

Circadian Rhythmicity and Photoperiodism in the Pitcher-Plant Mosquito: Adaptive Response to the Photic Environment or Correlated Response to the Seasonal Environment?

W. E. Bradshaw,* M. C. Quebodeaux,† and C. M. Holzapfel

Center for Ecology and Evolutionary Biology, University of Oregon, Eugene, Oregon 97403-5289

Submitted February 15, 2002; Accepted November 19, 2002;
Electronically published May 2, 2003

ABSTRACT: Many plants and animals use the length of day or photoperiod to cue their seasonal patterns of development, reproduction, dormancy, and migration. Among temperate arthropods, the median or critical photoperiod increases with latitude or altitude. Concomitantly, in beetles, moths, mites, flies, and mosquitoes, there is a declining expression of a rhythmic, presumably circadian-based, component of photoperiodic response. It has been proposed that the long summer days in the north select for a reduced response to light by the circadian clock, which results in this declining rhythmic expression and, consequently, longer northern critical photoperiods. However, these patterns might also be due to direct, seasonal selection on the critical photoperiod itself, which results in a correlated reduction in the rhythmic component as a result of internal physiological constraints within the organism. Using standard light duration and selection experiments, we show that evolution of photoperiodic time measurement in the mosquito, *Wyeomyia smithii*, results from the direct response of critical photoperiod to seasonal selection and a correlated response of the rhythmic component of photoperiodic time measurement. We conclude that expression of the circadian clock is necessary neither for the central mechanism of photoperiodic time measurement nor for the adaptive modification of critical photoperiod.

Keywords: adaptive dispersal, circadian rhythms, photoperiodism, evolution, biological clocks.

Herein, we examine the role of circadian rhythmicity (see glossary in appendix for expanded definitions of some of

the terms in this article) in the evolution of the response to day length used to cue seasonal development by a mosquito over the climatic gradient of North America. The most pervasive environmental variable at temperate latitudes is the changing of the seasons. These changes are regular and are highly correlated with local day length. Therefore, it is not surprising that a wide variety of plants, vertebrates, and arthropods use day length (photoperiodism; see glossary) to anticipate the changing seasons and to adjust their behavior, development, and reproduction (Vaartaja 1959; Withrow 1959; Frisch 1960; Aschoff 1965; Menaker 1971). Failure to accommodate to novel seasonality is the major cause of the failure of insect populations introduced for biological control (Stiling 1993); a mismatch between local seasonality and the photoperiodic response of introduced mammals has limited the northward distribution of rabbits in Australia (Cooke 1977) and is responsible for outbreeding depression in a managed population of the Hungarian ibex (Templeton 1986). Photoperiodic response can evolve rapidly in natural populations of successfully invading species (Hoy 1978; Tauber et al. 1986, pp. 238–245; Fochs et al. 1994) in response to climate change (Bradshaw and Holzapfel 2001a) or to altered predator phenology (Hairston and Walton 1986).

At northern latitudes, winter arrives early in the fall when local day length is still long. At southern latitudes, winter arrives late in the fall when local day length is short. Therefore, northern plants and animals switch from active development to dormancy when days are long; southern plants and animals switch from active development to dormancy when days are short. Indeed, one of the most robust ecogeographic “rules” is that the switching day length or critical photoperiod (CPP; see glossary) that mediates the onset of dormancy in arthropods (diapause) increases regularly with latitude and altitude (Andrewartha 1952; Danilevskii 1965; Bradshaw 1976; Taylor and Spalding 1986; Danks 1987, table 24). The critical photoperiod can vary

* Corresponding author; e-mail: bradshaw@darkwing.uoregon.edu.

† Present address: 2328 Meadows Lane, Castle Rock, Colorado 80104.

by 10 phenotypic standard deviations among populations within a single species, which thus represents an enormous capacity for adaptive evolution (Lair et al. 1997).

Photoperiodism has been known to Spanish chicken farmers for perhaps 200 yr (Farner 1959) and has been studied intensively by scientists since the early part of the previous century (Pittendrigh 1993). Originally, it was assumed that photoperiodic time measurement (PTM; see glossary) was accomplished by a day-interval timer, literally by measuring the length of day (i.e., by an hourglass mechanism). In 1936, Erwin Bünning proposed that the circadian clock played a causal (necessary, controlling) role in PTM. A causal role of circadian rhythmicity in PTM is intriguing because it would mean that the highly accurate, environmentally buffered circadian clock that regulates daily activities had assumed an additional role of also regulating seasonal activities. Because of both the enduring appeal and the elusive nature of Bünning's (1936) hypothesis, studies to distinguish it from an hourglass model have remained a central concern for more than 40 yr (Withrow 1959; Frisch 1960; Aschoff 1965; Menaker 1971; Saunders 1982; Takeda and Skopik 1997; Vaz Nunes and Saunders 1999).

In both hourglass and circadian models, a rise or decline in photosensitivity is triggered by dawn or dusk, and if light is present when photosensitivity exceeds some threshold, a long-day response ensues. In an hourglass model, the rise or decline of photosensitivity is initiated and terminates during each external light : dark (L : D) cycle; it does not repeat itself during a long dark period that lasts several days. In a circadian model, the rise or decline of photosensitivity is initiated by dawn or dusk but continues to cycle internally between photosensitive and photoin-sensitive phases during a long dark period that lasts several days.

The rhythmic component of PTM refers to the rhythmic behavior of long-day response in experiments with extended nights. The amplitude of this behavior is a measure of the responsiveness of the underlying physiology to light; the period of this behavior is a measure of cycle duration. Herein, we focus on amplitude. We consider the expression of a rhythmic, presumably circadian-based component of PTM, examine how that rhythmic expression co-varies with CPP over temperate climatic gradients, and resolve the general question, Does this covariation represent a correlative (ancillary, incidental) or a causal (necessary, controlling) role of circadian rhythmicity in the adaptive evolution of PTM? In a broader evolutionary sense, resolution of this question bears directly on understanding the functional mechanism by which many plants and animals have diversified throughout temperate seasonal environments.

Is Circadian Rhythmicity Causal or Correlative?

In Asian flies (Pittendrigh and Takamura 1993), North American moths and mosquitoes (Takeda and Skopik 1985; Wegis et al. 1997), and European beetles and mites (Thiele 1977; Vaz Nunes et al. 1990), the amplitude of a rhythmic component of PTM, suggestive of circadian involvement in PTM, declines with increasing latitude. This negative correlation therefore appears to be a general property of arthropod diversification into the North Temperate Zone. The question still remains as to whether this latitudinal covariation reflects a causal or a correlative role, or even no role at all, of circadian rhythmicity in the evolution of PTM. Next, we present our approach for resolving the issue.

Causal Role of Circadian Rhythmicity

This view has been proposed by Pittendrigh and coworkers (Pittendrigh and Takamura 1987, 1989, 1993; Pittendrigh et al. 1991) on the basis of the conviction that circadian rhythmicity is a causal element of insect photoperiodism. In this case, PTM at any latitude would require a functional circadian clock, but internal circadian rhythmicity typically tends to damp out or stop entirely at longer day lengths. In order to maintain circadian organization during the long summer days at high latitudes, northern organisms would evolve circadian clocks with a high-amplitude, robust oscillation. The idea is that as the magnitude of the oscillation increases, its endogenous inertia renders it less perturbable by light, much as a pendulum swinging in a wide arc is less perturbed by a given force than is a pendulum swinging in a narrow arc. The low perturbability of northern circadian oscillations would enable circadian rhythmicity to persist through the very long summer days at high latitudes but would also render circadian-mediated processes less responsive to light. As a result of a lower response to light (see glossary), northern populations should exhibit a lower long-day response to all day lengths from 0 to 24 h and a lower amplitude in the expression of a rhythmic component of PTM than do southern populations. This lower response to light would therefore result in longer critical photoperiods in northern populations than in southern populations. Hence, the evolution of longer critical photoperiods at higher latitudes would be the result of direct selection by longer summer days (the photic environment) on the circadian clock itself. This scenario predicts that the critical photoperiod and the amplitude of the rhythmic component of PTM should be negatively correlated over latitudinal gradients where summer day lengths increase and duration of the growing season decreases. With increasing altitude at a given latitude, the duration of the growing season decreases, but

summer day lengths remain the same. Consequently, over an altitudinal gradient, the CPP should increase with increasing altitude, but the amplitude of the rhythmic component of PTM should not change. The independent evolution of the CPP and the amplitude of the rhythmic component of PTM over altitudinal gradients also implies a loose genetic correlation or no genetic correlation between these two physiological processes within populations.

