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THE CONTRIBUTION OF AN HOURGLASS TIMER TO THE EVOLUTION OF PHOTOPERIODIC RESPONSE IN THE PITCHER-PLANT MOSQUITO, *WYEOMYIA SMITHII*

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Abstract.—Photoperiodism, the ability to assess the length of day or night, enables a diverse array of plants, birds, mammals, and arthropods to organize their development and reproduction in concert with the changing seasons in temperate climatic zones. For more than 60 years, the mechanism controlling photoperiodic response has been debated. Photoperiodism may be a simple interval timer, that is, an hourglasslike mechanism that literally measures the length of day or night or, alternatively, may be an overt expression of an underlying circadian oscillator. Herein, we test experimentally whether the rhythmic response in *Wyeomyia smithii* indicates a causal, necessary relationship between circadian rhythmicity and the evolutionary modification of photoperiodic response over the climatic gradient of North America, or may be explained by a simple interval timer. We show that a day-interval timer is sufficient to predict the photoperiodic response of *W. smithii* over this broad geographic range and conclude that rhythmic responses observed in classical circadian-based experiments alone cannot be used to infer a causal role for circadian rhythmicity in the evolution of photoperiodic time measurement. More importantly, we argue that the pursuit of circadian rhythmicity as the central mechanism that measures the duration of night or day has distracted researchers from consideration of the interval-timing processes that may actually be the target of natural selection linking internal photoperiodic time measurement to the external seasonal environment.

Key words.—Biological clocks, circadian rhythms, evolutionary physiology, geographical variation, photoperiodism, seasonality.

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The most pervasive environmental variable at temperate latitudes is the changing of the seasons. No life cycle at temperate latitudes is complete without the ability to exploit the favorable season, to avoid or mitigate the effects of the unfavorable season, and to switch in a timely manner between the two lifestyles. At temperate latitudes seasonal changes are regular and highly correlated with local day length. It is therefore not surprising that a wide variety of plants, vertebrates, and arthropods use day length (photoperiodism) to anticipate the changing seasons and to adjust their behavior, development, and reproduction (Vaartaja 1959; Withrow 1959; Anonymous 1960; Aschoff 1965; Menaker 1971). Photoperiodic response can affect the success of invading or introduced species (Cooke 1977), the escape of prey from a seasonal predator (Hairston and Walton 1986), outbreeding depression of managed populations (Templeton 1986), seasonal polyphenisms (Hazel 2002), and the adaptive response to rapid climate change (Bradshaw and Holzapfel 2001). Understanding the mechanism of photoperiodic adaptation therefore provides a means for understanding the evolutionary processes involved in the dispersal of organisms in the temperate zone and their potential to persist when confronted with environmental change.

At more northern latitudes, winter arrives earlier when days are longer than at more southern latitudes. One of the most robust ecogeographic ''rules'' is that the median or critical photoperiod (see Appendix for a glossary of terms) mediating 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 is then the phenotype undergoing selection and has repeatedly been shown to vary over wide geographic ranges in a manner consistent with its central role in the adaptive programming of seasonal development.

Originally, it was assumed that photoperiodic time measurement (PTM) was accomplished by a day-interval timer, literally by measuring the length of day; that is, by an hourglass mechanism. In 1936, Erwin Bünning proposed that PTM was a function of the central circadian pacemaker, that is, was the result of an endogenous rhythmic process. This proposition has been especially tantalizing because, if true, it would mean that the temporal organization of both daily and seasonal activity patterns of organisms were mediated by the common mechanism of circadian rhythmicity. Research and debate over a circadian versus interval-timer basis of photoperiodic response and its adaptive modification by seasonal selection have centered around three possible relationships: (1) The circadian clock comprises both the central mechanism of photoperiodic time measurement and the means by which output from the central mechanism is modified by evolution in response to geographically variable seasonal selection. An interval timer is not necessary for either process. (2) The central mechanism of photoperiodic time measurement is an interval timer but its output is modified by the circadian clock in response to geographically variable seasonal selection. (3) The central mechanism of photoperiodic time measurement is an interval timer whose output is modified independently from the circadian clock in response to geographically variable seasonal selection.

