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Rhythmic components of photoperiodic time measurement in the pitcher-plant mosquito, *Wyeomyia smithii*

Abstract Photoperiodic time measurement regulating larval diapause in the pitcher-plant mosquito, *Wyeomyia smithii*, varies in a close relationship with latitude. The critical photoperiod mediating the maintenance and termination of diapause is positively correlated with latitude ($r^2 = 0.977$) among six populations from southern (30–31° N), intermediate (40° N), and northern (46–49° N) latitudes in North America. The developmental response to unnaturally short and to unnaturally long photoperiods declines with increasing latitude, so that longer critical photoperiods are associated with a downward rather than a lateral shift in the photoperiodic response curve. Exotic light and dark cycles of varying period (T) with a short (10 h) photophase and a scotophase ranging from 14 (T = 24) to 62 (T = 72) h, reveal two geographic patterns: a decline in perturbability of the photoperiodic clock with increasing latitude, and no change with latitude in the 21-h period of rising and falling development with increasing T. These results show (1) that there is a rhythmic component to photoperiodic time measurement in *W. smithii*, (2) that the period of this rhythm is about 21 h in all populations, and (3) that more northern populations show decreasing responsiveness to photoperiod and increasing stability against perturbation by exotic period lengths (T > 24). Previous studies on *W. smithii* indicate that this single temperate species of a tropical and subtropical genus has evolved from south to north. We therefore conclude that the evolution of increasing critical photoperiod in *W. smithii* during its adaptive radiation into North America has more likely involved the amplitude and not the period of the underlying circadian pacemaker.

Key words Evolution · Phenology · Circadian · Biogeography · Diapause

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Introduction

Photoperiodism is a pivotal trait in hundreds of arthropods for synchronizing the life cycle with the changing seasons (Masaki 1983). The rapid evolution of photoperiodism in invading species (Hoy 1978; Tauber et al. 1986, pp. 238–245; Fochs et al. 1994) or in response to altered predator phenology (Hairston and Walton 1986) attests to its adaptive importance in maximizing fitness. One of the most powerful approaches to understanding the evolution of photoperiodic time measurement (PTM)[1] has been the examination of photoperiodic response curves (PPRCs) among populations of varying geographic origin (Danilevskii 1965; Bradshaw 1976; Taylor and Spalding 1986; Danks 1987, Table 24). More recently, these investigations have been extended to consider the mechanistic bases of PTM, especially by examining parallel variation in overt circadian rhythms and in components of PTM among populations of *Drosophila* (Lankinen 1986; Pittendrigh et al. 1991; Pittendrigh and Takamura 1993).

In temperate Japan, the incidence of diapause in *D. auraria* at a fixed temperature and photoperiod, as well as the critical photoperiod (CPP), increases with latitude (Kimura 1984, 1988; Pittendrigh and Takamura 1987). The similarity between the latitudinal cline of the eclosion pacemaker of *D. auraria* (Pittendrigh and Ta-

kamura 1987, 1989) and the latitudinal cline in the amplitude and temperature dependency of the PPRC of adult diapause lead Pittendrigh et al. (1991) to propose an "amplitude hypothesis" for PTM. Based on the analysis of Pavlidis' (1967) model of a circadian pacemaker, Pittendrigh et al. (1991) showed that as the amplitude of the circadian pacemaker increased, it became increasingly resistant to perturbation by light. The increase in CPP of northern populations could then be due to an increase in the amplitude of the circadian pacemaker with a concomitant decrease in its sensitivity to light and a vertical shift in the PPRC (Pittendrigh and Takamura 1989, 1993; Pittendrigh et al. 1991). There is a cautionary note here: cumulative evidence indicates that independent, or at least distinct, loosely coupled pacemakers underlie PTM and overt behavioral rhythms (Pittendrigh et al. 1970; Pittendrigh and Minis 1971; Saunders et al. 1989; Saunders 1990; Pittendrigh and Takamura 1987, 1993). Overt behavioral rhythms and the rhythmic component of PTM may show "parallel peculiarities" (Minis 1965) but the connection need not be causal. In *D. littoralis*, critical photoperiod over a latitudinal range of 30° "is not correlated with any of the eclosion rhythm parameters" in the same populations (Lankinen 1986, p. 140). As a consequence, the contribution of circadian rhythmicity to photoperiodic time measurement can be evaluated reliably only by examining the formal properties of photoperiodism itself, and we use this approach.