Correlative Role of Circadian Rhythmicity

This view is the usual one taken by evolutionary physiologists concerned more with the ecology and evolution of seasonality than with the mechanism of PTM (Andrewartha 1952; Danilevskii 1965; Tauber et al. 1986; Taylor and Spalding 1986; Danks 1987; Leather et al. 1993). Shorter growing seasons in the north than in the south should select for the initiation of migration or dormancy earlier in the year when days are longer. Seasonal selection should therefore have a direct effect on critical photoperiod. Internal functional constraints (as yet unknown) imposed by the evolution of longer critical photoperiods are then responsible for the latitudinal covariation between critical photoperiod and the rhythmic component of PTM across arthropods in general. Consequently, over an altitudinal gradient, the CPP should increase with increasing altitude, and as a correlated response, the amplitude of the rhythmic component of PTM should decrease with increasing altitude. This scenario necessarily requires a tight genetic correlation between these two physiological processes within populations.

Resolving the Alternatives

To resolve the role, if any, of circadian rhythmicity in the evolution of PTM, we use the pitcher-plant mosquito, *Wyeomyia smithii*. Both the historical biogeography and the known properties of PTM in this species make it ideally suited for resolving the outlined alternatives. *Wyeomyia smithii* completes its entire preadult development only within the water-filled leaves of the purple pitcher plant, *Sarracenia purpurea* L. The range of the mosquito closely follows that of its host plant from about 30° to 54°N in North America. *Wyeomyia smithii* invaded North America from South America and since becoming established in North America has diversified northward (Ross 1964; Bradshaw and Lounibos 1977; Istock and Weisburg 1987; Bradshaw and Holzapfel 1990). Throughout its range, *W. smithii* overwinters in the evergreen leaves of *S. purpurea* in a larval diapause that is initiated and maintained by short days and is averted or terminated by long days (Smith and Brust 1971; Bradshaw and Lounibos 1972,

1977; Evans and Brust 1972). The critical photoperiod that mediates the onset and maintenance of diapause increases with latitude and altitude (Bradshaw 1976; Hard et al. 1993a; Lair et al. 1997; Bradshaw and Holzapfel 2001a); at the same time, the amplitude of the rhythmic component of PTM declines with latitude from southern to intermediate to northern latitudes (Wegis et al. 1997). Latitudinal variation in photoperiodic response of *W. smithii* is therefore representative of the other arthropods whose critical photoperiods and rhythmic components of PTM co-vary similarly across temperate latitudes in Asia, North America, and Europe (Bradshaw and Holzapfel 2001b).

We use this background with *W. smithii* to resolve the alternatives. First, we determine the covariation of CPP and the rhythmic component of PTM over latitudinal and altitudinal gradients. Second, we select on divergent critical photoperiods within a single population from an intermediate latitude. We then compare the direct response of critical photoperiod and the correlated response in the rhythmic component of PTM with their respective geographic patterns among populations. Finally, we test two contrasting, explicit predictions: First, if the declining expression of a rhythmic component of PTM in northern populations is a consequence of direct adaptation by the circadian clock to the external photic environment (long summer days), then the amplitude of any rhythmic component of PTM should decrease with increasing latitude but not with increasing altitude, and any rhythmic component of PTM should be only loosely genetically correlated or uncorrelated within populations. Second, if the declining expression of a rhythmic component of PTM in northern populations is a consequence of direct adaptation by the CPP to seasonality, then the amplitude of any rhythmic component of PTM should decrease both with increasing latitude and with increasing altitude, and any rhythmic component of PTM should be tightly genetically correlated within populations.

Methods

Collection and Maintenance

We collected larvae of *Wyeomyia smithii* during the overwintering generation from 14 localities in eastern North America in the spring of 1996 (fig. 1). At least 2,000 larvae were collected from each locality. Before the start of any experiments, in order to minimize field effects, the 14 populations were raised for three generations on long days (18L:6D) with a smooth, sine-wave thermoperiod that ranged from 13° to 29°C each day.

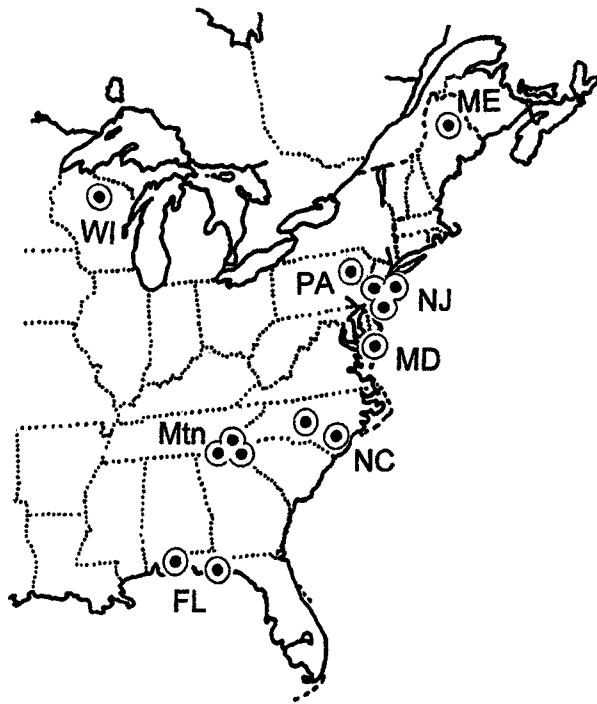


Figure 1: Origin of experimental populations

Common Experimental Procedures

In unchilled larvae, the critical photoperiod in a given population is the same for both the initiation and the maintenance of diapause; once initiated, diapause is always photoreversible by exposure to long days (Bradshaw and Lounibos 1972). Experimental animals were sampled from the continuously reproducing stock population. Larvae used in experiments were reared on short days (8L : 16D) at $21^{\circ} \pm 0.5^{\circ}\text{C}$ for at least 30 d before the start of an experiment to synchronize development and to ensure that animals were in diapause. For each experiment, within any one population, diapausing larvae were pooled from a number of petri dishes into a large pan, stirred, and then haphazardly allocated to 150×25 -mm petri dishes to make up three replicate cohorts of 35 larvae per dish for each treatment. Experiments were carried out in a controlled-environment room at $23^{\circ} \pm 0.5^{\circ}\text{C}$ inside light-tight photoperiod cabinets where larvae were exposed to a 4-W cool-white fluorescent lamp at a distance of 10–20 cm. To avoid an unintentional thermoperiod, the ballasts were located outside the cabinets, and each lamp was housed in a clear polycarbonate tube through which 28 L/min forced air was blown. At the beginning of the experiment and twice a week thereafter, the dishes of larvae from each of the 14 populations were haphazardly arranged within each photoperiod cabinet without regard to

latitude or altitude of the population. Each experiment was carried out as a single block with all populations experiencing a given treatment concurrently in the same cabinet. Experimental animals were fed with a 3 : 1 by volume mixture of ground Geisler guinea pig chow and Bay Brand brine shrimp. They were fed, cleaned, and checked for pupae, which were removed, twice a week for the duration of the experiment.

We used experiments with a fixed total period (T ; see glossary; $T = L + D$) of 24 h in which we varied the day length from 0 to 24 h and experiments with a variable total period of $T = 24$ to 72 h in which we fixed the day length at 10 h and varied night length. In experiments with $T = L + D = 24$ h, all larvae were exposed to the experimental treatments for 30 d and then were transferred to short days (8L : 16D) at $21^{\circ} \pm 0.5^{\circ}\text{C}$ for two additional weeks to allow development of larvae that had been stimulated by the L : D cycle during the experiment but that had not pupated by day 30. At the end of the 2 wk on short days, the remaining larvae were censused and then discarded. In experiments that varied $T = L + D$ from 24 to 72 h, larvae were exposed to the experimental treatments for 8 wk (to allow for a minimum of 19 L : D cycles when $T = 72$) and then transferred to short days for 2 wk as previously described. Percentage development (long-day response) was calculated for each replicate as (total number of larvae having pupated) \div (total number of larvae having pupated + number of larvae remaining alive) at the end of the experiment (44 d when $T = 24$; 70 d when $T = 24$ to 72). Statistical analyses and graphics were performed using Axum (TriMetrix 1992).

