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Mini-review

# Phenotypic evolution and the genetic architecture underlying photoperiodic time measurement☆

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**Abstract**

A wide variety of higher plants, vertebrates, and arthropods use the length of day to synchronize growth, development, reproduction, dormancy, and migration with the changing seasons. In the pitcher-plant mosquito, *Wyeomyia smithii*, critical photoperiod mediating the onset and maintenance of larval diapause has evolved about 10 standard deviations in mean critical photoperiod between the ancestral, Gulf Coast populations, and the derived, Canadian populations. We are seeking to understand how this evolution has been accomplished at both the genetic and the physiological levels.

At the genetic level, average heterozygosity at protein-coding loci decreases with latitude of origin, while genetic variation for photoperiodic response increases with latitude of origin, particularly within the formerly glaciated regions of North America. Hybridization experiments reveal widespread genetic differences in critical photoperiod due to epistasis. We ascribe the increase in genetic variation in photoperiodic response, despite directional and stabilizing selection to the contrary, to the release of additive from epistatic variance during successive founder events in *W. smithii*'s northward dispersal following recession of the Laurentide Ice Sheet, and to the resulting genetic drift and reorganization of genetic architectures in descendent populations.

At the physiological level, northern populations of *W. smithii*, as well as northern populations of spider mites, flies, moths, and beetles in both North America and Europe show a declining expression of the rhythmic component of photoperiodic response.

In *Drosophila melanogaster*, when the epistatic coupling between the *period* locus and photoperiodic response is disrupted, the critical photoperiod is shifted towards shorter daylengths; analogously in *W. smithii*, when epistatic interactions are disrupted in the recombining generations of hybrid populations, the critical photoperiod is shifted towards shorter daylengths. The implications here are (1) that in *W. smithii*, it is the epistatic modification of the photoperiodic response curve by the circadian clock that is being disrupted in the recombining generations and (2) that post-glacial range expansion into the North-Temperate Zone by arthropods in general may involve uncoupling of the circadian and photoperiodic clocks.

Despite the tremendous advances that have been made in understanding circadian rhythmicity at the molecular level, virtually nothing is known about how or whether any of the downstream 'clock-controlled genes' connect with photoperiodic time measurement. Except in *W. smithii*, little is known about how this connection changes with seasonal adaptation of photoperiodic response through evolutionary time. It is our goal and desire that our top–down approach to the evolution of photoperiodic time measurement will meet and mesh with the bottom–up approach that is being developed so fruitfully for circadian clocks. 

*Keywords:* Circadian clocks; Evolutionary genetics; Geographic strains; Photoperiodism; *Wyeomyia smithii*

## 1. Introduction

The ability to evaluate and use daylength or nightlength (photoperiodism) to regulate seasonal changes in behavior, appearance, growth, development, reproduction, dormancy, and migration is widespread among plants and animals in the temperate zone. Hundreds of insect species are known to use photoperiodism to regulate their seasonal activities (Masaki, 1983; Saunders, 1982, Appendix 4). Initially, photoperiodic response and its geographic variation were envisioned as a species-level attribute. "Species from low latitudes (as compared with species from high latitudes) must be expected to respond to shorter critical photoperiods,

☆ Dedicated to David Saunders in celebration of his enduring contributions to our understanding of insect clocks and with deep appreciation for his friendship

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otherwise diapause would not be averted during the favourable season" (Lees, 1955, p 17). Danilevskii (1965, p 141) recognized the importance of intraspecific variation in photoperiodic response that anticipated Pittendrigh's (Pittendrigh et al., 1991) amplitude hypothesis: "Thus not only the critical day-length, but also the intensity of the photoperiodic reaction are not characteristic of a species. These factors are primarily characteristic of zonal-geographic adaptations in intraspecific populations." 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. 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; Vaz Nunes et al., 1990); these studies have shown a repeated, consistent increase of critical photoperiod with increasing temperate latitudes. This pattern does not, then, represent a selection-mediated response idiosyncratic to a particular species, but rather an arthropod-wide, recurrent adaptation to the ecoclimatic gradient of temperate latitudes. The genetic and physiological processes that underlie this widespread adaptation remain largely unknown. Herein, we first examine geographic variation of photoperiodic response and its genetic variation in the pitcher-plant mosquito, *Wyeomyia smithii* (Coq.). Second, we consider geographic variation at the physiological level by examining the contribution of a rhythmic component (circadian rhythm?) to photoperiodic time measurement. Finally, we draw conclusions that follow directly from our data and then propose an explicit evolutionary-genetic process to explain both our results and those of others who have described geographic variation in the rhythmic component of photoperiodic time measurement.