The first proposition now seems unlikely. The circadian

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clock in *Drosophila melanogaster* consists of an autoregulatory, negative feedback loop centered around the *period* gene on the X chromosome (Young 2000; Panda et al. 2002). Null mutations at the *period* locus render flies behaviorally arrhythmic but still result in a robust photoperiodic response curve (Saunders 1990). Similarly, in *Chymomyza* (Drosophilidae), selection for nonphotoperiodic diapause results in an autosomal mutation (*npd*) as well as a six-base-pair deletion in the *period* transcript (per^{npd}) (Koštál and Shimada 2001). Selected flies (i.e., whose males are per^{npd}/Y, *npd*/*npd*) are arrhythmic and nonphotoperiodic. Hybrid *per*+/Y, *npd*+/*npd* males, are normally rhythmic and have a normal photoperiodic response, whereas hybrid per^{npd}/Y, *npd*+/*npd* males are weakly rhythmic but still have a normal photoperiodic response. These results indicate that a wild-type *npd* allele can partially rescue rhythmic behavior but a dysfunctional *period* gene does not interfere with normal photoperiodic response. Both of these studies show that a functional circadian clock is not necessary for photoperiodic response.

In Saunders' (1990) experiments, the critical photoperiod in the *period* null mutants was shifted toward shorter day lengths, meaning that the circadian clock may serve as an epistatic modifier of an interval-timer-based photoperiodic response (Bradshaw and Holzapfel 2001). However, disfunction of one or more of the central circadian rhythm genes causes pervasive arrythmicity of cycling transcripts throughout the genome (Claridge-Chang et al. 2001). The shifted critical photoperiod in Saunders' (1990) experiments may then have been the manifestation of physiological stress concomitant with a breakdown in circadian organization generally, rather than the disruption of the adaptive connection between circadian rhythms and a photoperiodic interval timer specifically. Hence, Saunders' results, while eliminating the first proposition from consideration, do not discriminate between the second and third propositions, above. Herein, we devise a test to discriminate between these two propositions. We show that in the pitcher-plant mosquito, *Wyeomyia smithii*, an interval timer is sufficient and a circadian modifier insufficient, and, in fact, inappropriate to explain the evolution of critical photoperiod over altitudinal and latitudinal clines in seasonality.

Experimental Approach

Historically, the most common experiment used to infer a circadian contribution to photoperiodic time measurement is the Nanda-Hamner or *T* experiment (Takeda and Skopik 1997; Vaz Nunes and Saunders 1999; Tauber and Kyriacou 2001). In this experiment, organisms are exposed to a short day and, in separate experiments, to increasing night lengths to produce a total light:dark cycle from $T = L + D = 24$–72 h. Each of the test regimens consists of a short day and a long night. The idea, then, is that if photoperiodic time measurement were an interval timer, all the L:D cycles from 10:14 to 10:62 would result in a short-day response. By contrast, if photoperiodic time measurement were a process of the circadian clock, then there would be a rhythmic sensitivity to light that would continue to cycle between photo-sensitive and photo-insensitive phases during the progressively longer nights. If at the end of the long night, dawn fell in a photosensitive phase of the cycle, a long-day response would ensue; if at the end of the long night dawn fell in a photoinsensitive phase of the cycle, a short-day response would ensue. Consequently, the response to increasing night lengths should be a cyclic switching between short- and long-day responses. Indeed, this cyclic response to *T* experiments is the one obtained in most experiments involving plants, birds, mammals, and arthropods (Pittendrigh 1981); but these results still do not distinguish between the disruption of the adaptive connection between circadian rhythms and a photoperiodic interval timer specifically and the consequences of a breakdown in circadian organization generally. Hence, when the external light:dark cycle ''resonates'' or cycles in phase with the internal circadian clock, the short day and long night of a *T* experiment are correctly interpreted as a short-day; when the external short day and long night are discordant or out of phase with the internal circadian clock (analogous to jet lag), the temporal organization of the whole system is disrupted and the short day and long night of a *T* experiment are misinterpreted as a long day (Pittendrigh 1972; Vaz Nunes and Saunders 1999; Veerman 2001). In the latter case, a rhythmic response to *T* experiments constitutes an artifact of disorganized physiology, that is, a false indicator of circadian involvement in normal photoperiodic response.