Photoperiodic Response Curves

To determine critical photoperiods, three dishes of diapausing larvae from each population were subjected to light regimens ranging from 11 to 16.75 h of light per day in 15-min increments. The experiments were run as a single block with all populations and replicates exposed concurrently in the same cabinet to a given photoperiod. The critical photoperiod for each population was estimated as the 50% intercept on the photoperiodic response curve (see glossary) for the pooled replicates.

Increasing northern CPPs could be the result of an increasing delay in peak photosensitivity during the L : D cycle or could be the result of a decreasing overall response to light. If the increasing northern CPPs are due to a decreasing response to light, then there should be a reduced long-day response to exotic (unnatural, ecologically irrelevant) short or long day lengths (0–8 or 20–24 h), as well as in the vicinity of the CPP (Pittendrigh and Takamura 1993; Wegis et al. 1997). We therefore use area under the curve of long-day response to exotic short or long days

Table 1: Origin of populations used in this study, their critical photoperiods (CPP), and the period (τ) of the rhythmic component of photoperiodic response

| Origin | Reference | Latitude (°N) | Altitude (meters) | CPP | τ (a) (hours) | τ (b) (hours) | Symbol |
|---------|-----------|------------------|----------------------|-------|-----------------------|-----------------------|--------|
| FL 1 | WI | 30 | 10 | 12.14 | 22 | 21.23 | ● |
| FL 2 | CR | 31 | 40 | 12.39 | 21 | 20.98 | ○ |
| NC 1 | GS | 34 | 20 | 12.72 | 21 | 20.50 | ● |
| NC 2 | PM | 35 | 90 | 13.04 | 21 | 20.74 | ○ |
| NC Mtn1 | DB | 35 | 900 | 13.88 | Undefined | Undefined | ● |
| NC Mtn2 | HK | 35 | 900 | 13.88 | Undefined | Undefined | ○ |
| NC Mtn3 | CB | 36 | 900 | 14.04 | Undefined | Undefined | ◇ |
| MD | NP | 38 | 20 | 13.41 | 22 | 21.71 | ● |
| NJ 1 | HV | 40 | 10 | 13.44 | 24 | 19.29 | ○ |
| NJ 2 | MM | 40 | 10 | 13.44 | 21 | 22.67 | ◇ |
| NJ 3 | PB | 40 | 10 | 13.50 | 20 | 20.50 | □ |
| PA | TH | 41 | 600 | 14.38 | 23 | 21.47 | × |
| ME | KC | 46 | 370 | 15.43 | 20 | 18.57 | ● |
| WI | ML | 46 | 500 | 14.99 | 22 | 23.16 | ○ |

Note: References show designation of the specific population for cross-reference with earlier papers from our lab. τ indicates period of the underlying oscillator estimated from peak-to-peak distances in long-day response from T experiments by the actual position of the highest points— τ (a)—or from peak values of the response curves fitted by locally weighted regression— τ (b). For NC Mtn1–3, τ (a) and τ (b) were undefined because there were no peaks for comparison. The symbols provided are those used for each population in figures 3 and 4.

as a measure of response to light (Wegis et al. 1997). To determine the response to light at exotic long and short photoperiods, larvae were exposed to 0–8 h and 20–24 h per day of light in 1-h increments.

Rhythmic Component of PTM

The most common experiment used to infer a circadian influence on PTM is the resonance or T experiment (see glossary) where organisms are exposed to a short day length and, in separate experiments, night lengths are increased in duration to produce a total light + dark period ranging from $T = L + D = 24$ to 72 h. Note that each of these experimental regimens provides a short day and a long night. If PTM is caused by a simple interval or hourglass timer, these experiments should yield a consistent short-day response as illustrated in the vetch aphid, *Megoura viciae* (Lees 1973). If there is a rhythmic component to PTM, these experiments should produce a succession of peaks and valleys in long-day response as T increases in duration and as the underlying rhythm oscillates between photosensitive and photoinsensitive phases during the long night. The peak-to-peak or valley-to-valley interval should then represent τ (see glossary), the period of the underlying rhythm of sensitivity to light (Pittendrigh 1981; Saunders 1982; Takeda and Skopik 1997; Vaz Nunes and Saunders 1999).

To test for rhythmic responses to varying night lengths, we exposed larvae to a 10-h day followed by varying night lengths of 14–62 h to create $T = 24$ to 72 h in 24 separate

experiments as in Wegis et al. (1997). The experiments were run as a single block with all populations and replicates exposed concurrently in the same cabinet to a given T . The positions of replicates and populations in each cabinet or in the subsequent short-day room were maintained haphazardly throughout the 10 wk of the experiment.

We ran three controls: the usual long-day (18L:6D) and short-day (10L:14D) controls to ensure that long days promoted development and short days maintained diapause in all populations and a supplemental long-day (17L:55D) regimen to control for the minimum number of inductive cycles when $T = 72$ h. During the 8 wk of exposure to experimental conditions, the number of potentially diapause-terminating cycles decreased from 56 when $T = 24$ to 19 when $T = 72$. Hence, a lack of development in response to the longer T 's might reflect the requirement of more long-day cycles to terminate diapause rather than the cycle being perceived as a short day. The 17L:55D regimen controls for this effect because if the minimum number of 19 cycles is sufficient, the 17L:55D regimen should elicit a full long-day response in all populations.

We plotted the mean long-day response for three replicates for each population as a function of T . We estimated the period of the underlying rhythm (τ) from peak-to-peak distances (table 1) on the basis of the actual highest points of the two peaks (τ [a]) or on the basis of the positions of the peaks after smoothing with a locally weighted linear regression (τ [b]; Wegis et al. 1997).

Genetic Correlations

The correlated response (*CR*) in trait *Y* to direct selection on trait *X* (Falconer 1981, p. 286) is given by $CR_Y = i_X h_X h_Y r_A \sigma_{PY}$, where i_X is the intensity of selection; h_X and h_Y are the square roots of the heritabilities of *X* and *Y*, respectively; r_A is the additive genetic correlation between *X* and *Y*; and σ_{PY} is the phenotypic standard deviation of *Y*. An important consequence of this relationship is that if one selects on *X* and obtains a correlated response in *Y*, then the heritabilities of *X* and *Y* and the genetic correlation between *X* and *Y* must all be nonzero, with the sign of the correlated response providing the sign of the genetic correlation. To test for a genetic correlation between critical photoperiod and the rhythmic component of PTM, we selected on critical photoperiod and then examined both the direct and correlated responses to this selection.

We collected from a single locality in New Jersey at 40°N (NJ 3, table 1) that exhibits an intermediate CPP and intermediate expression of the rhythmic component of PTM (Wegis et al. 1997). To increase independence of the replicate lines, we collected from three different sublocalities spaced 100–300 m apart. The first was along a stream that ran through a cedar swamp, the second from a backwater of that stream, and the third from a raised, sandy bog 300 m from the first two. Lab populations from each sublocality were reared and maintained separately in a controlled-environment room with a smooth, sine-wave daily thermoperiod (12°–29°C) and an unambiguous long-day photoperiod (18L : 6D). For rearing and maintenance, the thermoperiod lagged the photoperiod by 3 h; for selecting on critical photoperiod, the thermoperiod coincided with the photoperiod.

After rearing for three generations to minimize field effects, we started truncation selection. At generation 0 of selection within each of the populations from each separate sublocality, we split the population into two selected lines and a control line. Hence, the division between up, down, and unselected lines was made at generation 0, and the nine resulting lines were maintained separately thereafter.

During each generation of selection, each line was synchronized in diapause. In the short-selected lines, ~1,000 larvae were reared on unambiguous short days (8L : 16D) for 25 d to induce diapause. They were then exposed to the test day length; those that developed must have had a CPP shorter than the test day length and were used to found the next generation. In the long-selected lines, ~1,000 larvae were reared at the test day length for 25 d; those that did not develop must have had a CPP longer than the test day length and were used to found the next generation (after returning them to unambiguous long days [18L : 6D] to induce resumed development). In the

control lines, we reared 200–600 larvae on unambiguous short days (8L : 16D) to induce diapause and maintained the larvae in diapause to achieve synchrony with the selected lines before returning them to unambiguous long days (18L : 6D) to induce resumed development.