[1] Abbreviations, symbols, and terms: ***Diapause***; arthropod dormancy, herein assumed to be hibernal. ***CPP***; critical photoperiod, hours of light per day that stimulates 50% development and initiates or maintains 50% diapause in a sample population. ***L:D=X:Y***; a cycle consisting of X hours of light and Y hours of darkness. ***PPRC***; photoperiodic response curve, a dose–response relationship between percent development and hours of light per day; this curve is usually sigmoid in shape at ecologically relevant photoperiods; extreme long or extreme short days may elicit intermediate responses. ***PTM***; photoperiodic time measurement, the ability of organisms to assess the duration or length of day or night. ***T***, the period of an external driving cycle, usually L+D; $\tau$=***Tau***, the period of an organism's free-running (unentrained) rhythm or oscillator. ***Photophase*** and ***Scotophase***; the periods of light and dark, respectively, in an L:D cycle.

## 2. Geographic variation of photoperiodic response and its genetic variation in *W. smithii*

The mosquito, *Wyeomyia smithii*, completes its entire preadult development only within the water-filled leaves of the purple pitcher plant, *Sarracenia purpurea* L., and the range of the mosquito closely approximates that of its host plant from about 30–54°N in North America (Fig. 1). *Wyeomyia smithii* invaded North America from South America and, since becoming established in North America, has diversified northwards (Ross, 1964; Bradshaw and Lounibos, 1977; Istock and Weisburg, 1987; Bradshaw and Holzapfel, 1990). Genetically, populations of *W. smithii* fall into two major clades, a southern clade extending from the Gulf of Mexico to central North Carolina (30–36°N), and a northern clade extending from southern Maryland northwards to Newfoundland and westward to Saskatchewan (38–54°N)(Armbruster et al., 1998). From central New Jersey northwards, the northern clade occupies exclusively areas covered by the Laurentide Ice Sheet, so that these populations must have been established since recession of the glaciers from 21,000 to 8,000 yr ago (Andrews, 1987). Because *W. smithii* is a weak- flying species highly prone to death by desiccation (Istock and Weisburg, 1987), the forest habitats separating the wetlands that harbor pitcher plants are inimical to adult mosquitoes. It is not therefore surprising that average gene diversity at allozyme loci remains high in the southern clade, but, in the northern clade, declines with increasing distance northwards from New Jersey (Fig. 2). This pattern is not unusual for animals invading formerly glaciated areas and is usually interpreted as the loss of genetic variation during successive founder events, each of which carries with it only a subset of its ancestral genome (Armbruster et al., 1998).

Throughout their range, *W. smithii* overwinter in the evergreen leaves of *S. purpurea* in a larval diapause that is initiated and maintained by short days and averted or terminated by long days (Smith and Brust, 1971; Evans and Brust, 1972; Bradshaw and Lounibos 1972, 1977). In unchilled larvae, the critical photoperiod is the same for the initiation and the maintenance of diapause, so that, once initiated, diapause is always photoreversible by exposure to long days (Bradshaw and Lounibos, 1972). Ordinarily, critical photoperiod is determined by exposing a number of replicated cohorts of insects to a range of different static photoperiods in short increments of time, plotting percentage diapause or development as a function of photoperiod, and from logistic regression or by interpolation, defining the critical photoperiod as the 50% intercept. By this technique, critical photoperiod is a property of a sample population, not of individuals. In *W. smithii*, because diapause is always reversible by long days, we are able to determine the critical photoperiod of individuals. First we rear large populations of

