techniques of Bradshaw (1972), except that the white light source consisted of a single 4 Watt, cool-white fluorescent lamp at a distance of 5–10 cm. Light intensities were measured with a Gamma 2400 quantum photometer. The Gamma 2400 photometer was calibrated by comparing readings made simultaneously with a Gamma 3000 R spectroradiometer (Munz and McFarland, 1973) of an Osram 70259 6 V, 15 W, lamp at a distance of 1.08 m. The action spectrum cabinet consisted of 12 chambers, 10 with monochromatic light, one long-day control, and one short-day control. For the short-day control, a 5×5 cm coverglass wrapped with black electrical tape intervened between the spectral lamps and the animals; in the long-day control, the coverglass was clear so that the larvae received unattenuated white light from the spectral lamp (General Electric, 120 VAC, 100 W, colorimeter lamp). Diapausing third instars were placed in a 60×20 or 100×20 mm Falcon polypropylene culture dish which was filled to a depth of about 12 mm, with no water extending above the lower portion of the lid. The culture dishes were placed so that the beam of monochromatic light struck them horizontally. Larvae were exposed to a 12.75 h white-light short day which was either preceded (dawn) or followed (dusk) by a 2.25 h pulse of monochromatic light. Thus, if larvae "saw" the monochromatic light, the otherwise short day would be extended into a long day and development would result. Larvae received 5 or 6 days of white plus monochromatic light at dawn or dusk followed by 5 or 6 white-light short days at 22 ± 1° C. The durations of exposure to monochromatic light and subsequent short days were adjusted so as to obtain maximum differentiation between long- and short-day responses. After 10–12 days total experimental time, development was scored and the experiment terminated. Percent development in response to the monochromatic light, $\%\lambda$, was then standardized against the percentages responding to the long- and short-day controls, %LDC and %SDC, respectively:

$$\text{relative \% response} = 100(\%\lambda - \%\text{SDC})/(\%\text{LDC} - \%\text{SDC}).$$

Relative responses ranged from -17 to $+117\%$; consequently, the upper asymptote was set at 120% and the lower asymptote at -20%; approximately sigmoid dose-response curves were then transformed to linear:

$$\text{Transformed \%} = \ln((120 - \text{relative \% response})/(\text{relative \% response} + 20)).$$

Transformed % response was then regressed on the $\log_{10}$ of flux density (photons/cm^2 s) to which the larvae had been exposed. Backtransformation,

$$\text{Backtransformed \%} = (120 + 20\exp(\alpha + \beta\log(\text{photons})))/(1 + \exp(\alpha + \beta\log(\text{photons}))),$$

where α and β are the constants found by regression, yielding the dose-response curves from which the 50% intercepts were read. Eleven to 17 replicas of 20 larvae each were exposed to various flux densities at each wavelength. Altogether, the action spectra are based on the developmental responses of over 6000 larvae.

The Photic Environment

To measure light available to *W. smithii* during twilight in nature, the fiber optic probe of the Gamma 2400 radiometer was used to record the light in a green leaf of *S. purpurea*. The leaf was placed in the middle of a 0.12 ha field with its orifice pointed towards the zenith. The bottom of the leaf was cut off, leaving a 5 mm opening. The probe was alternately inserted into this opening or pointed directly at the zenith. To calculate percent transmission of the leaf, the flux density of photons of zenith light at each wavelength was plotted as a function of time after sunset. Since the readings in the leaf were made alternately with the zenith readings, interpolated zenith flux densities were compared with direct readings in the leaf at a given time. This method included scattered light entering the orifice of the leaf as well as light transmitted through it; hence, the transmission readings obtained represent a more accurate picture of light available to *W. smithii* than could be obtained from the transmission spectrum of a piece of leaf in a spectrophotometer.

Results and Discussion

Critical Daylengths

Percent development among larvae from Forge Village increased consistently with photoperiod at all temperatures (Table 1). For the termination of diapause among larvae captured in the field on April 1, the critical photoperiod was longer at higher temperatures than at lower ones, the critical photoperiod at 25.0° C,

Table 1. Percentage of development (non-diapause) in response to various photoperiods at 16.5, 21, and 25° C for the initiation (init) and termination (term) of diapause among *Wyeomyia smithii* from Forge Village