Herein, we ask whether a higher CPP at more northern latitudes reflects primarily the evolution of the amplitude or the period of the underlying circadian rhythm, using the pitcher-plant mosquito, *Wyeomyia smithii*. *W. smithii* is photoperiodic for the initiation, maintenance, and termination of larval diapause (Smith and Brust 1971; Evans and Brust 1972; Bradshaw and Lounibos 1972; Lounibos and Bradshaw 1975). The critical photoperiod for the initiation and maintenance of diapause is tightly correlated with latitude and altitude ($R^2 = 0.96$) and with length of the frost-free period ($r^2 = 0.92$) at the locality of origin (Bradshaw 1976). Selection due to changing seasonality during its evolutionary expansion has resulted in the divergence from southern to northern populations of over 9 standard deviations in critical photoperiod (Hard et al. 1993a). Photoperiodic response represents the major, evolutionarily dynamic life history trait yet identified in *W. smithii* (Bradshaw and Holzapfel 1990; Hard et al. 1993b) and this response is tightly correlated with the evolutionary trajectory of *W. smithii* in North America. Laboratory stocks of *W. smithii* can be stored for 6 months as diapausing larvae on short days at 21°C. Diapause is always photoreversible and diapausing stocks may be stimulated to resume development rapidly by exposing them to long days. Unlike *Ostrinia nubilalis* (Skopik and Takeda 1986), the critical photoperiod in *W. smithii* is the same for both the onset and the maintenance of diapause (Bradshaw and Lounibos 1972). Diapausing larvae are therefore able to accumulate photoperiodic information over an indefinitely long period, in contrast to the relatively brief sensitive period determining the onset of diapause (Campbell and Bradshaw 1992).

Amplitude of the rhythm

Direct evidence for the involvement of the pacemaker amplitude in photoperiodic response would come from an inverse amplitude in the photoperiodic phase response curve. To our knowledge, the photoperiodic phase response curve has been determined only for *Sarcophaga argyrostoma* (Saunders 1976) and relies on the response of this species to light breaks throughout a short-day, long-night cycle with a period of T = 72 h. In *W. smithii*, response to 1-h light breaks during the scotophase of a (Light:dark) L:D = 12:60 regimen occurs only during the very early and very late portions of the scotophase (W.A. Hawley, unpublished results) so that determination of a photoperiodic response curve is, at present, not possible in this species. Consequently, we test indirectly for geographic variation in the amplitude of the underlying oscillator by examining the entire PPRC from 0–24 h of light per day (Fig. 1). The latitudinal increase in critical photoperiod might be accomplished by a straightforward shift in the phase angle between the external photoperiod and the internal rhythm of photosensitivity [the inducible phase of Pittendrigh and Minis (1964)]. In this case (Fig. 1A), the entire PPRC should shift laterally towards longer daylengths at higher latitudes; there should be no change, and there may even be an increase, in responsiveness to unnaturally longer or short photoperiods. Alternatively, the latitudinal increase in critical photoperiod might be accomplished by an increasing amplitude of the underlying circadian pacemaker (Fig. 1B). As the amplitude of the pacemaker increases, its endogenous inertia renders it less perturbable by light (Pittendrigh et al. 1991) and the response to all daylengths should decrease. In this case, the entire PPRC should shift vertically downwards at higher latitudes. We then test an explicit prediction of the amplitude hypothesis: developmental response to both unnaturally short (0–8 h) and unnaturally long (18–24 h) photophases should be lower in northern than southern populations.

Period of the rhythm

Perhaps the most powerful means of detecting a rhythmic component of PTM are Nanda and Hamner (1959) or "T" experiments, so called because the period (T) of the L:D cycle is systematically varied. Here, we expose diapausing larvae to a fixed, short photophase and vary the scotophase to create T = 24–72 h in separate experiments. If PTM in *W. smithii* consisted of an interval timer or hourglass process, then larvae should maintain diapause regardless of T. Alternatively, if PTM involved a rhythmic component of photosensitivity, then diapause should be a periodic function of T, occurring and
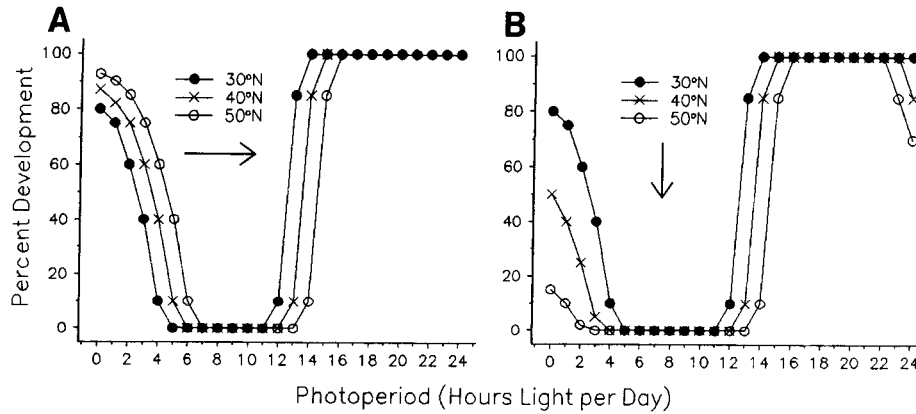
A **B**

Percent Development

Photoperiod (Hours Light per Day)

Fig. 1A, B Alternative predictions for two mechanisms explaining the latitudinal increase in critical photoperiod. **A** A change in the phase angle between the external photoperiod and the internal rhythm of photosensitivity causes a lateral shift in the entire photoperiodic response curve to longer daylengths at higher latitudes. **B** An increase in the amplitude of the underlying circadian pacemaker renders it less perturbable by light, thereby reducing responsiveness to all day-lengths, and causing a vertical, downward shift in the enitre photoperiodic response curve at higher latitudes

reoccurring at circadian intervals equal to τ. We then test the explicit prediction that if the evolution of PTM in *W. smithii* involves the period of the underlying circadian oscillation, τ should vary systematically with latitude.