During the first three generations of selection (0–2), we did not obtain a sufficient response to selection that would change the test day length from the original 14 h (fig. 2). In each successive generation of selection (3–12), we increased the test day length in the long-selected lines and decreased the test day length in the short-selected lines. In each line in each generation of selection, we sexed the adults that emerged after selection and estimated the effective population size (*Ne*) of mosquitoes that passed the selection criteria as (Falconer 1981, p. 65)

$$Ne = \frac{4N_m N_f}{N_m + N_f},$$

where N_m and N_f are the number of male and female pupae. We maintained $Ne > 100$ in each selected line for each generation by reversing selection by 0.1 h when Ne was small (mean[number of instances] \pm SD = 117[1] \pm 0), we did not modify the day length if Ne was modest (155[8] \pm 29), and we increased selection by 0.1 or 0.2 h if Ne was large (191[23] \pm 56 or 305[11] \pm 29, respectively). At the same time, we culled the control lines to maintain Ne at approximately the same levels as in the selected lines. Selection commenced in the fourth lab generation and continued for 13 generations of selection (fig. 2). Inbreeding each generation (F_g) with effective population size Ne_g was estimated as (Hartl and Clark 1989, p. 76)

$$F_g = \frac{1}{2Ne_g} + \left(1 - \frac{1}{2Ne_g}\right)F_{g-1}.$$

Cumulative inbreeding after 13 generations of selection was <4% and did not differ between control and selected lines (one-way ANOVA: $F = 3.29$, $df = 2, 6$, $P = .108$). After the last generation of selection, we reared the selected and control lines through two generations on unambiguous long days. We then determined the direct response of photoperiodic response curves in the third generation after selection and the correlated response to *T* experiments in the fifth generation after selection under the same conditions and with the same experimental protocols as described for determining the photoperiodic response curve and response to *T* experiments among populations.

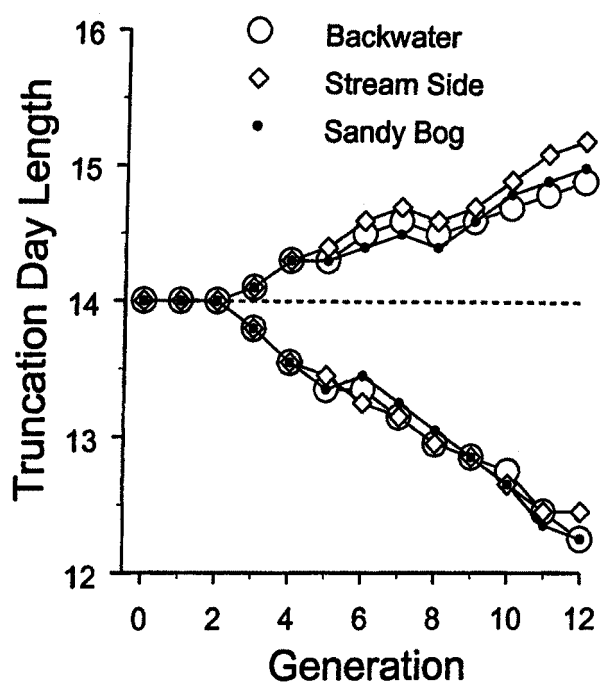


Figure 2: Divergent selection for long and short critical photoperiods. Truncation selection was imposed by selecting the individuals that developed (short critical photoperiod) or remained in diapause (long critical photoperiod) at the truncation day length, which was decreased (short selected) or increased (long selected) in each generation to maintain $N_c \geq 100$ after selection in each generation in each line. Selection was imposed independently on three subpopulations collected from a backwater, along the stream side, or in a raised sandy bog, all within 300 m of each other in New Jersey (NJ 3, table 1).

Results

Photoperiodic Response Curves

At ecologically relevant day lengths (11–16 h), all populations, regardless of geographic origin, showed a long-day response ranging from <5% to >90% (fig. 3). The critical photoperiod (table 1) increased linearly with latitude (slope \pm SE: $b = 0.148 \pm 0.007$; $P < 10^{-5}$) and altitude ($b = 0.00114 \pm 0.00012$; $P < 10^{-5}$) of origin ($R^2 = 0.975$).

Recall that the magnitude of long-day response to exotic day lengths (unlikely to be encountered in nature) provides a measure of photoperiodism as a response to light (Pittendrigh and Takamura 1993; Wegis et al. 1997). At exotic short (0–8 h) or long (20–24 h) day lengths, long-day response declined with latitude or altitude. Response to short ($r^2 = 0.68$; $P = .002$) but not long ($r^2 = 0.33$; $P = .064$) exotic day lengths was negatively correlated with critical photoperiod (fig. 3F). The increase of critical photoperiod with latitude (fig. 3E to 3C to 3A to 3B) or

altitude (fig. 3C to 3D) was therefore associated with a decreasing response to light. These results show that mountain populations exhibited a reduced response to light that is characteristic of extreme northern populations rather than a response similar to that of lowland populations at the same latitude.

Rhythmic Component of PTM

Short-day controls elicited 0%–4% development; long-day controls elicited 95%–100% development. Hence, short days maintained and long days terminated diapause. The 17L : 55D control elicited 89%–100% development (fig. 4), which showed that the minimum number of experimental T cycles was sufficient to elicit a robust long-day response in all populations.

Response to progressively longer night lengths (14–62 h) showed two peaks (long-day response) and two valleys (short-day response). The amplitude of the underlying photosensitivity rhythm was estimated from peak heights. Peak height declined with increasing latitude (fig. 4E to 4C to 4A to 4B) and altitude (fig. 4C to 4D). The period of the underlying photosensitivity rhythm (τ) was estimated from peak-to-peak distances. Peaks in long-day response in the mountain populations were barely discernible or not apparent and were not sufficiently resolved to assign distances. We obtained estimates of the positions of both peaks from the other 11 populations between 30° and 46°N. Actual peak-to-peak distances ($\tau[a]$; table 1) averaged (\pm SE) 21.55 ± 0.38 h and were not significantly correlated with critical photoperiod ($r^2 = 0.002$, $t = 0.124$, $df = 9$, $P = .904$); peak-to-peak distances from smoothed curves ($\tau[b]$; table 1) averaged 20.98 ± 0.40 h and were not significantly correlated with critical photoperiod ($r^2 = 0.004$, $t = 0.189$, $df = 9$, $P = .854$). Area under the long-day response curves (fig. 4F) declined with increasing critical photoperiod ($r^2 = 0.72$; $P < .001$).

These results show that increasing critical photoperiods are not associated with any discernible variation in the period of an underlying rhythm but are associated with a dramatically reduced response to light and lower amplitude of the rhythmic component of PTM. Mountain populations exhibit a reduced response to light and a reduced amplitude of the rhythmic component of PTM that are characteristic of extreme northern populations, unlike lowland populations at the same latitude.

Genetic Correlations

Selection on critical photoperiod resulted in shorter and longer critical photoperiods in the short- and long-selected lines, respectively, than in the control lines (fig. 5). The long-day responses to exotic (0–8 or 20–24 h) day lengths