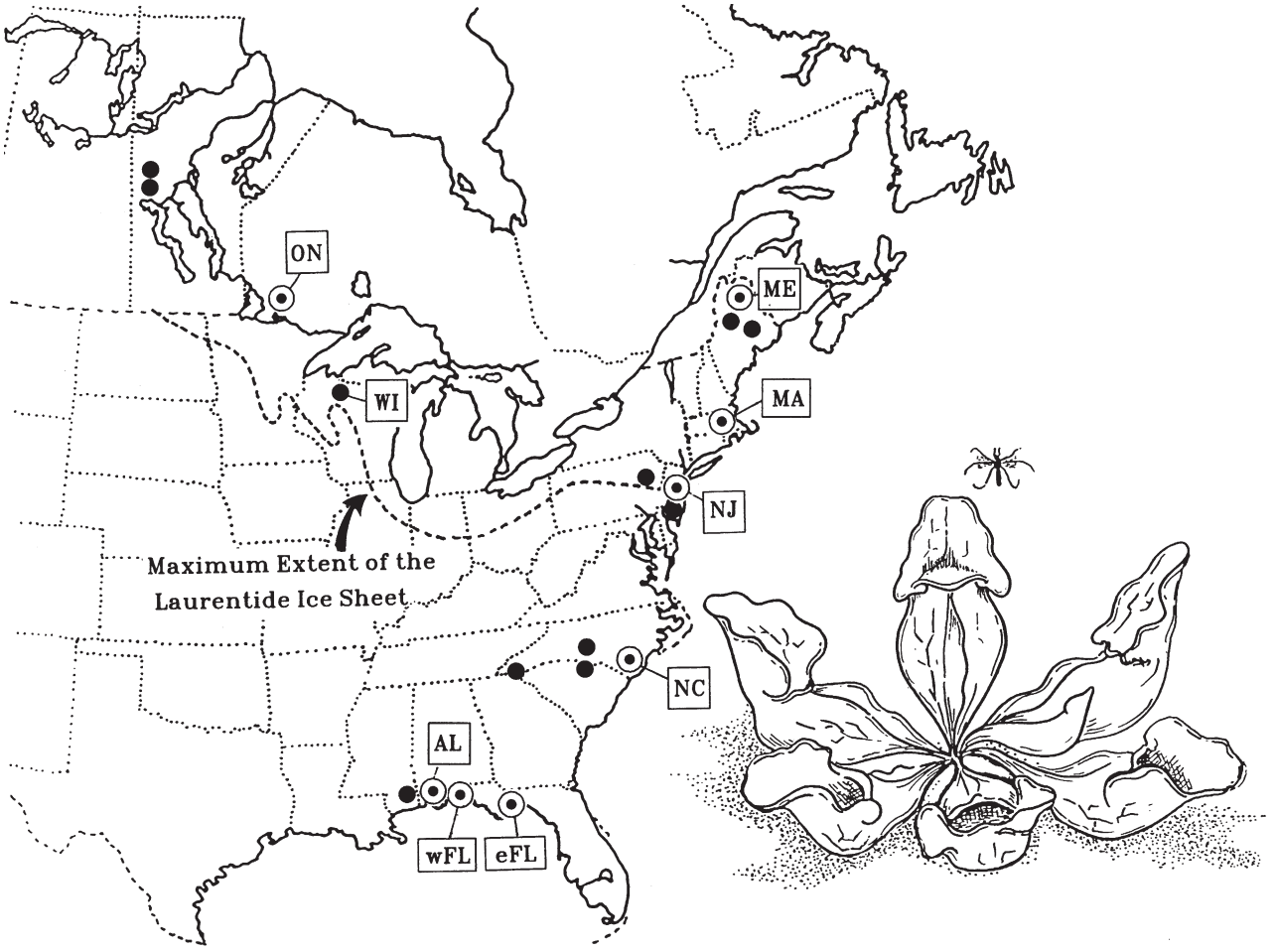


Fig. 1. The pitcher plant, *Sarracenia purpurea*, the mosquito, *Wyeomyia smithii*, and the origin of mosquito populations referred to in the text. (◉) Populations used in experimental studies; (●) additional populations used to evaluate allozyme variation (Fig. 2). Note that some localities may be close enough to be covered by a single symbol.