Materials and methods

Collection and maintenance

We collected overwintering *W. smithii* from six localities in North America in spring 1993 (Fig. 2). Populations from Alabama (AL, 31° N), Florida (FL, 30° N), New Jersey (NJ, two populations at
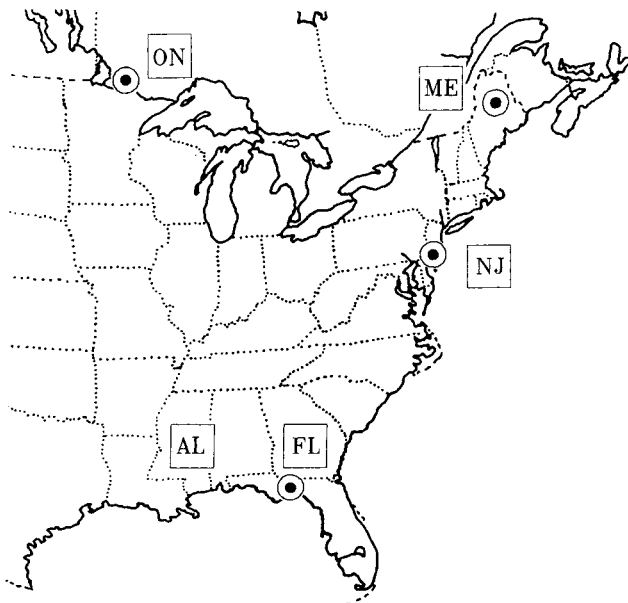

Fig. 2 Origin of *Wyeomyia smithii* populations in North America

40° N), Maine (ME, 46° N), and Ontario (ON, 49° N) correspond to LI, WI, MM, PB, KC, and DL, respectively, of earlier publications from this laboratory. All populations were reared for at least two but no more than five generations in the laboratory prior to the start of experiments. Experiments used larvae reared in a short-day photoperiod (L:D = 8:16) at 21 ± 0.5°C and that had been on short days for 1 month prior to initiating the experiment. Within any one population, diapausing larvae were pooled from a number of Petri dishes into a large pan, stirred, and then allocated to make up two replicate cohorts of 50 animals each for each experimental treatment. Experiments were run with 25 larvae per 150 × 25 mm Petri dish. Prior to the start of experiments, two of the dishes (50 total larvae) were assigned to an A replicate and two additional dishes to a B replicate.

All experiments were run in a controlled-environment room at 23 ± 0.5°C inside light-tight photoperiod cabinets where diapausing larvae were exposed to a 4-W, cool-white fluorescent lamp at a distance of 10–20 cm. To avoid a parallel thermoperiod, the ballasts were located outside the cabinet and each lamp was housed in a clear polycarbonate tube through which was blown 100 ft³/min forced air.

Photoperiodic response curves

Four dishes of 25 larvae each from each population were exposed to 0–24 h light per day in 1-h increments. Larvae were exposed to the experimental photoperiod for 30 days, then transferred to short days (L:D = 8:16) at 21 ± 0.5°C for 15 additional days to permit development of stimulated larvae. At the end of 45 days, percentage development was calculated for each replicate as (cumulative number of larvae having pupated) ÷ (cumulative number of larvae having pupated + number of larvae alive on day 45). For each replicate for each population, we calculated response to unnaturally short photoperiods as log (area under the PPRC from 0–8 h) and response to unnaturally long photoperiods as log (area under the PPRC from 18–24 h). Log (area) was then subjected to nested ANOVA with differences among latitudes (north, intermediate, south) evaluated from $F_{2,3}$ = mean square$_{latitude}$/mean square$_{localities\ within\ latitudes}$.

To determine the ecologically relevant critical photoperiod with greater precision, the above experiment was repeated, except that diapausing larvae were exposed to 4–6 photoperiods in half-hour increments ranging from 1.5 h shorter to 1.5 h longer than the critical photoperiod estimated from the above experiment. The ecological critical photoperiod was then estimated as the 50% intercept on the PPRC for each replicate.

Period of the rhythm underlying the photoperiodic response

To estimate the period of the rhythmic component of PTM, we used Nanda-Hamner-type experiments. In these experiments, the photophase was held constant at 10 h, a short, diapause-maintaining photophase on a L:D = 10:14 regimen, and the dark or

scotophase was varied. We ran these experiments in two blocks. In each block, two replicates of 50 diapausing larvae each from each population were exposed to each treatment.