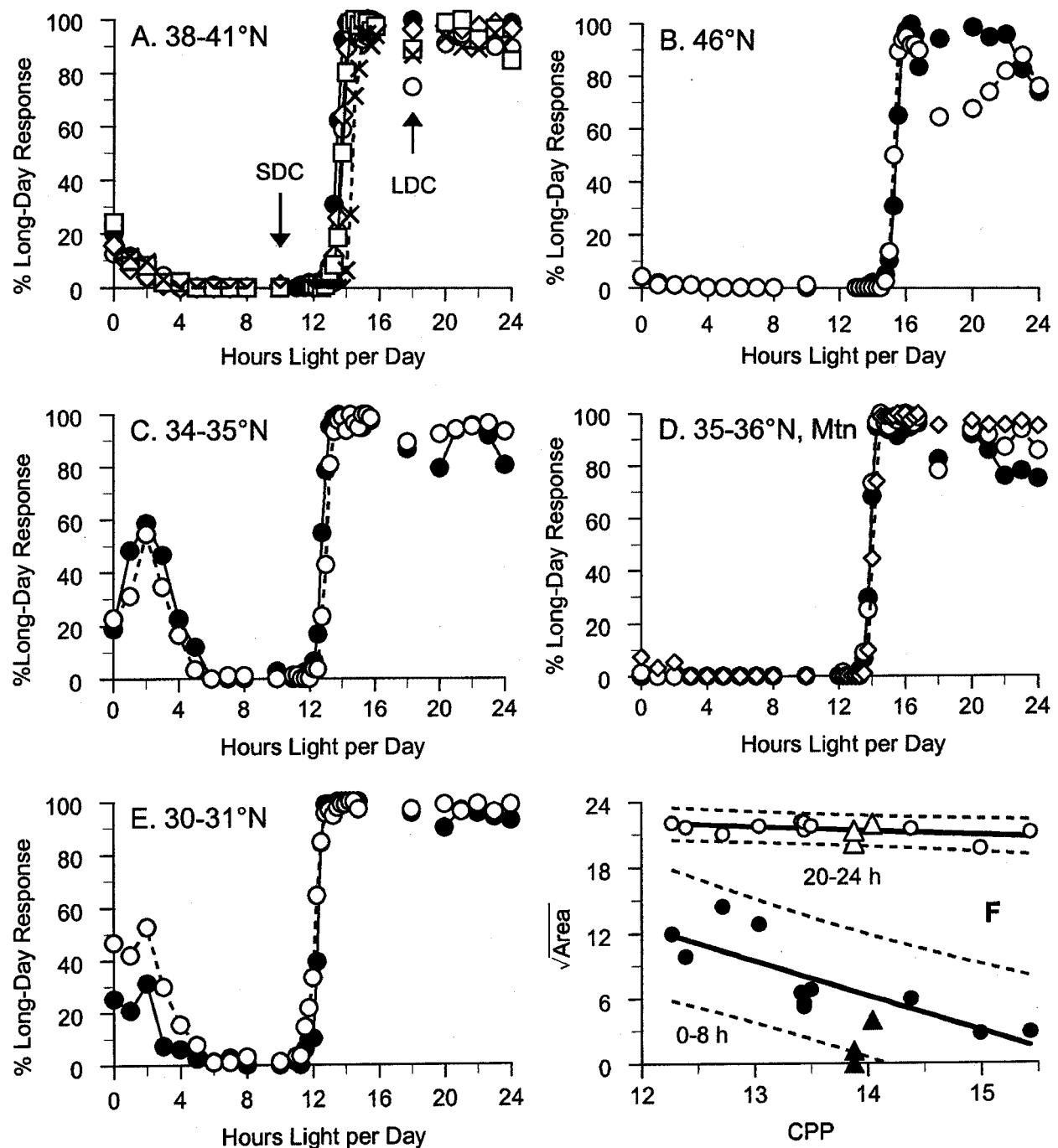


Figure 3: A–E, Developmental (long-day) response of *Wyeomyia smithii* to ecologically relevant (11–16 h) and to exotic, unnatural, short (0–8 h) or long (20–24 h) day lengths. The area under the curves at exotic short or long day lengths is used to measure a population’s response to light (Wegis et al. 1997). This figure and figure 4 are plotted to show increasing latitude from E to C to A to B and the comparison of lowland (C) with mountain (D, Mtn) populations at similar latitudes in North Carolina. The isolated points at 10-h (SDC) and 18-h (LDC) day lengths show the short- and long-day controls, respectively. F, Relationship between CPP from table 1 and area under the response curves to exotic long or short day lengths. The regressions show the 95% confidence belts and do not include the mountain populations, shown as triangles. Specific populations corresponding to the different symbols are given in table 1.

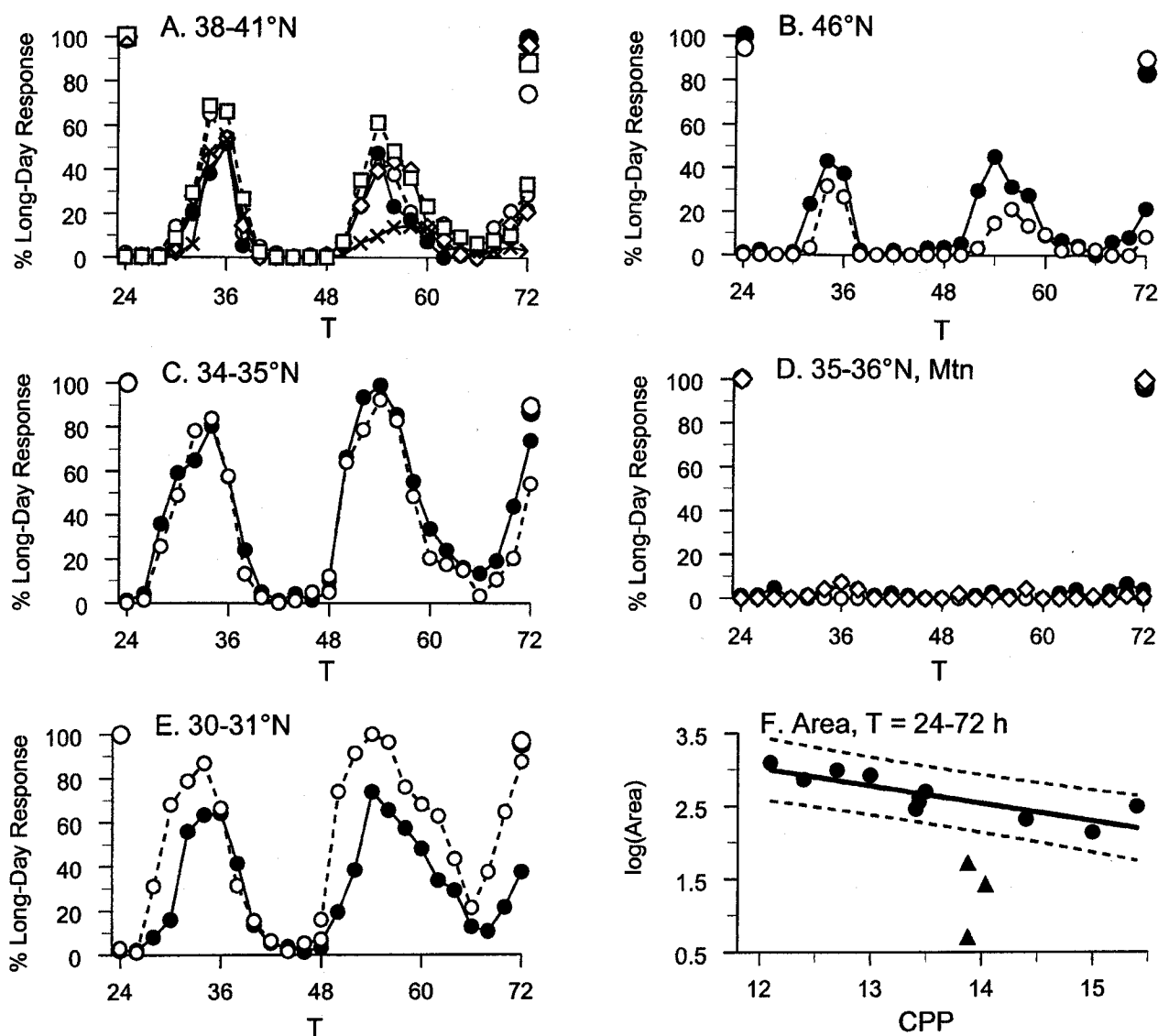


Figure 4: A–E, Developmental (long-day) response of *Wyeomyia smithii* to T experiments with a 10-h day and variable night length to create $T = L + D = 24$ to 72 h. This figure and figure 3 are plotted to show increasing latitude from E to C to A to B. In each graph (A–E), the lower points on the line at 24 h show the 10L:14D short-day control; the higher, isolated points at 24 h show the 18L:6D long-day control; and the higher isolated points at 72 h show the 17L:55D control. F, Relationship between CPP from table 1 and area under the response curves in A–E. The regression shows the 95% confidence belts and does not include the mountain populations, shown as triangles. Specific populations corresponding to the different symbols are given in table 1.

provide another measure of the response to light. At exotic short day lengths (0–8 h), area under the long-day response curve decreased in the long-selected lines and increased in the short-selected lines relative to the control lines (fig. 5; Kruskal-Wallis: $H = 7.20$, $df = 2$, $P = .027$). At exotic long day lengths (20–24 h), area under the long-day response curve did not differ among the long-selected, short-selected, and control lines (Kruskal-Wallis: $H = 3.82$, $df = 2$, $P = .148$). These results mean

that critical photoperiod was negatively genetically correlated with response to light in the New Jersey population.