Wyeomyia smithii is the sole temperate representative out of 50 or more species in its otherwise tropical and subtropical genus (Stone et al. 1959). Throughout their range from the Gulf of Mexico to northern Canada, *W. smithii* are photoperiodic for the initiation, maintenance, and termination of larval diapause (Bradshaw and Lounibos 1977). Like the many arthropods cited above, the critical photoperiod of *W. smithii* increases regularly ($R^2 \geq 92\%$) with latitude and altitude and constitutes the major adaptation to the temperate climate of North America (Bradshaw 1976; Hard et al. 1993; Lair et al. 1997; Bradshaw and Holzapfel 2001). *Wyeomyia smithii* exhibits a typical, rhythmic response to *T* experiments (Fig. 1); the amplitude of this response declines with increasing latitude or altitude (Wegis et al. 1997; Bradshaw et al. 2003) analogously to Asian flies, North American moths, and European mites and beetle (Thiele 1977; Takeda and Skopik 1985; Vaz Nunes et al. 1990; Pittendrigh and Takamura 1993). The specific question then is whether this rhythmic response and its geographic variation have a circadian basis or the mechanism is that of an interval timer.

Evolution by adaptive modification of an independent interval timer

If geographic variation in critical photoperiod represents modification of a day-interval timer independently of the circadian clock, then the photoperiodic response curves observed when $T = 72$ h and day length is varied should be independent of night length. Hence, a day-interval timer predicts that with *T* fixed at 72 h, long-day response should *increase* as the day length increases; the open squares on the photoperiodic response curve when $T = 24$ h in Figure 2A provide the quantitative prediction for that particular population.

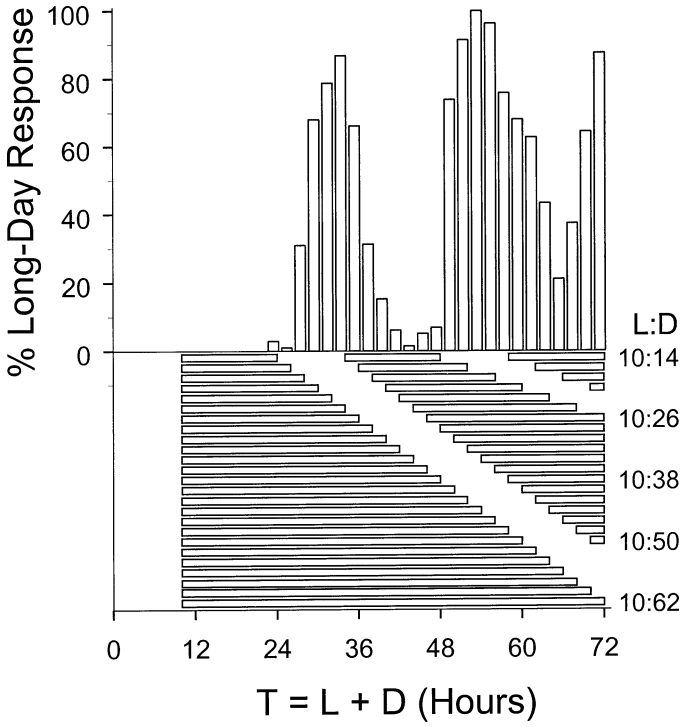

FIG. 1. Developmental (long-day) response of *Wyeomyia smithii* to *T* experiments with a 10-h day and variable night length to create $T = L + D = 24–72$ h. The rhythmic long-day response indicates a rhythmic sensitivity to light and historically has implicated circadian involvement in photoperiodic time measurement. The curve shows the long-day response of a southern (30°N) population. Plotted from data in Bradshaw et al. (2003).

Evolution by circadian modification of the output from an interval timer

If geographic variation in critical photoperiod represents modification of an interval timer by the circadian clock, then the photoperiodic response curves observed when $T = 72$ h and day length is varied should be dependent on night length. In this case, the rhythmic fluctuations of long-day response in Figure 1 supposedly represent an underlying photosensitivity rhythm that initiates at lights-off and continues to cycle during the long night. Consequently, a delay in lightsoff should effect a concomitant delay in that sensitivity rhythm (see Saunders 1973, fig. 5). Figure 2B illustrates this model for *W. smithii*. The bold line plots the response of a single southern population and the lighter lines plot, from left to right, the projected, delayed sensitivity rhythm effected by increasing the day length from 10 to 13 to 14.5 and to 17 h, respectively, while *T* is fixed at 72 h. Hence, a circadian-based model predicts that, with *T* fixed at 72 h, long-day response should *decrease* as the day length increases and the open squares at 72 h provide the quantitative prediction for that particular population.