		Hours light per day							Critical photoperiod
		12.0	12.5	13.0	13.5	14.0	14.5	15.0	
init	25° C	0	0	0	0	24	33	100	14.63
	21° C	0	0	0	0	0	17	97	14.71
term	25° C	–	–	7	17	35	93	–	14.13
	21° C	–	0	7	20	64	100	–	13.84
	16.5° C	–	22	50	100	–	–	–	13.00

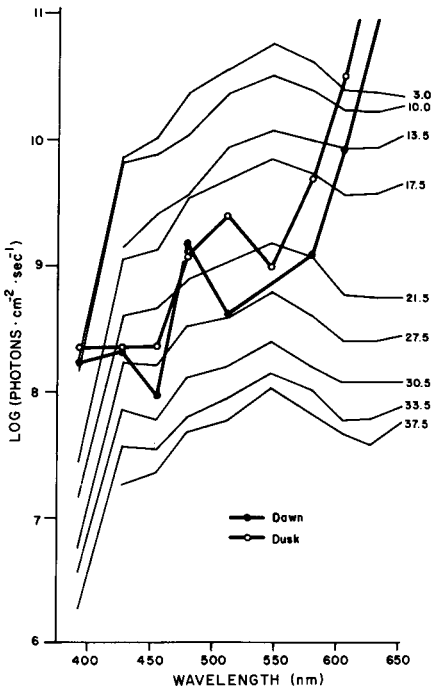


Fig. 1. Action spectra for photoperiodic response and light available in a pitcher-plant leaf during twilight. Pulses of monochromatic light were provided immediately prior to (Dawn) or following (Dusk) an otherwise short, white-light day. The heavy lines plot the flux density of photons necessary to elicit 50% long-day response; narrow lines plot the flux density of photons present at various times after sunset (the numbers to the right of each line give the time in minutes after local sunset)

14.13 h, being an hour longer than that at 16.5° C, 13.00 h. For the initiation of diapause among laboratory reared larvae the opposite situation occurred: the critical photoperiod was longer at 21° C than at 25° C. At both 21 and 25° C, the critical photoperiods were longer for the initiation than for the termination of diapause. Bradshaw and Lounibos (1972) found that the critical photoperiod for the initiation of diapause among larvae reared in the laboratory was identical to that for its maintenance among larvae caught in nature during the fall. The present disparity between the initiation and termination of diapause suggests that environmental factors during the winter and early spring are affecting the responsiveness of the larvae. In several insects, extended chilling may affect both the depth of diapause and the critical photoperiod maintaining it (Bradshaw, 1974; Tauber and Tauber, 1975, 1976; Williams and Adkisson, 1964; Vinogradova, 1975; Bradshaw and Holzapfel, 1977). Chilling may serve a similar role in *W. smithii*. In addition to the effects of winter and spring weather, the photoperiodic clock appears to overcompensate for temperature and the critical photoperiod is shorter at 21 or 25° C than at 16.5° C (Table 1). Environmental effects on the overwintering larvae and lower spring than fall temperatures should result in larvae terminating diapause in nature at considerably shorter daylengths than required for the initiation of dormancy.

Action Spectra and the Photic Environment

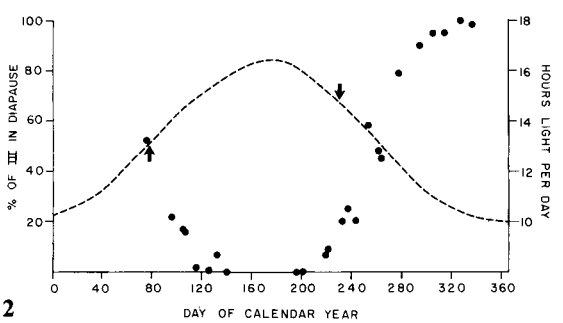
Figure 1 shows the action spectra for photoperiodic maintenance of diapause superimposed on light available during twilight. During the dawn and during the dusk, larvae were most responsive to light in the blue portion of the spectrum, 393–454 nm. Responsiveness declined with wavelength but there was a distinct shoulder in the blue-green and green at 478–547 nm. Flux densities in excess of 10^{12} photons/cm^2 s were required to elicit 50% standard response at 632 or 654 nm. At all wavelengths, larvae were slightly more sensitive to light in the dawn than in the dusk. Light available in the leaves of *S. purpurea* declined about three orders of magnitude during the first half hour of twilight (Fig. 1). Throughout twilight, the highest flux density of available light occurred at 540 nm, declining towards 600–630 nm, and rising slightly thereafter.