Selection on critical photoperiod also resulted in a correlated response in the rhythmic component of PTM (fig. 5). Area under the long-day response curves differed among short, long, and unselected lines ($H = 7.20$, $df = 2$, $P = .027$). Relative to the unselected lines, the lines selected for short critical photoperiods showed an increase in the amplitude of the rhythmic response to

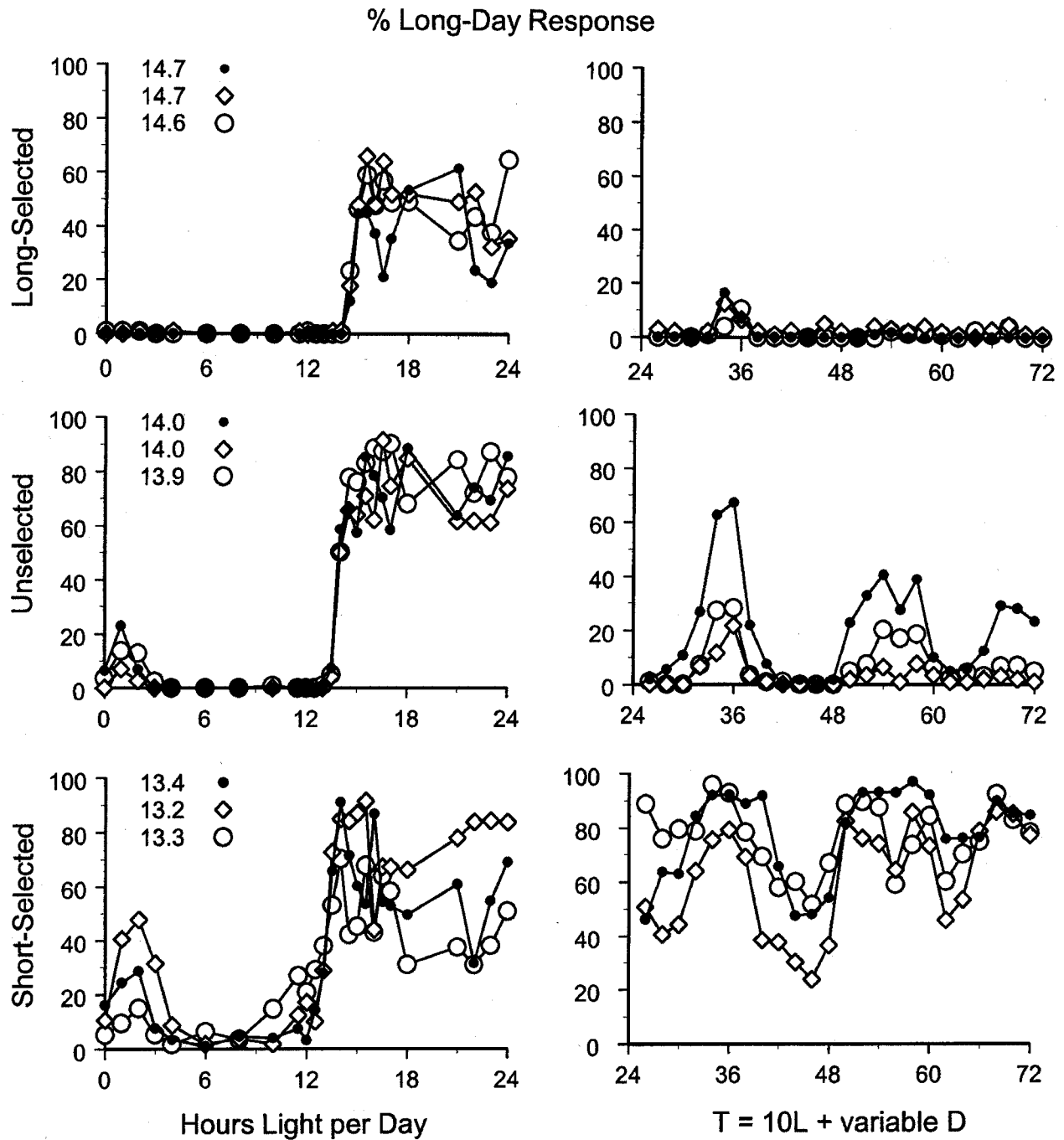


Figure 5: Direct and correlated long-day response (% development) to selection on critical photoperiod (CPP) in a latitudinally intermediate population. The left column shows the long-day response of selected and control lines to varying hours of light per day ($T = 24$ h) analogous to figure 3; the right column shows the long-day response of selected and control lines to a 10-h day and variable night length from 14 to 62 h ($T = 24$ to 72 h), analogous to figure 4. *Top row*, Lines selected for long CPP; *middle row*, unselected lines; *bottom row*, lines selected for short CPP. In the upper left of each plot in the left column, CPPs are shown. Symbols correspond to the separate subpopulations shown in figure 2.

varying T ; the lines selected for long critical photoperiod showed a greatly reduced amplitude of the rhythmic response to varying T . The interval between the first and second peaks did not differ between the short and unselected lines ($H = 3.43$, $df = 1$, $P = .064$). The second peak in the long-selected lines was reduced to baseline variation, so its position could not be determined reliably. These results mean that critical photoperiod was negatively genetically correlated with the amplitude and uncorrelated with the period of the rhythmic component of PTM within the New Jersey population.

In sum, these results show that genetic correlations within the New Jersey population parallel the phenotypic correlations among populations. The CPPs are negatively correlated with a response to light and with the amplitude of the rhythmic component of PTM but are uncorrelated with the period of the rhythmic component of PTM.

Discussion

In seasonal environments, no organism can thrive without the ability to exploit the favorable season, to avoid or mitigate the unfavorable season, and to anticipate those seasonal changes (Hard et al. 1993*b*). At temperate latitudes, the close correlation between the local changes in day length and the local changes in the seasons provides the opportunity for such an anticipatory cue. The number of arthropod species known to be photoperiodic measures in the hundreds (Masaki 1983). When confronted with novel seasonal environments, evolution of photoperiodic response can take place over a few generations (Hoy 1978; Hairston and Walton 1986; Tauber et al. 1986, pp. 238–245; Fochs et al. 1994; Bradshaw and Holzapfel 2001*a*); the repeatedly observed correlation between critical photoperiod and geography among insects and mites (Taylor and Spalding 1986; Danks 1987, table 24) attests to its adaptive importance over longer evolutionary time. Understanding the processes by which arthropod populations modify photoperiodic response is therefore a major element in understanding adaptive dispersal into temperate environments.

Circadian rhythms are ubiquitous to eukaryotes, and they have a pervasive ability to orchestrate daily temporal organization throughout an organism (Pittendrigh 1960; Aschoff 1965; Meanker 1971; Bünning 1973). Bünning's (1936) hypothesis that circadian rhythmicity is also central to photoperiodic time measurement (PTM) and, consequently, that circadian rhythms are also responsible for regulating seasonal as well as daily activities, has therefore proved to be a captivating if elusive proposition. Historically, the most common approach used to implicate a

circadian influence on PTM is the T experiment, which has revealed a rhythmic response to variable night length in plants, birds, mammals, and insects (Pittendrigh 1981; Saunders 1982; Takeda and Skopik 1997; Vaz Nunes and Saunders 1999). The strikingly similar decline in the amplitude of the rhythmic response to T experiments with increasing latitude among mites, moths, mosquitoes, flies, and beetles in Asia, Europe, and North America (Thiele 1977; Takeda and Skopik 1985; Vaz Nunes et al. 1990; Pittendrigh and Takamura 1993; Wegis et al. 1997) suggests that a similar underlying physiological process is common to the adaptive dispersal of temperate arthropods in general. Investigators with an ecological and evolutionary perspective have ascribed the increase in CPP with latitude to a straightforward consequence of direct selection on PTM by local seasonality without being much concerned about the underlying mechanism. By contrast, Pittendrigh and coworkers, having pursued Bünning's (1936) hypothesis for several decades, proposed that long summer days at high latitudes (i.e., the photic environment) select directly on the circadian clock for a high-amplitude oscillation with a reduced response to light and, consequently, an increased critical photoperiod (reduced long-day response).