MATERIALS AND METHODS

Our methods for animal husbandry, for generating photoperiodic response curves, and for setting up the *T* experiments are presented in detail in Bradshaw et al. (2003); we provide below an abbreviated description for the methods required to understand our results.

Procedures Common to All Experiments

Overwintering larvae were collected from 14 localities in North America from 30–46°N (Table 1) and maintained in

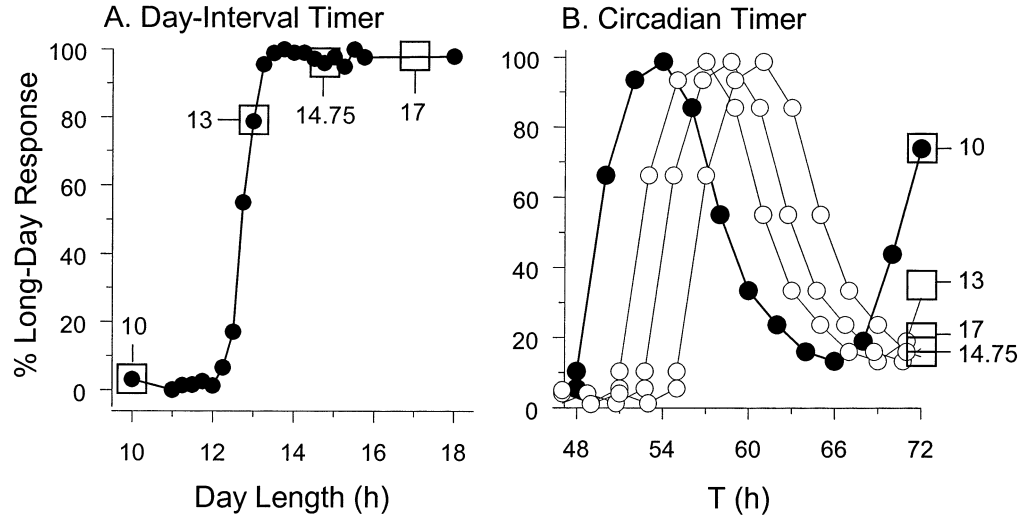

FIG. 2. Predicted responses in a southern population to variable L:D cycles when $L + D = T$ is fixed at 72 h. (A) Predictions for an hourglass or day-interval timer. The plot in A shows the long-day response curve for 10–18-h day lengths when $T = 24$ h. The open squares then provide the predictions of long-day response to day lengths of 10, 13, 14.75, and 17 h when $T = 72$ h. (B) Predictions for a circadian-based timer. The plot in B shows a long-day response curve (bold curve) from a *T* experiment for a 10-h day and night lengths of 38 to 62 h to create $T = 48–72$ h. If photoperiodic time measurement is accomplished by a circadian sensitivity to light that continues to cycle during the long dark period of a *T* experiment, then when *T* is fixed at 72 h and day lengths vary from 10 to 17 h, the initiation of that rhythm at lights-off is progressively delayed, and the long-day response curve should shift progressively to the right (light curves). The open squares at $T = 72$ h then provide the predictions of long-day response to day lengths of 10, 13, 14.75, and 17 h when $T = 72$ h.

TABLE 1. Origin of populations used in this study. Shown are: state of origin; regions referred to in the text and figures as southern (South), midlatitude (Mid), northern (North), and mountain (Mtn); and specific population acronym for cross reference with earlier papers from our laboratory.

Origin	Region	Reference	Latitude (°N)	Altitude (m)
FL	southern	CR	31	40
FL	southern	WI	30	10
NC	southern	GS	34	20
NC	southern	PM	35	90
NC	mountain	DB	35	900
NC	mountain	HK	35	900
NC	mountain	CB	36	900
MD	midlatitude	NP	38	20
NJ	midlatitude	HV	40	10
NJ	midlatitude	MM	40	10
NJ	midlatitude	PB	40	10
PA	midlatitude	TH	41	600
ME	northern	KC	46	370
WI	northern	ML	46	500

the laboratory for at least three but no more than 10 generations prior to experimentation. Experimental animals were sampled from the continuously reproducing stock population. Larvae used in experiments were reared on short-days (L:D = 8:16) at 21 ± 0.5°C for at least 30 days 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 into a large pan, stirred, and then haphazardly allocated to one of three replicate cohorts of 35 larvae for each treatment. Experiments were carried out in a controlled-environment room at 23 ± 0.5°C inside light-tight photoperiod cabinets. At the beginning of the experiment and twice a week thereafter, the dishes of larvae from each of the 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.