In the first block, the scotophase was varied from 14 to 62 h (T = 24–72 h) in 2-h increments. Larvae were exposed to the fixed experimental T for 30 days and then transferred to short days (L:D = 8:16) at 21 ± 0.05°C for 15 additional days. After a total of 45 experimental days, percent development was calculated as above. A plot of percentage development as a function of T revealed two minima of low developmental response (high maintenance of diapause) in the vicinity of T = 45 and T = 66 h. At the same time, the developmental response was lower in more northern populations, resulting in a lower resolution of the minima. The second block of experiments was designed to overlap these minima and to increase developmental response in more northern populations by extending the experimental time.

In the second block, the scotophase varied from T = 39 to T = 49 h in 2-h increments and T = 63 to T = 68 h in 1-h increments. Larvae were exposed to the experimental treatments for a total of 8 weeks without supplemental exposure to short days, but censused after 30 days and after 8 weeks so that the relative developmental response could be evaluated after a short treatment time (30 days) in the southern populations and after prolonged stimulation (8 weeks) in the more northern populations.

For both the first and the second block, we plotted percentage development, calculated as above, as a function of T for each replicate and for each block within each population. The plot gave us four potential estimates for the position of a minimum development in the vicinity of 45 and in the vicinity of 66 h for each population. We scored the position (T) of a minimum in a hierarchical manner. First, if in five sequential points, the first three plotted as consistently declining development and the last three as consistently increasing points, we scored the T corresponding to the middle point as a minimum. Second, if no such minimum was

defined, we used a locally weighted linear regression ["lowess plots" TriMetrix (1993)] of percent development with T = 35–52 h or T = 60–72 h as the predictor variable. In lowess plots, an iterative, nonparametric algorithm is used to obtain smoothed values for each value of y, given the values for x. The regression becomes more locally weighted as the number of iterations increases and the percentage of total points used in smoothing each value of y decreases. We obtained the best definition of local minima with no iteration and using 75% of the points for each local regression. This procedure took into account variability in the vicinity of a local minimum as well as nearby rises to higher maxima. If the smoothed plot through the interval T = 35–52 or T = 60–72 displayed a distinct minimum, we scored the corresponding T as a minimum. Third, if neither of the above procedures defined a minimum, the replicate was scored as indeterminate and treated as a missing value in the subsequent analyses.

Response to these regimens resulted in an unbalanced data set. To evaluate geographic variation in the position of minima, we treated each replicate as an independent observation, regardless of population within latitudinal zone, and performed separate one-way ANOVA of minima T = 35–52 and T = 60–72 using geography (south, intermediate, north) as treatments.

Results

Photoperiodic response curves

The photoperiodic response curves of each population are shown in Fig. 3. The ecological critical photoperiod increased with latitude (Fig. 4). Developmental response to unnaturally short photoperiods was greater in southern than in northern populations. Nested

Fig. 3 Photoperiodic response curves (PPRCs) of *W. smithii*. Circles show the PPRC from 0–24 h in 1-h increments; *squares* show the PPRC in the vicinity of the ecological critical photoperiod in half-hour increments. *Open* and *closed* symbols illustrate replicate experiments
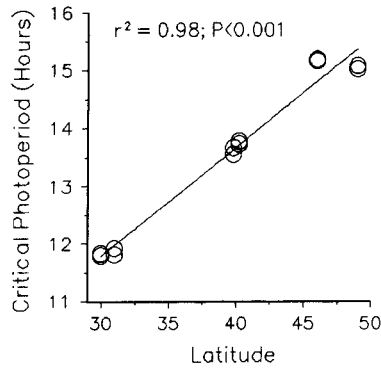

Hours of Light per Day
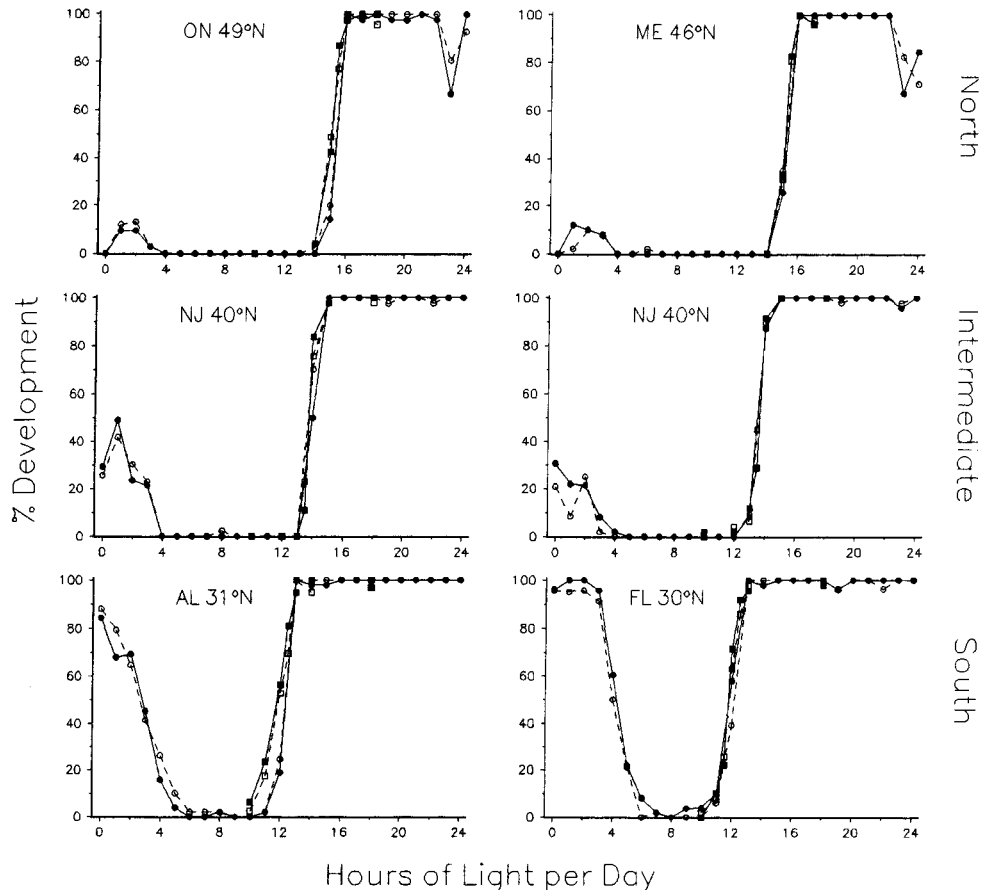
Fig. 4 Critical photoperiod determined as the daylength corresponding to the 50% intercept of the PPRC (Fig. 3, *squares*) at ecologically relevant daylengths