Zenith Angles Defining a Photoperiodic Day

The action spectra for photoperiodic response combined with the spectra of light available within the leaves of *S. purpurea* provide the means for estimating the astronomic units (zenith angle of the sun) which comprise a photoperiodic day for *W. smithii*. The spectra of available light in Fig. 1 were taken during evening twilight; light available in a leaf during morning twilight is assumed to be the same as during evening twilight. In an unshaded leaf, 50% response would be elicited about 28 min before sunrise at 450 nm and continue until about 25 min after sunset. Because the spectral distribution in available light corresponds to the general shape of the photoperiodic action spectra, a 50% response will be elicited and sustained by light in the 480–540 nm region of the spectrum within 5 min of excitation at 450 nm. Since the duration of twilight is not constant from one latitude to another or between different times of the year, it is important to convert these twilight times to the sun's zenith angle upon which illumination at twilight is directly dependent (Rozenberg, 1966; Nielsen, 1963). The dawn and dusk zenith angles corresponding to 28 min before sunrise and 25 min after sunset at 44°N latitude on September 26, in Eugene, are 95°48′ and 94°52′, respectively. Thus, *W. smithii* should experience a symmetric day which would begin shortly after the onset of civil twilight in the dawn and continue until late civil twilight in the dusk. This symmetric day contrasts with that estimated for another mosquito (Beach and Craig, 1979), a midge (Bradshaw, 1972, 1974), and for a parasitic wasp (Saunders, 1975) all of whose days were estimated to be asymmetric, beginning at about the onset of civil twilight and ending about sunset.

Development in Nature

The expected dates of development for *W. smithii* at Forge Village, Massachusetts, can now be calculted using the above zenith angles and the critical photoperiod values in Table 1. Figure 2 shows the number of hours of light in a photoperiodic day and the percentages of development observed during 1972 and 1973 by Lounibos and Bradshaw (1975). The upward pointing arrow in Fig. 2 indicates that the critical photoperiod for the termination of diapause at 16.5° C should occur on March 20; the downward pointing arrow shows the critical photoperiod for the initiation of diapause at 21–25° C which should occur on August 12. Since there will be a lag time between the imposition of diapause-terminating stimuli and the actual termination of diapause, 50% termination of diapause should not be observed until some time just after the upwards pointing arrow. In 1972, the first sample was



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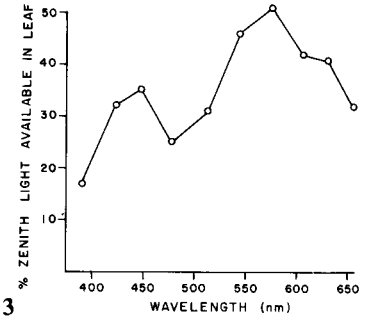

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Fig. 2. Proportion of a natural population of *W. smithii* in third instar diapause (after Lounibos and Bradshaw, 1975) and hours of light defining a photoperiodic day in nature. The upwards and downwards pointing arrows indicate the critical photoperiods for diapause termination at 16.5° C and initiation at 21–25° C, respectively

Fig. 3. Percentage of zenith light available inside a pitcher-plant leaf 16 min after sunset

taken on April 16, when 17% of the larvae were in diapause. On March 16, 1973, exactly 50% of the third instars remained in diapause and percentage diapause declined in subsequent samples. The data in Table 1 show that short days at 25° C, the temperature used by Lounibos and Bradshaw (1975), maintained diapause in 100% of the third instars caught on April 1, 1974. Hence, 50% termination of diapause at Forge Village may occur as early as March 16 or be delayed until after April 1.

The accuracy of the prediction for the initiation of diapause is harder to assess. Since the diapausing status of *W. smithii* is determined in the first or very early second instar (Evans and Brust, 1972), the larvae actually in the third instar on August 12, as well as many in the second instar, will have experienced sufficiently long days so that diapause will be averted. Consequently, it is those larvae in the first or early second instar which will be affected on August 12. For some time after this date, the population of third instars will be mixed as to their diapausing status. As non-diapausers develop and diapausing larvae accumulate, the percentage of dormant thirds should rise. In fact, up to 10% of the third instar larvae are in diapause on or before August 12 (Fig. 2) but percentage does rise steadily thereafter. Even without actually recording temperatures in nature, the critical photoperiods in nature deduced from the action spectra and light availability in pitcher-plant leaves provide reasonable predictions of diapause in nature.