We have resolved these viewpoints by comparing the rhythmic component of PTM over latitudinal gradients, where both the photic and seasonal environments closely co-vary, with altitudinal gradients at the same latitude where the photic environment remains constant and only the seasonal environment varies. We found that with increasing altitude, response to light and the rhythmic component of PTM paralleled their latitudinal pattern. With increasing altitude, there was a decreased response to light and an extreme decrease in the rhythmic component of PTM. The CPP and the rhythmic component of PTM closely co-vary over a seasonal gradient with a constant photic environment. We therefore reject the proposition by Pittendrigh and coworkers (Pittendrigh and Takamura 1987, 1993; Pittendrigh et al. 1991) that direct selection by the photic environment on the circadian clock constitutes an important factor that affects the evolution of PTM in *Wyeomyia smithii*.

Similarly, we selected for divergent long and short CPPs and obtained a strong correlated response in the rhythmic component of PTM in the absence of any selection by the photic environment (fig. 5). Although the CPPs of the selected lines (fig. 5) fell within the range of CPPs represented by the southern and northern populations (table 1), the rhythmic component of the short-selected lines (fig. 5) exceeded that of the southernmost population (fig. 4), and the rhythmic component of the long-selected lines was reduced below that of the northernmost population. Hence, the correlated response of the rhythmic component

of PTM to direct selection on CPP exceeded the covariation between CPP and the rhythmic component of PTM in nature where both the seasonal and photic environments are acting in concert. While the genetic architecture underlying CPP can vary among populations of *W. smithii* (Hard et al. 1993a; Lair et al. 1997; Bradshaw and Holzapfel 2000, 2001b), within a typical population from an intermediate latitude with an intermediate CPP and intermediate rhythmic component of PTM, direct selection on CPP is driving the evolution of the rhythmic component of PTM, not the reverse.

Several observations in photoperiodic organisms with strong, overt circadian rhythms lead us to regard suspiciously any study that seeks to establish a causal connection between circadian rhythms and photoperiodic response from their parallel behavior. In the moth, *Pectinophora gossypiella*, red light (600 nm), at intensities sufficient to regulate the photoperiodic induction of diapause, has no ability to synchronize or set its circadian clock (Pittendrigh and Minis 1971). In *Drosophila littoralis*, when the direct effects of latitude are factored out, photoperiodic control of adult diapause is uncorrelated with any parameters of the adult eclosion rhythm. "Diapause may still be influenced by the period of the eclosion rhythm, even though its minor contribution may be masked by a more variable, eclosion rhythm independent system in the determination of diapause" (Lankinen 1986, p. 123). In the photoperiodic Canton-S strain of *Drosophila melanogaster*, a double, overlapping deletion mutant (*per*⁻) eliminates the *period* locus, renders the flies behaviorally arrhythmic, and results in a chaotic response to *T* experiments but leaves intact a robust photoperiodic response curve, albeit with a shorter CPP (Saunders 1990). As a consequence, the contribution of circadian rhythmicity to PTM can be evaluated reliably only by examining the formal properties of photoperiodism itself, and that approach is the one we have used.

In nature, seasonal adaptation of critical photoperiod, and not adaptation by the circadian clock to the photic environment, explains the geographic covariation of critical photoperiod and the rhythmic component of PTM in *W. smithii*. Selection on critical photoperiod in the absence of selection by the photic environment drives the evolution of the rhythmic component of PTM in excess of that observed in nature where both the seasonal and the photic environments are acting in concert. Finally, we observed perfectly robust photoperiodic responses in the mountain populations under the same conditions in which they lack any detectable rhythmic component to PTM. We therefore conclude that the circadian clock may modify the output of mechanisms underlying PTM but that the expression of the circadian clock is neither a central mechanism of PTM nor necessary for the adaptive modification of the

critical photoperiod and, hence, the adaptive dispersal of arthropods into novel seasonal environments.

Acknowledgments

We thank J. Brandt, P. Fernando, M. Lynch, T. Miller, B. Roy, D. Saunders, D. Todd, N. Tublitz, and J. Willis for helpful commentary on previous versions of this manuscript and L. Andrijasevich, J. Getty, D. Hill, C. Kleckner, Y.-J. Kwon, A. Puls, L. Reed, and C. Riemer for their logistical support. These studies were supported by National Science Foundation grants IBN-9513834 and IBN-9814438.

APPENDIX

Glossary

Circadian rhythm: An endogenous, internally maintained rhythm with a period of about a day. Circadian rhythms are usually highly temperature compensated and repeat indefinitely under the conditions of constant darkness and constant temperature without external time-setting cues.

CPP: Critical photoperiod; hours of light per day that stimulate 50% development and initiate or maintain 50% diapause in a sample population.

Photoperiodism: The ability to use the length of day (or night) to control behavioral, physiological, or developmental events, usually related to seasonality.

Photoperiodic response curve: The relationship between a behavioral, physiological, or developmental event and day length. The 50% intercept at ecologically relevant day lengths, usually in the region of 10–18 h, defines the critical photoperiod; however, very short (0–8 h) or very long (20–24 h) exotic, unnatural day lengths may also stimulate various levels of photoperiodic response.

PTM: Photoperiodic time measurement; the ability of organisms to assess the duration or length of day or night.

Response to light: The magnitude of long-day response to various L : D cycles, including exotic (not normally encountered in nature) short (0–8 h) or long (20–24 h) day lengths, ecologically relevant day lengths, or L : D cycles longer than 24 h as used in *T* experiments.

Rhythmic component of PTM: The portion of photoperiodic time measurement that is attributable to an endogenous, cyclically repeating sensitivity to light, as opposed to a nonrepeating hourglass timer that must be reset by light each day. The rhythmic component of PTM is defined by the rhythmic behavior of a long-day

response in experiments with extended nights. Herein, the amplitude of a rhythm refers to peak height; the period of a rhythm refers to the time for one complete cycle, peak-to-peak.

T : The period of the external L : D cycle, $T = L + D$.

T experiments: Exposure of individuals to a fixed short day followed, in separate experiments, by varying long nights; they are called T experiments because T is used as the symbol to denote the duration of the total $L + D$ cycle.

τ (τ): The period of an organism's internal rhythm, the time interval, peak-to-peak of rhythmic behavior expressed under constant conditions in the absence of an external time-setting stimulus; τ is usually assessed in constant darkness at a constant temperature.