ANOVA of area under the curve 0–8 h showed very highly significant differences among latitudes ($F_{2,3} = 174.74$; $P < 0.001$). Since the comparison was planned a priori, we compared means using the least significant difference (Fig. 5A). Response to unnaturally short photoperiods then declined consistently ($P < 0.001$) with increasing latitude. Nested ANOVA of area under the curves 18–24 h showed very highly significant differences among latitudes ($F_{2,3} = 92.19$; $P < 0.001$). Response to unnaturally long photoperiods (Fig. 5B) then remained the same from southern to intermediate latitudes ($P > 0.05$) but declined ($P < 0.001$) between the in-
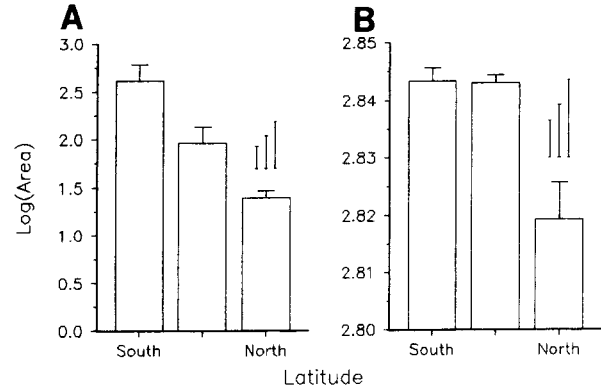

Fig. 5A, B Photoperiodic response [log (area under the PPRC)] **a** to short (0–8 h) and **b** long (18–24 h) photoperiods. The columns plot means ± 2SE; the *vertical bars* show the least significant difference corresponding to the 5.0, 1.0, and 0.1% levels of protection

termediate and northern latitudes. These results show that the increase in CPP at northern latitudes was associated with a decline in developmental response to both unnaturally short and unnaturally long photoperiods, i.e., a vertical, downward shift in the entire PPRC.

Developmental response to varying T

Developmental responses to a short photophase and varying scotophase are shown in Fig. 6. Developmental