Photoperiodic Response Curves

To determine the photoperiodic response curves at ecologically relevant photoperiods when $T = 24$, diapausing larvae from each population were subjected to day lengths ranging from 10 to 18 h. All larvae were exposed to the experimental treatments for 30 days, and then transferred to short days (L:D = 8:16) at 21 ± 0.5°C for two additional weeks to allow and record 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 two weeks on short days, the remaining larvae were censused and then discarded. Percentage development (long-day response) was calculated at the end of the experiment for each replicate as total number of larvae having pupated ÷ (total number of larvae having pupated + number of larvae remaining alive on day 44).

T Experiments

To determine the rhythmic responses to varying night lengths when $T = 72$, larvae were exposed to a 10-h day length followed by a varying night length of 54 to 62 h to create $T = 64$–72 h in five separate experiments. All larvae were exposed to the experimental treatments for eight weeks, and then transferred to short days (L:D = 8:16) at 21 ± 0.5°C for two additional weeks to allow and record development of larvae that had been stimulated by the L:D cycle during the experiment but that had not pupated by day 56. At the end of the two weeks on short days, the remaining larvae were censused and then discarded. Percentage development (long-day response) was calculated at the end of the experiment for each replicate as total number of larvae having pupated ÷ (total number of larvae having pupated + number of larvae remaining alive on day 70). We plotted the mean long-day response for three replicates for each population as a function of T, and then used these response curves as predictors of development using a circadian-based model.

Circadian versus Hourglass Timers

To test the predictions of photoperiodic response generated by hourglass-based and circadian-based models (Fig. 2A,B), we exposed larvae to day lengths of 10, 13, 14.75, and 17 h of light followed by night lengths of 62 to 55 h, respectively, to create $T = 72$. These day lengths were chosen so as to provide short days for all populations (10 h), long days for southern but not midlatitude, mountain, or northern populations (13 h), long days for all but the northern populations (14.75 h), and long days for all populations (17 h) assuming a day-interval model. We tested these predictions by correlating observed developmental (long-day) response with developmental response predicted from each population's respective response to variable day length when $T = 24$ h (Fig. 2A) or rhythmic response to a short day and variable night length (Fig. 2B). Experimental procedures followed those of the T experiment, above. In each case, the points were not independent and we tested for the significance of the correlation of observed with expected values using the number of populations ($n = 14$) as our sample size and calculated the significance of r with $n - 2 = 12$ df.

RESULTS

Photoperiodic Response Curves

In the vicinity of the ecological critical photoperiods (10–18 h), all populations, regardless of geographic origin, showed robust, sigmoid dose-response curves of development (long-day response), ranging from less than 5% to more than 90% (Fig. 3A). The intercepts of the dashed vertical lines on each of the response curves provide the percentage of long-day responses expected from a day-interval model when $T = 72$ and day lengths are set at 10, 13, 14.75, and 17 h.

T Experiments

At long night lengths following a 10-h day length, developmental (long-day) responses decreased from $T = 64$–66 h and then increased from $T = 66$–72 h. Long-day response declined from southern to midlatitude to northern populations to mountain populations (Fig. 3B). The intercepts of the dashed vertical lines on each of the response curves provide