*W. smithii* on short days to induce diapause and then, when the population has become synchronized in diapause, we expose them to short days that increment by 3 min per day. At some point, the increasing daylength is interpreted as a development-promoting long day by each larva and it resumes development and pupates. Even though a concatenation of covert and overt developmental events take place between the first perception of long days and pupation, it is the recognition of long days that is the major rate-limiting step. We then take the daylength on the day of pupation as that individual's 'critical photoperiod.' In this manner, we are able to treat critical photoperiod as a parametric trait with a mean and variance (and the latter's derivatives, the standard deviation and standard error).

The mean individual critical photoperiod mediating the maintenance and termination of larval diapause increases with latitude and altitude [Fig. 3(A)], just as does the critical photoperiod determined as a population-level trait with static photoperiods (Bradshaw, 1976; Bradshaw and Lounibos, 1977). If critical photoperiod at a given latitude ($CPP_{Lat}$) is expressed as a divergence from the ancestral Gulf Coast (30–31°N) populations in units of mean standard deviation (SD) of the Gulf Coast populations,

$$\text{CPP in SD units} = \frac{CPP_{Lat} - \frac{1}{2}(CPP_{30°N} + CPP_{31°N})}{\frac{1}{2}(SD_{30°N} + SD_{31°N})} \quad (1)$$

then [Fig. 3(A) right axis] the evolution of mean critical photoperiod in *W. smithii* has evolved in excess of 10 standard deviations between the Gulf of Mexico and Canada (30–50°N). This value represents an enormous evolutionary response to the climatic gradient of North America. We now consider the components of genetic variation in photoperiodic response within and between populations.

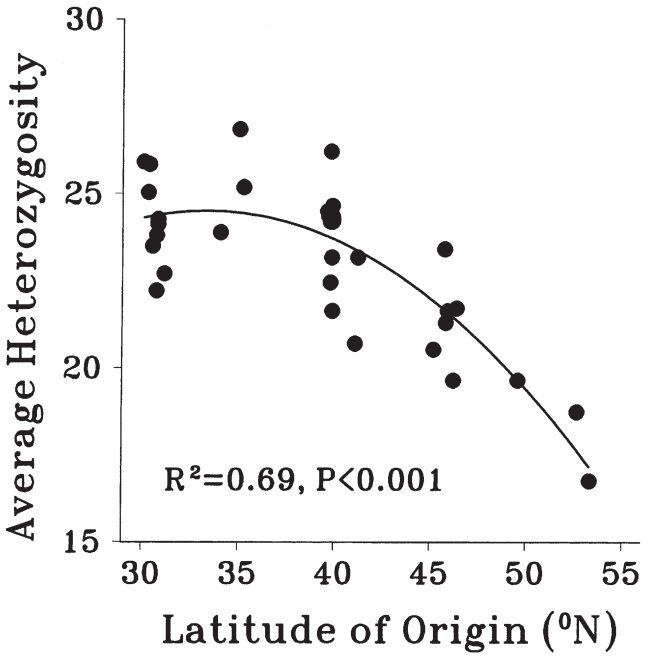


Fig. 2. Geographic variation in average heterozygosity at 10 polymorphic allozyme loci (from Armbruster et al., 1998).