Fig. 6 Developmental response to a 10 h (short) photophase and varying scotophases from 14 (T = 24) to 62 (T = 72) h. Replicates from the first and second block of experiments are plotted as *circles* and *squares*, respectively. For the second block, the plots show responses after 30 days in the two southern populations and after 8 weeks in the four more northern populations.
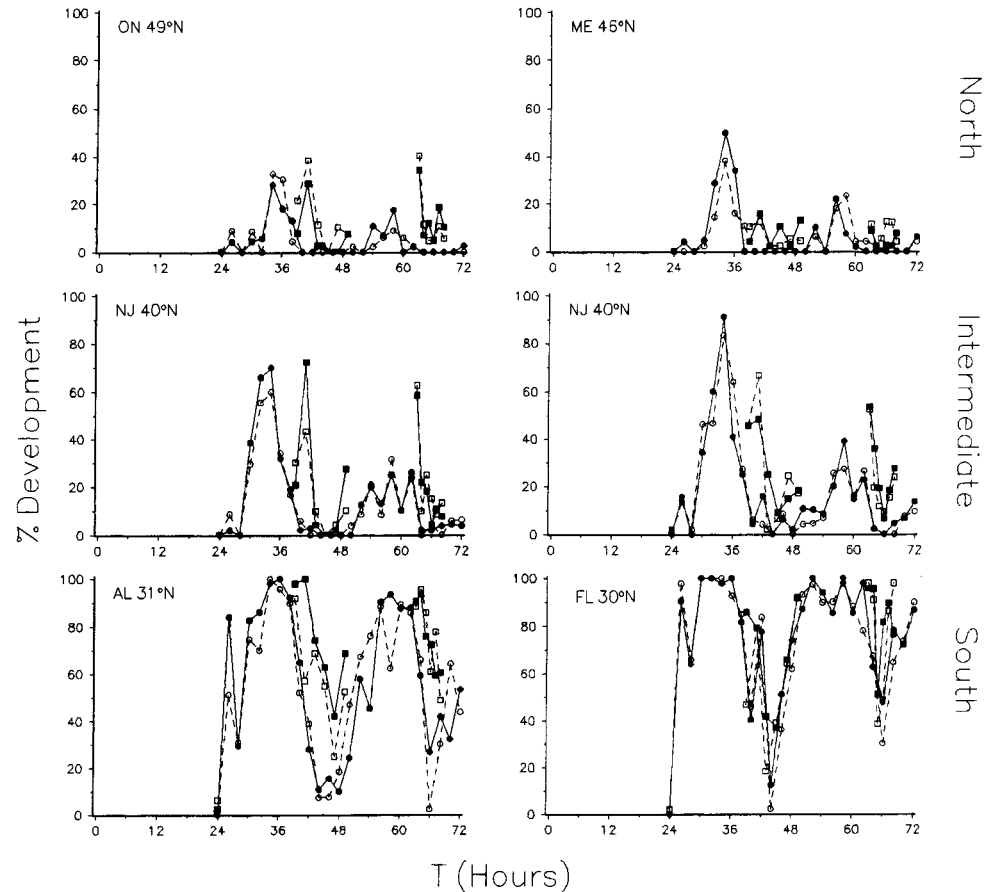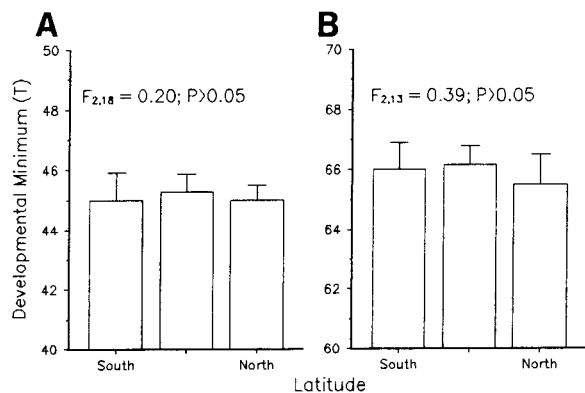
Fig. 7A, B Minima of developmental response to a short photophase and variable scotophase (Fig. 6). The columns plot means ± 2 SE. Since ANOVA did not show significant differences among means, no least significant difference is provided

responses to all Ts and resolution of minima in this response were higher in southern than in northern populations. When $T = 24$, the $L:D = 10:14$ cycle is interpreted as a short-day photoperiod and larvae remain in diapause. At longer Ts, the developmental response shows two successive "peaks" and "valleys," with minima in the vicinity of 45 and 66 h. We were able to score 21 of the 24 possible minima in the region 35–52 h; missing were one intermediate and two northern replicates. Scorable local minima averaged $T = 45.1$ h and ranged from $T = 43$ to $T = 47$ h. One-way ANOVA (Fig. 7A) showed no significant differences in local minima due to geography. We were able to score 14 of the 24 possible minima in the region $T = 60$–72 h; missing were two replicates from the southern, two from intermediate, and six from northern localities. Scorable local minima averaged $T = 66.0$ h and ranged from $T = 65$ to $T = 68$ h. One-way ANOVA showed no significant differences in local minima due to geography (Fig. 7B). These results indicate a declining developmental response to all cycles greater than $T = 24$ h with increasing latitude but no geographic trend in the position of either the first or the second minimum in developmental response.

Discussion

Evaluation of the PPRCs (Fig. 3) reveals a consistent pattern: the ecological critical photoperiod increases with latitude (Fig. 4) and this increase is accompanied by a decline in response both to unnaturally short and to unnaturally long photoperiods (Fig. 5). The shift in critical photoperiod reflects the climatic gradient over which the mosquitoes were collected (Bradshaw 1976; Bradshaw and Lounibos 1977) but it is the responses to exotic photoperiods that provide insight into the underlying mechanisms. As seen in *Acronycta rumicis* (Danilevskii 1965, p, 131), *D. auraria* (Pittendrigh and Takamura 1987), and *W. smithii* (Fig. 3), the increase in

critical photoperiod is accompanied by a vertical (downward), not a lateral, shift of the PPRC. These results support Pittendrigh's (Pittendrigh et al. 1991; Pittendrigh and Takamura 1993) hypothesis that latitudinal increases in CPP result from an increase in the amplitude of the underlying circadian pacemaker. As the amplitude of the pacemaker increases, its endogenous inertia renders it less perturbable by light, a property that might be highly adaptive at far-northern localities where very long summer days might otherwise disrupt internal temporal organization (Pittendrigh et al. 1991). As discussed earlier, this pattern of the PPRCs in *W. smithii* is consistent with the amplitude hypothesis but does not constitute definitive evidence for it.