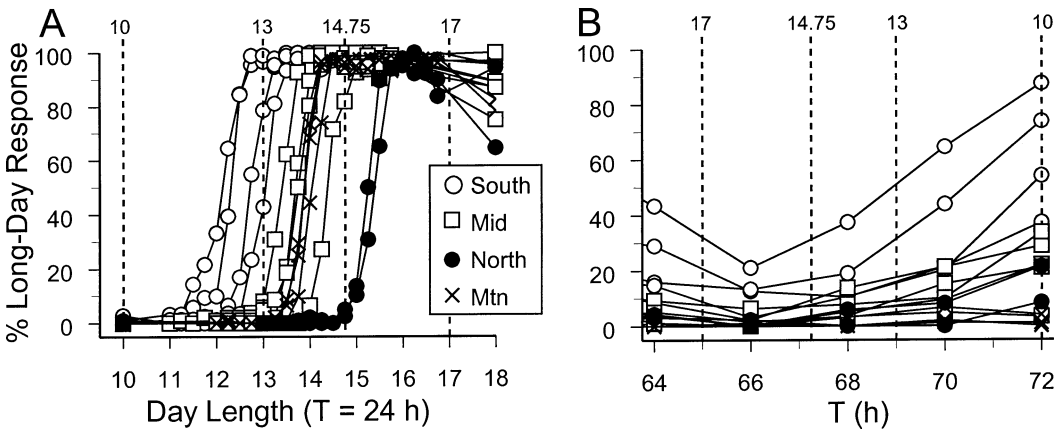

FIG. 3. Long-day responses of *Wyeomyia smithii* (A) to day lengths of 10 to 18 h when $T = 24$ h and (B) to a 10-h day length when $T = 64$–72 h. In both plots, the intercepts on the vertical dashed lines represent the expected long-day responses predicted (Fig. 2) from (A) a day-interval, hourglass timer and (B) a rhythmic circadian sensitivity to light. Plotted from data in Bradshaw et al. (2003).

the long-day responses expected from a circadian model when $T = 72$ and day lengths are set at 10, 13, 14.75, and 17 h.

Circadian versus Hourglass Timers

When exposed to varying day lengths with $T = 72$ h, all populations showed typical, sigmoid photoperiodic response curves (Fig. 4A). The critical photoperiod when $T = 72$ h was positively correlated with the critical photoperiod when $T = 24$ h (Fig. 4B; $r^2 = 0.87$, $P < 10^{-5}$). The slope of the regression was not significantly different from one ($b \pm$ SE $= 1.17 \pm 0.13$, $t = 1.31$, $P = 0.215$) and the intercept was not significantly different from zero ($a \pm$ SE $= -2.43 \pm 1.80$, $t = 1.35$, $P = 0.202$). Fitting the data to a second-order polynomial did not return a significant quadratic regression coefficient ($b \pm$ SE $= -0.164 \pm 0.117$, $t = 1.41$, $P = 0.188$). The critical photoperiods determined with $T = 24$ h provided a highly accurate prediction of critical photoperiods with $T = 72$ h.

Figure 5 compares developmental (long-day) responses predicted from a day-interval timer (Figs. 2A, 3A) with those predicted from a circadian timer (Figs. 2B, 3B) when $T = 72$ h and day lengths are varied from 10 to 17 h. The predictions based on a day-interval timer (Fig. 5A) provided a close positive correlation between observed and expected long-day response ($r^2 = 0.91$, $P < 10^{-7}$). The predictions based on a rhythmic circadian timer (Fig. 5B) did not provide a significant correlation between observed and expected long-day response ($r^2 = 0.05$, $P = 0.45$) and the sign of the nonsignificant correlation was negative, not positive as expected.

The above results incorporated the responses of southern populations showing a strong rhythmic expression to T experiments with northern and mountain populations showing a reduced rhythmic expression (Bradshaw et al. 2003). To control for the possibility that pooling of the northern and southern data may have occluded expression of a significant rhythmic component that was indeed present, we repeated the above analyses using only the four southern populations. Again, the predictions based on a day-interval timer (Fig. 5C) provided a close positive correlation between observed