## 3. Genetic variation of photoperiodic response within and between populations

### *3.1. Heritability and the components of genetic variation within populations*

Evolutionary response ($R$) depends upon the genetic variability to respond (heritability, $h^2$) as well as the strength ($S$) of selection applied to a population

$$R=h^2S \quad (2)$$

but, at the same time, response to selection should erode genetic variation in populations. To determine the amount of residual genetic variation in the northern compared with the southern populations, we estimated the heritability of critical photoperiod in geographically disparate populations. The phenotypic variance ($V_P$) is the sum of both the genetic ($V_G$) and environmental ($V_E$) variances:

$$V_P=V_G+V_E \quad (3)$$

and the genetic variance is composed of

$$V_G=V_A+V_D+V_I \quad (4)$$

where

$V_A$ the additive variance, genetic variation due to the independent expression of alleles regardless of locus

$V_D$ the dominance variance, genetic variation due to allelic interactions within loci

$V_I$ the interaction or epistatic variance, genetic variation due to genic interactions among loci.

The heritability (narrow sense) is the proportion of phenotypic variation due to the independent (additive) effects of alleles

$$h^2=V_A/V_P. \quad (5)$$

Heritability is based on the resemblance between relatives and is estimated most directly from the regression

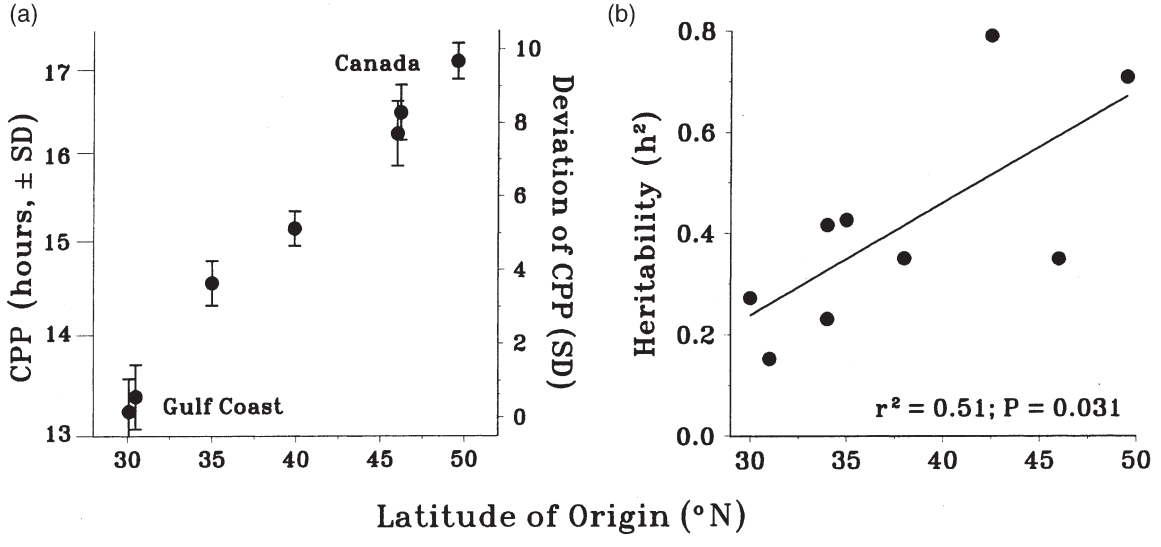


Fig. 3. Geographic variation in critical photoperiod (CPP) and its heritability. (a) Critical photoperiods (CPP) determined with incrementing daylengths plotted on the left axis as mean log (hours light per day) ±1 standard deviation and on the right axis as deviation from the Gulf Coast (ancestral populations) in units of average standard deviation in CPP of the two Gulf Coast populations (from Lair et al., 1997). (b) Heritability of CPP determined from weighted regression of offspring on parent means (Extreme 6 points from Hard et al., 1993; middle 3 points at 35–38°N original data from subsequent experiments).