The pattern of peaks and valleys seen in response to varying T (Fig. 6) provides the most conclusive evidence that PTM in all populations of *W. smithii* involves a rhythmic component. The interval from 24 h to the first minimum in developmental response (Fig. 6) and the interval from the first to the second minimum in developmental response both provide estimates of the period of the underlying endogenous rhythm of sensitivity to light (τ). In the case of *W. smithii*, both the earlier and the later intervals indicate a period of $\tau = 21$ h for the circadian component of photoperiodic time measurement in this species. This period does not vary among populations (Fig. 7). We therefore exclude changes in the period of the underlying pacemaker as a causal component of geographic variation in photoperiodic time measurement of *W. smithii*.

The only comparable study in a native (nonintroduced) insect comes from *D. auraria*, in which the incidence of diapause is higher at lower temperatures and the CPP increases consistently with latitude (Kimura 1984; Pittendrigh and Takamura 1993). At 15°C, the PPRC of *D. auraria* resembles that of *W. smithii* and there is clear evidence that the latitudinal increase in CPP is associated with a vertical, downward shift of the PPRC. At an unspecified temperature, the diapause response to a short photophase and variable scotophase to create $T = 24$–72 was strong in a northern (43° N) population and weak in a southern (34° N) population but τ appeared to be about 24 h in both populations (Pittendrigh and Takamura 1993). Hence, in the pattern of its basic response, *W. smithii* very much resembled *D. auraria*: relative to southern populations, northern populations exhibited a vertical, downward shift in the PPRC (when plotted as percent development), increased stability of diapause at $T > 24$ h, and similar if not identical τ.

Wyeomyia is a tropical genus of Sabethene mosquito comprising of over 80 species, of which only 1, *Wyeomyia smithii*, survives at temperate latitudes. A variety of characters including behavior, morphology, and physiology, as well as biogeography, indicate that southern populations are ancestral and northern populations are derived (Bradshaw and Lounibos 1977; Istock and Weisburg 1987; Bradshaw and Holzapfel 1990). Hence, unlike previous studies with *D. auraria* (Pittendrigh and Takamura 1993), the geographic patterns we

have shown in the photoperiodic response of *W. smithii* indicate the direction in evolution of the rhythmic component of photoperiodic time measurement. Over its historical invasion of North America, the critical photoperiod has increased with its northward advance, producing a pattern common to most hibernal-diapausing temperate arthropods (Danilevskii 1965; Taylor and Spalding 1986; Danks 1987) including *W. smithii* (Fig. 4). What we have shown that is new, however, is that the evolution of increasing critical photoperiod results from a decreased responsiveness to photo-stimulation (Fig. 3) and a decreased perturbability by light of the underlying pacemaker (Fig. 6), but without involving the period of that pacemaker (Fig. 7). We conclude that photoperiodic adaptation to increasingly more northern seasonal patterns has been accomplished through increasing robustness (amplitude?) of the circadian pacemaker independently of its period. This independence also implies that there is no causal physiological connection or genetic correlation between the period and amplitude of the circadian pacemaker underlying photoperiodic time measurement in *W. smithii.*

References

Bradshaw WE (1976) Geography of photoperiodic response in a dispausing mosquito. Nature 262: 384–386

Bradshaw WE, Holzapfel CM (1990) Evolution of phenology and demography in the pitcher-plant mosquito, *Wyeomyia smithii.* In Gilbert F (ed) Insect life cycles: genetics, evolution, and coordination. Springer, Berlin Heidelberg New York, pp 47–67

Bradshaw WE, Lounibos LP (1972) Photoperiodic control of development in the pitcher-plant mosquito, *Wyeomyia smithii.* Can J Zool 50: 713–719

Bradshaw WE, Lounibos LP (1977) Evolution of dormancy and its photoperiodic control in pitcher-plant mosquitoes. Evolution 31: 546–567

Campbell MD, Bradshaw WE (1992) Genetic coordination of diapause in the pitcherplant mosquito, *Wyeomyia smithii.* Ann Entomol Soc Am 85: 445–451

Danilevskii AS (1965) Photoperiodism and seasonal development in insects. Oliver and Boyd, Edinburgh

Danks HV (1987) Insect dormancy: an ecological perspective. Biological Survey of Canada (Terrestrial Arthropods), Ottawa

Evans KW, Brust RA (1972) Induction and termination of diapause in *Wyeomyia smithii* (Diptera: Culicidae), and larval survival studies at low and subzero temperatures. Can Entomol 104: 1937–1950

Fochs DA, Linda SB, Craig GB Jr, Hawley WA, Pumpuni CB (1994) *Aedes albopictus* (Diptera: Culicidae): a statistical model of the role of temperature, photoperiod, and geography in the induction of egg diapause. J Med Entomol 31: 278–286

Hairston NG Jr, Walton WE (1986) Rapid evolution of a life history trait. Proc Natl Acad Sci USA 83: 4831–4833