Comparison of W. smithii with other Insects

The spectra in Fig. 1 do not permit speculation as to the underlying chromophore. However, the slopes of the dose-response curves at 390–450 nm were steeper than those at 480–540 nm in the dusk (analysis of covariance: $F_{1,87}=15.51$; $P<0.005$), but not in the dawn (analysis of covariance: $F_{1,78}=2.27$; $P>0.10$). The shoulder at 480–540 nm as well as the difference in the slopes of the saturation curves at dusk between the 390–450 nm peak and this shoulder do suggest that there may be two pigments involved. Lees (1971) described a two-pigment system in the vetch aphid, *Megoura viciae*. Instead of dawn and dusk pulses, Lees used light breaks during an otherwise long night and showed that greater energies were required to evoke 50% development in the late than in the early night. Unlike the dawn and dusk responses of *W. smithii*, there was a marked change in the spectra. Light pulses in the early night were most effective at 450–470 nm and, while maximum sensitivity remained at 450–470 nm in the late night, there was also a considerable increase in the effectiveness of longer wavelengths from 500–600 nm.

The above discussion suggests that different insects use different receptor pigment systems, in sharp contrast to the relative uniformity illustrated by the phytochrome system among higher plants (Mitrakos and Shropshire, 1972; Galston, 1974; Hendricks and Borthwick, 1976). Of the available spectra where both energy or flux density and wavelength are varied to give a standard response, the response of *W. smithii* is qualitatively most similar to that of the codling moth, *Laspeyresia pomonella*, during dusk (Hayes, 1971; Norris et al., 1969). In the latter case, no dawn spectra were determined and during dusk, *W. smithii* exhibits approximately a 20–25 fold greater sensitivity. Both the phantom midge, *Chaoborus americanus* (Bradshaw, 1972, 1974) and the parasitic wasp, *Nassonia vitripennis* (Saunders, 1975), show greatest sensitivity further towards the red end of the spectrum at around 540 nm and 550–585 nm, respectively. Not only do these differences in spectral sensitivity apply among orders of insects, but within them as well. Hayes (1971) and Norris et al. (1969), showed very different spectra for two Lepidoptera, *Antheraea pernyi* and *L. pomonella*. Likewise, the action spectra for *W. smithii* (Fig. 1) are distinct from those of *C. americanus* (Bradshaw, 1972, 1974), another nematocerous Dipteran. The disparity between the action spectra for photoperiodic response of two such closely related species indicates a lack of conservatism of the underlying chromophore and suggests that similarities which are found between more divergent groups such as *W. smithii* and *L. pomonella* may be superficial or the result of convergent evolution.

Effect of Moonlight

The great sensitivity of *Wyeomyia* to light raises the question as to the effect of moonlight on its photoperiodic system. Bünning (1971) and Saunders (1975) have addressed this problem in plants and an insect, respectively. Both considered light in terms of lux rather than regarding the spectral distribution of moonlight vis á vis their systems. At 454 nm, the flux density necessary to elicit 50% development in *W. smithii* is about 9×10^7 photons/cm^2 s in the dawn and 3×10^8 photons/cm^2 s in the dusk. Munz and McFarland (1973) found that moonlight on a clear night consisted mainly of red light and at 400–500 nm emitted no more than 1×10^8 photons/cm^2 s nm. The sensitive chromophore of *W. smithii*, were it exposed directly to this light, might be subjected to some stimulation by moonlight. *Wyeomyia*, however, lives

within the confines of the pitcher-plant leaf which transmits only about 1/3 of incident light at 420–450 nm (Fig. 3). The transmissions in Fig. 3 are for a green leaf fully exposed to the open sky. Normally, the leaves of *S. purpurea* on the open bog mat or in exposed pine savannahs are moderately to heavily anthocyanic and transmit even less light during twilight. The lightest green leaves are found on plants beneath the heath surrounding bogs or in savannahs where the plants are heavily shaded by wire-grass. Thus, although the green leaves of *S. purpurea* transmit more incident light, they tend to occur where less light is available.

There is a basic conflict in the selective forces determining the sensitivity of the photoperiodic clock to light. On the one hand, the clock would be least affected by varying weather conditions if the underlying chromophore was stimulated during late civil twilight when light intensity is changing at its maximum rate (Rozenberg, 1963; Bünning, 1971). On the other hand, too great a sensitivity to light would subject the clock to perturbation by the light of the full moon. The sensitivity of the *Wyeomyia* photoperiodic clock may thus represent an adaptive compromise to these conflicting forces of selection.

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