of mean offspring on mean or mid-parent phenotype. The slope and standard error of this regression provide an estimate of the heritability and its standard error, respectively. Offspring–parent regression of critical photoperiod in *W. smithii* [Fig. 3(B)] shows that the heritability of critical photoperiod increases with latitude of origin between the Gulf of Mexico and Canada, in marked contrast to the decrease in average allozyme heterozygosity over the same range (Fig. 2). We have concluded consistently (Hard et al. 1992, 1993; Lair et al., 1997; Bradshaw and Holzapfel, 2000) that this contrast results from the genetic consequences of successive founder events, followed by isolation and drift, during the dispersal of *W. smithii* into northern North America. Because of its high host specificity, *W. smithii* can invade a new locality only after its host plant has become established. Consequently, colonizing mosquitoes enter a wide-open habitat where isolation and drift should result in reduced heterozygosity as seen in Fig. 2. But, for quantitative traits, the same isolation and drift can break up formerly favorable epistatic interactions, thereby not only changing the average effect of alleles on quantitative traits, but also releasing additive from epistatic variance. Hence, the contrasting pattern in increasing latitude of decreasing heterozygosity at protein-coding loci (Fig. 2) and increasing heritability for critical photoperiod [Fig. 3(B)] can be explained by isolation and drift following successive founder events, if epistasis has been involved in the evolution of photoperiodic response.

### 3.2. Components of genetic variation between populations

The components of genetic differences between populations can be estimated from the means and variances of hybrid phenotypes (Hard et al. 1992, 1993; Lair et al., 1997). If two populations differ in critical photoperiod, and all of these differences are due to the independent (additive) effects of alleles, then the $F_1$, $F_2$, and backcross ($B_1=F_1\times P_1$;$B_2=F_1\times P_2$) generation means should all fall on the straight line joining the two parent means (Fig. 4). The 'additive expectation' is then that all six generations will not deviate from this line (Fig. 4, solid line). If the differences in mean critical photoperiod involve dominance at some loci, there should be a deviation from the additive expectation in the $F_1$. Because of independent assortment in the recombining ($F_2$, $B_1$, and $B_2$) generations, the $F_2$ and backcross generations lose half of their heterozygosity and, in the absence of epistasis, the 'additive-dominance expectations' are (Fig. 4, dashed line):

$$F_2=\frac{1}{4}(2F_1+P_1+P_2)$$

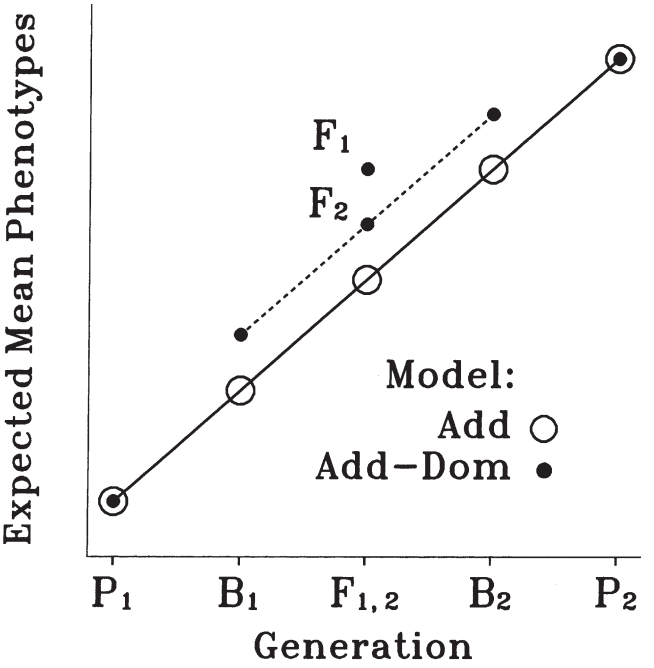


Fig. 4. Expected mean phenotypes in the $F_1$, $F_2$, and backcross ($B_1$ and $B_2$) generations of hybrids between two populations ($P_1$ and $P_2$) under the assumption that populations differ only in (○) the additive effects of genes or (●) the additive and dominance effects of genes. Significant departure from the additive-dominance expectation indicates genetic differences between populations due to epistasis.

$$B_1=\frac{1}{2}(P_1+F_1)$$

$$B_2=\frac{1}{2}(P_2+F_1)$$

The $F_1$ possesses a full haploid set of genes from each parent and therefore preserves whatever favorable epistatic interactions had been present in the parent generation. During independent assortment and recombination in the $F_1$, however, these favorable interactions are disrupted and, if epistatic interactions differ between the parent populations, then the means of the recombining $F_2$ and backcross generations should deviate from the additive-dominance expectation.