Literature Cited

- Andrewartha, H. G. 1952. Diapause in relation to the ecology of insects. *Biological Reviews* 27:50–107.
- Aschoff, J., ed. 1965. *Circadian clocks*. North Holland, Amsterdam.
- Bradshaw, W. E. 1976. Geography of photoperiodic response in a diapausing mosquito. *Nature* 262:384–386.
- Bradshaw, W. E., and C. M. Holzapfel. 1990. Evolution of phenology and demography in the pitcher-plant mosquito, *Wyeomyia smithii*. Pages 47–67 in F. Gilbert, ed. *Insect life cycles: genetics, evolution, and coordination*. Springer, London.
- . 2000. The evolution of genetic architectures and the divergence of natural populations. Pages 245–263 in J. B. Wolf, E. D. Brodie III, and M. J. Wade, eds. *Epistasis and the evolutionary process*. Oxford University Press, New York.
- . 2001a. Genetic shift in photoperiodic response correlated with global warming. *Proceedings of the National Academy of Sciences of the USA* 98:14509–14511.
- . 2001b. Phenotypic evolution and the genetic architecture underlying photoperiodic time measurement. *Journal of Insect Physiology* 47:809–820.
- Bradshaw, W. E., and L. P. Lounibos. 1972. Photoperiodic control of development in the pitcher-plant mosquito, *Wyeomyia smithii*. *Canadian Journal of Zoology* 50:713–719.
- . 1977. Evolution of dormancy and its photoperiodic control in pitcher-plant mosquitoes. *Evolution* 31:546–567.
- Bünning, E. 1936. Die endogene Tagesrhythmik als Grundlage der photoperiodischen Reaktion. *Berichte der Deutschen Botanischen Gesellschaft* 54:590–607.
- . 1973. *The physiological clock*. Springer, New York.
- Cooke, B. D. 1977. Factors limiting the distribution of the wild rabbit in Australia. *Proceedings of the Ecological Society of Australia* 10:113–120.
- Danilevskii, A. S. 1965. *Photoperiodism and seasonal development in insects*. Oliver & Boyd, Edinburgh.
- Danks, H. V. 1987. *Insect dormancy: an ecological perspective*. Biological Survey of Canada (Terrestrial Arthropods), Ottawa.
- Evans, K. W., and R. A. Brust. 1972. Induction and termination of diapause in *Wyeomyia smithii* (Diptera Culicidae), and larval survival studies at low and subzero temperatures. *Canadian Entomologist* 104:1937–1950.
- Falconer, D. S. 1981. *Introduction to quantitative genetics*. Longman, London.
- Farner, D. S. 1959. Photoperiodic control of annual gonadal cycles in birds. Pages 717–750 in R. B. Withrow, ed. *Photoperiodism and related phenomena in plants and animals*. American Association for the Advancement of Science, Washington, D.C.
- Fochs, D. A., S. B. Linda, G. B. Craig, Jr., W. A. Hawley, and C. B. Pumpuni. 1994. *Aedes albopictus* (Diptera: Culicidae): a statistical model of the role of temperature, photoperiod, and geography in the induction of egg diapause. *Journal of Medical Entomology* 31:278–286.
- Frisch, L., ed. 1960. *Biological clocks*. Cold Spring Harbor Symposia on Quantitative Biology, vol. 25.
- Hairston, N. G., Jr., and W. E. Walton. 1986. Rapid evolution of a life-history trait. *Proceedings of the National Academy of Sciences of the USA* 83:4831–4833.
- Hard, J. J., W. E. Bradshaw, and C. M. Holzapfel. 1993a. The genetic basis of photoperiodism and evolutionary divergence among populations of the pitcher-plant mosquito, *Wyeomyia smithii*. *American Naturalist* 142:457–473.
- . 1993b. Genetic coordination of demography and phenology in the pitcher-plant mosquito, *Wyeomyia smithii*. *Journal of Evolutionary Biology* 6:707–723.
- Hartl, D. L., and A. G. Clark. 1989. *Principles of population genetics*. Sinauer, Sunderland, Mass.
- Hoy, M. A. 1978. Variability in diapause attributes of insects and mites: some evolutionary and practical implications. Pages 101–126 in H. Dingle, ed. *Evolution of insect migration and diapause*. Springer, New York.
- Istock, C. A., and W. G. Weisburg. 1987. Strong habitat selection and the development of population structure in a mosquito. *Evolutionary Ecology* 1:348–362.
- Lair, K. P., W. E. Bradshaw, and C. M. Holzapfel. 1997. Evolutionary divergence of the genetic architecture underlying photoperiodism in the pitcher-plant mosquito, *Wyeomyia smithii*. *Genetics* 147:1873–1883.
- Lankinen, P. 1986. Geographical variation in circadian eclosion rhythm and photoperiodic adult diapause in

- Drosophila littoralis*. *Journal of Comparative Physiology A* 159:123–142.
- Leather, S. R., K. F. A. Walters, and J. S. Bale. 1993. The ecology of insect overwintering. Cambridge University Press, Cambridge.
- Lees, A. D. 1973. Photoperiodic time measurement in the aphid *Megoura viciae*. *Journal of Insect Physiology* 19: 2279–2316.
- Masaki, S. 1983. Introduction. Pages 1–8 in V. K. Brown and I. Hodek, eds. *Diapause and life cycle strategies in insects*. Junk, The Hague.
- Menaker, M., ed. 1971. *Biochronometry*. National Academy of Sciences, Washington, D.C.
- Pittendrigh, C. S. 1960. Circadian rhythms and the circadian organization of living systems. *Cold Spring Harbor Symposia on Quantitative Biology* 25:159–184.
- . 1981. Circadian organization and the photoperiodic phenomena. Pages 1–35 in B. K. Follett and D. E. Follett, eds. *Biological clocks in seasonal reproductive cycles*. John Wright, Bristol.
- . 1993. Temporal organization: reflections of a Darwinian clock-watcher. *Annual Review of Physiology* 55: 17–54.
- Pittendrigh, C. S., and D. H. Minis. 1971. The photoperiodic time measurement in *Pectinophora gossypiella* and its relation to the circadian system in that species. Pages 212–250 in M. Menaker, ed. *Biochronometry*. National Academy of Sciences, Washington, D.C.
- Pittendrigh, C. S., and T. Takamura. 1987. Temperature dependency and evolutionary adjustment of critical night length in insect photoperiodism. *Proceedings of the National Academy of Sciences of the USA* 84: 7169–7173.
- . 1989. Latitudinal clines in the properties of a circadian pacemaker. *Journal of Biological Rhythms* 4: 217–235.
- . 1993. Homage to Sinzo Masaki: circadian components in the photoperiodic responses of *Drosophila auraria*. Pages 288–305 in M. Takeda and S. Tanaka, eds. *Seasonal adaptation and diapause in insects (in Japanese)*. Bun-ichi Sôgô Shuppan, Tokyo.
- Pittendrigh, C. S., W. T. Kyner, and T. Takamura. 1991. The amplitude of circadian oscillations: temperature dependence, latitudinal clines, and the photoperiodic time measurement. *Journal of Biological Rhythms* 6:299–313.
- Ross, H. H. 1964. The colonization of temperate North America by mosquitoes and man. *Mosquito News* 24: 103–118.
- Saunders, D. S. 1982. *Insect clocks*. Pergamon, Oxford.
- . 1990. The circadian basis of ovarian diapause regulation in *Drosophila melanogaster*: is the *period* gene causally involved in photoperiodic time measurement? *Journal of Biological Rhythms* 5:315–331.
- Smith, S. M., and R. A. Brust. 1971. Photoperiodic control of the maintenance and termination of larval diapause in *Wyeomyia smithii* (Coq.) (Diptera: Culicidae) with notes on oogenesis in the adult female. *Canadian Journal of Zoology* 49:1065–1073.
- Stiling, P. 1993. Why do natural enemies fail in classical biological control programs? *American Entomologist* 39:31–37.
- Takeda, M., and S. D. Skopik. 1985. Geographic variation in the circadian system controlling photoperiodism in *Ostrinia nubilalis*. *Journal of Comparative Physiology A* 156:653–658.
- . 1997. Photoperiodic time measurement and related physiological mechanisms in insects and mites. *Annual Review of Entomology* 42:323–349.
- Tauber, M. J., C. A. Tauber, and S. Masaki. 1986. *Seasonal adaptations of insects*. Oxford University Press, New York.
- Taylor, F., and J. B. Spalding. 1986. Geographical patterns in the photoperiodic induction of hibernial diapause. Pages 66–85 in F. Taylor and R. Karban, eds. *The evolution of insect life cycles*. Springer, New York.
- Templeton, A. R. 1986. Coadaptation and outbreeding depression. Pages 105–116 in E. Soulé, ed. *Conservation biology: the science of scarcity and diversity*. Sinauer, Sunderland, Mass.
- Thiele, H. U. 1977. Differences in measurement of daylength and photoperiodism in two stocks from subarctic and temperate climates in the carabid beetle *Pterosticus nigrata* F. *Oecologia (Berlin)* 30:349–365.
- TriMetrix. 1992. *Axum: technical graphics and data analysis*. TriMetrix, Seattle.
- Vaartaja, O. 1959. Evidence of photoperiodic ecotypes in trees. *Ecological Monographs* 29:91–111.
- Vaz Nunes, M., and D. Saunders. 1999. Photoperiodic time measurement in insects: a review of clock models. *Journal of Biological Rhythms* 14:84–104.
- Vaz Nunes, M., D. S. Koveos and A. Veerman. 1990. Geographical variation in photoperiodic induction of diapause in the spider mite (*Tetranychus urticae*): a causal relationship between critical nightlength and circadian period? *Journal of Biological Rhythms* 14:84–104.
- Wegis, M. C., W. E. Bradshaw, T. E. Davison, and C. M. Holzapfel. 1997. Rhythmic components of photoperiodic time measurement in the pitcher-plant mosquito, *Wyeomyia smithii*. *Oecologia (Berlin)* 110:32–39.
- Withrow, R. B., ed. 1959. *Photoperiodism and related phenomena in plants and animals*. American Association for the Advancement of Science, Washington, D.C.