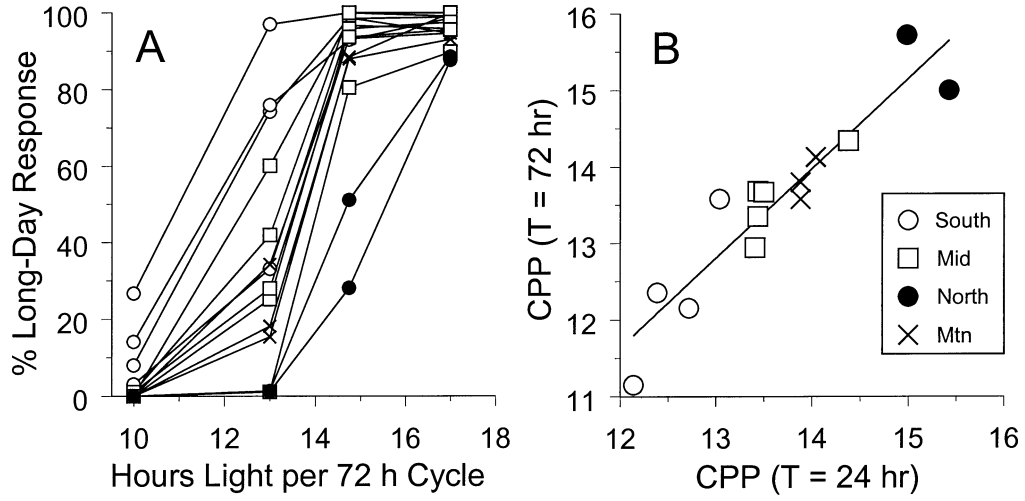

FIG. 4. (A) Long-day response of *Wyeomyia smithii* to day lengths of 10 to 17 h when $T = 72$ h, and (B) the relationship in critical photoperiod (CPP) between when $T = 24$ h and when $T = 72$ h.

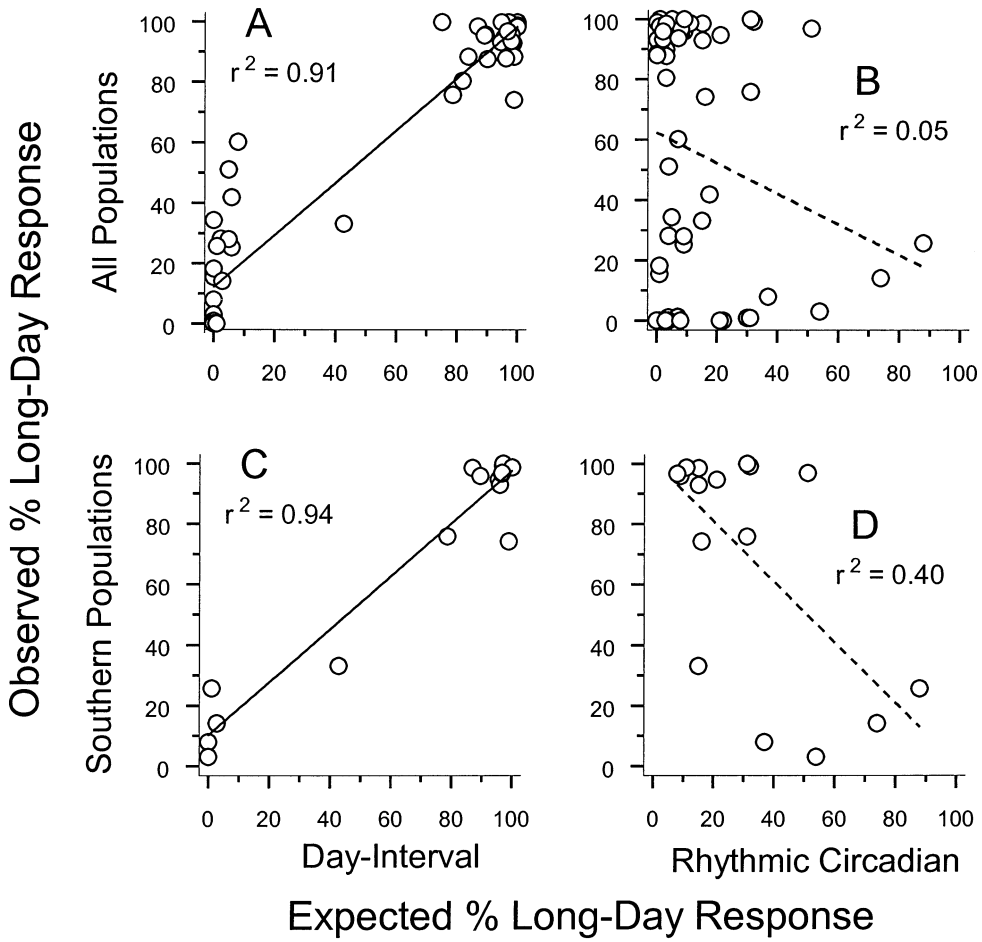

FIG. 5. Expected and observed long-day responses of *Wyeomyia smithii* predicted by a day-interval timer (A, C) or a rhythmic circadian sensitivity to light (B, D) in all populations (A, B: 30–46°N) or in southern populations only (C, D: 30–36°N). Expected responses are based on the rationale outlined in Figure 2 and the intercepts on the dashed lines in Figure 3.

and expected long-day response ($r^2 = 0.94$, df = 2, $P = 0.015$). The predictions based on a rhythmic circadian timer (Fig. 5D) did not provide a significant correlation between observed and expected long-day response ($r^2 = 0.40$, df = 2, $P = 0.24$) and the sign of the nonsignificant correlation was still negative, not positive as expected.