We evaluate the significance of non-additive genetic differences among populations by performing line crosses between pairs of populations, testing for significant departures from the additive and additive-dominance expectations using the joint-scaling test derived from Mather and Jinks (1982), Hayman (1958, 1960) and Lynch and Walsh (1997). The joint-scaling test uses the method of least squares to test for goodness of fit ($\chi^2$ statistic) of generation means and variances to models incorporating purely additive, or additive and dominance effects (Fig. 4). We proceed with the joint-scaling test in a hierarchical manner. First, we test for goodness-of-fit to a model incorporating only additive effects. Acceptance of the additive model indicates that additive

effects alone are sufficient to explain the genetic divergence of the parental populations. Rejection of the additive model leads us to test for goodness-of-fit to an additive-dominance model. Acceptance of the additive-dominance model indicates that additive and dominance effects without epistasis are sufficient to explain the genetic divergence of the parent populations. Rejection of the additive-dominance model indicates the presence of epistatic genetic differences between the parent populations. Detection of differences due to dominance between parent populations requires both parents and their $F_1$ hybrids; detection of differences due to epistasis requires, additionally, at least the $F_2$ generation. The inclusion of the first backcross generations ($B_1=F_1\times P_1$ and $B_2=F_1\times P_2$) permits controlling for maternal effects as well (Lair et al., 1997).

There are several caveats concerning this approach. (1) In the presence of substantive epistasis, estimation of differences due to additive or dominance effects is unreliable (Hayman, 1960a). (2) Estimates of the number of loci, or, minimum number of effective factors, contributing to the differences in mean phenotype between two populations are biased by non-additive differences between them (Lande, 1981; Zeng et al., 1990; Mather and Jinks, 1982). Consequently, in the presence of significant dominance or epistasis between populations, estimates of the minimum number of effective factors are unreliable. (3) Hybrid means are a composite of all genetic effects and, if these effects differ in sign, they may cancel each other out. Only when there is net directional dominance or directional epistasis does the joint-scaling test identify significant differences due to dominance or epistasis, respectively. Joint scaling tests are therefore prone to Type II error because they only detect net directional dominance or net directional epistasis. This caveat does mean, however, that the detection of non-additive difference between populations is very robust.

The contribution of non-additive effects to the genetic differences between populations of *W. smithii* depends on the region from which the parent populations originated (Table 1). For crosses between populations entirely within the southern clade, or for crosses between the southern and northern clade, genetic differences consistently involved epistasis. For crosses entirely within the northern clades, only the cross between ME and ON showed differences due to epistasis. The cross between ME and WI showed differences due to dominance but not epistasis and the most distant cross within the northern clade (NJ×ON), both geographically and phenotypically, showed only additive differences between the parent populations. From this latter cross, we estimated that the minimum number of effective factors (±SE) by which critical photoperiod differed between the parent populations was 5±1/2. The minimum number of effective factors is an estimate of the number of loci involved, if each locus has the same effect on the phenotype. It is more likely, however, that there are a few loci of major effect and multiple other loci of lesser effect. In addition, our estimate of the minimum number of effective factors concerns only those genes contributing to differences among populations; even more genes are probably involved in creating the components of photoperiodic response that are common to all populations.