Hard JJ, Bradshaw WE, Holzapfel CM (1993a) The genetic basis of photoperiodism and its evolutionary divergence among popu-

lations of the pitcher-plant mosquito, *Wyeomyia smithii.* Am Nat 142: 457–473

Hard JJ, Bradshaw WE, Holzapfel CM (1993b) Genetic coordination of demography and phenology in the pitcher-plant mosquito, *Wyeomyia smithii.* J Evol Biol 6: 707 723

Hoy MA (1978) Variability in diapause attributes of insects and mites: some evolutionary and practical implications. In: Dingle H (ed) Evolution of insect migration and diapause. Springer. Berlin Heidelberg New York, pp 101–126

Istock CA, Weisburg WG (1987) Strong habitat selection and the development of population structure in a mosquito. Evol Ecol 1: 348–362

Kimura MT (1984) Geographic variation of reproductive diapause in the *Drosophila auraria* complex (Diptera: Drosophilidae). Physiol Entomol 9: 425–431

Kimura MT (1988) Adaptations to temperate climates and evolution of overwintering strategies in the *Drosophila melanogaster* species group. Evolution 42: 1288–1297

Lankinen P (1986) Geographical variation in circadian eclosion rhythm and photoperiodic diapause in *Drosophila littoralis.* J Comp Physiol A 159: 123–142

Lounibos LP, Bradshaw WE (1975) A second diapause in *Wyeomyia smithii*: seasonal incidence and maintenance by photoperiod. Can J Zool 53: 215–221

Masaki S (1983) Introduction. In: Brown VK, Hodek I (eds) Diapause and life cycle strategies in insects. Junk, The Hague, pp 1–5

Minis DH (1965) Parallel peculiarities in the entrainment of a circadian rhythm by light cycles. In: Aschoff J (ed) Circadian clocks. North-Holland, Amsterdam, pp 333–343

Nanda KK, Hamner KC (1959) Photoperiodic cycles of different lengths in relation to the flowering of *Biloxi* soybean. Planta 53: 45–52

Pavlidis T (1967) A mathematical model for the light affected system in the *Drosophila* eclosion rhythm. Bull Math Biophys 29: 291–310

Pittendrigh CS, Minis DH (1964) The entrainment of circadian oscillations by light and their role as photoperiodic clocks. Am Nat 98: 261–294

Pittendrigh CS, Minis DH (1971) The photoperiodic time measurement in *Pectinophora gossypiella* and its relation to the circadian system in that species. In: Menaker M (ed) Biochronometry. National Academy of Sciences, Washington, pp 212–250

Pittendrigh CS, Takamura T (1987) Temperature dependence and evolutionary adjustment of critical night length in insect photoperiodism. Proc Natl Acad Sci USA 84: 7169–7173

Pittendrigh CS, Takamura T (1989) Latitudinal clines in the properties of a circadian pacemaker. J Biol Rhythms 4: 217–235

Pittendrigh CS, Takamura T (1993) Homage to Sinzo Masaki: circadian components in the photoperiodic responses of *Drosophila auraria.* In: Takeda M, Tanaka S (eds) Seasonal adaptation and diapause in insects (in Japanese). Bun-ichi Sôgô Shuppan, Tokyo, pp 288–305

Pittendrigh CS, Eichhorn JH, Minis DH, Bruce VG (1970) Circadian systems. VI. Photoperiodic measurement in *Pectinophora gossypiella.* Proc Natl Acad Sci USA 66: 758–764

Pittendrigh CS, Kyner WT, Takamura T (1991) The amplitude of circadian oscillations: temperature dependence, latitudinal clines, and the photoperiodic time measurement. J Biol Rhythms 6: 299–313

Saunders DS (1976) The circadian eclosion rhythm in *Sarcophaga argyrostoma*: some comparisons with the photoperiodic "clock". J Comp Physiol 110: 111–133

Saunders DS (1990) The circadian basis of ovarian diapause regulation in *Drosophila melanogaster*: Is the *period* gene causally involved in photoperiodic time measurement? J Biol Rhythms 5: 315–331

Saunders DS, Henrich VC, Gilbert LI (1989) Induction of diapause in *Drosophila melanogaster*: photoperiodic regulation and the impact of arrhythmic clock mutations on time measurement. Proc Natl Acad Sci USA 86: 3748–3752

Skopik SD, Takeda M (1986) Photoperiodic control of diapause induction and termination in *Ostrinia nubilalis*: two different clocks? J Biol Rhythms 1: 137–143

Smith SM, Brust RA (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. Can J Zool 49: 1065–1073

Tauber MJ, Tauber CA, Masaki S (1986) Seasonal adaptations of insects. Oxford University Press, New York

Taylor F, Spalding JB (1986) Geographical patterns in the photoperiodic induction of hibernal diapause. In: Taylor F, Karban R (eds) The evolution of insect life cycles. Springer, Berlin Heidelberg New York, pp 66–85

TriMetrix (1993) Axum. Technical graphics and data analysis. Reference. TriMetrix, Seattle