DISCUSSION

Herein we show that predictions based on an hourglass or day-interval timer (Figs. 2A, 3A) are sufficient (Fig. 5A,C) and predictions based on a rhythmic circadian timer (Figs. 2B, 3B) are inadequate (Fig. 5B,D) to explain the evolutionary modification of photoperiodic time measurement in *Wyeomyia smithii*. Our results do not mean that the central circadian clock does not regulate a myriad of important physiological, developmental, and behavioral events in the daily life of organisms and that *T* experiments are not useful for examining circadian regulation of these daily events. Our results do mean that *T* experiments can be false indicators of a connection between the circadian clock regulating daily events and the photoperiodic timer regulating seasonal events. As we pointed out in the introduction, circadian organization can break down when the period of the external L:D cycle is discordant with the period of the internal circadian clock. Our results indicate that rhythmic responses to *T* experiments may constitute an artifact of disrupted circadian organization generally rather than a specific regulatory connection between circadian rhythmicity and seasonal adaptation. Circadian rhythmicity simply does not regulate the geographic, adaptive modification of photoperiodic time measurement in *W. smithii*.

In drosophilid flies, several genes have been identified as essential to the normal functioning of circadian rhythmicity (Young 2000; Panda et al. 2002). Mutations at the *period* locus render flies in two different genera arrhythmic at both the molecular and behavioral levels but do not interfere with the expression of a robust photoperiodic response curve (Saunders 1990; Claridge-Chang et al. 2001; Koštál and Shimada 2001). Our results with *W. smithii* at the physiological level show that invoking a circadian clock to explain evolutionary modification of the output from a photoperiodic timer is unnecessary and misleading. From the above, we conclude that an hourglasslike mechanism, independent of the central circadian clock, is responsible for both the physiological architecture of PTM and the adaptive modification of its output.

In retrospect, it would seem maladaptive to couple the central circadian pacemaker to the degree of evolutionary flexibility required for PTM. A functional circadian clock is responsible for the coordinated timing of hundreds of transcriptional events during a day (Claridge-Chang et al. 2001; McDonald and Rosbash 2001). In *W. smithii*, critical photoperiod increases by about one standard deviation in mean phenotype per increase of two degrees in latitude between Florida and Canada (Lair et al. 1997; Bradshaw and Holzapfel 2000). If this dramatic change in critical photoperiod were effected by a concomitant modification of either the period or amplitude of the circadian clock, that change would have pervasive, discordant effects on the daily timing of biochemical events throughout the organism. Instead, we propose that PTM is a process separate from the central circadian pacemaker and capable of independent evolutionary modification without disrupting the organism-wide temporal organization of daily events.

We believe that the tantalizing appeal of circadian rhythmicity's orchestration of both daily and seasonal events in the life history of organisms has distracted investigators (including ourselves) away from pursuing the independent nature of PTM. It is now time to focus on the phenomenon of photoperiodism itself and inquire as to the genetic basis for its essential function and for its adaptive modification. Only then can we understand the mechanistic processes underlying seasonal adaptation of diverse organisms dispersing within the temperate zone or confronted with climate change.

Acknowledgments

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Appendix

Circadian rhythm.—An endogenous, internally maintained rhythm with a period of about a day. Circadian rhythms repeat indefinitely under constant conditions, that is, without external, time-setting cues, usually constant darkness at a constant temperature.

Critical photoperiod.—Hours of light per day that stimulate 50% development and initiate or maintain 50% diapause in a sample population; the day length at which organisms switch from a long-day to a short-day response or vice versa.

Diapause.—Arthropod dormancy, herein assumed to be hibernal.

L:D.—Light:dark cycle; L:D = 10:14 represents 10 h of light and 14 h of darkness in a single 24-h cycle.

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.—Usually sigmoidal in shape, is the relationship between a behavioral, physiological, or developmental event and day length. The 50% intercept at ecologically relevant day lengths in the region of 10 to 18 h, defines the critical photoperiod.

Photoperiodic time measurement.—The ability of organisms to assess the duration or length of day or night.

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.