Table 1
Genetic differences among populations of *Wyeomyia smithii* due to additive, dominance, and epistatic effects[a]

| | Add | Dom | Epistasis | Ref |
|---|---|---|---|---|
| South×South[b] | | | | |
| AL×FL | – | – | ** | L |
| eFL×NC | – | – | ** | H |
| wFL×NC | – | – | ** | H |
| South×North | | | | |
| eFL×MA | – | – | ** | H |
| wFL×MA | – | – | ** | H |
| eFL×ME | – | – | ** | H |
| wFL×ME | – | – | ** | H |
| eFL×ON | – | – | ** | H |
| wFL×ON | – | – | ** | H |
| North×North[c] | | | | |
| WI×ME | ** | ** | * | L |
| ON×ME | – | – | ** | L |
| NJ×ON | ** | * | * | L |

[a] Entries in the table denote probabilities of a significant effect: * $P\geq 0.05$; ** $P<0.05$; (–) undefined because additive (Add) and dominance (Dom) effects are estimated unreliably in the presence of substantial epistasis (Hayman, 1960a). Significant epistasis is signified by rejection of the additive-dominance model in separate, reciprocal crosses (Ref H, Hard et al., 1993) or rejection of an additive-dominance-maternal model in the combined reciprocal crosses (Ref L, Lair et al., 1997).

[b] Crosses defined by region as shown in Fig. 1; South=southern, more ancient populations 30–35°N; North=northern, more recently derived populations 38–50°N.

[c] Separated by ≥20° in longitude.

As described above, the detection of epistatic differences between populations is possible only when there is net directional epistasis. We measured directional epistasis as the difference between the additive-dominance expectation and the observed phenotypic mean in the recombining generations ($F_2$ and backcross, $B_1$ and $B_2$). Fig. 5 shows that this deviation was significantly negative; i.e. the breakup of epistatic interactions relating to critical photoperiod in the parent populations resulted in a shift of the mean phenotype towards shorter critical photoperiods.

From these results, we make several conclusions.

1. Genetic differences among ancestral (southern clade) populations, or between ancestral and derived (northern clade) populations all involve epistasis; genetic differences among derived (northern clade) populations involved epistasis in only one of three crosses.

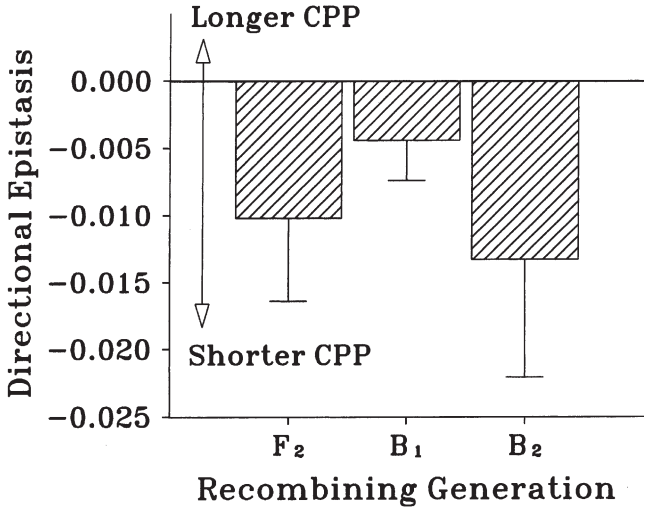


Fig. 5. Directional epistasis, calculated as deviation of the phenotypic means in the recombining generations ($F_2$, $B_1$ and $B_2$) from the additive-dominance expectation (Fig. 4). The bars plot mean ±2 standard errors in the six crosses performed by Hard et al. (1993) (Table 1).

2. The northern, derived populations are not yet in drift-migration equilibrium and constitute a much younger lineage than the ancestral, southern populations (Armbruster et al., 1998). Consequently, we also conclude that differences due to additive and dominance effects arise early in the genetic differentiation of populations and differences due to epistasis arise over longer periods of evolutionary time (Lair et al., 1997).
3. The number of genes involved in programming both the evolutionarily static and the evolutionary dynamic components of photoperiodic response in *W. smithii* is probably greater than the 6–7 loci known to form the basis of circadian rhythmicity within populations of *Drosophila melanogaster* (Young, 2000). Elucidation of the molecular basis for photoperiodic time measurement is therefore likely going to be a more formidable undertaking than identifying the molecular basis for circadian rhythmicity.
4. The decline in epistatic differences in photoperiodic response among the more recently evolved (northern) populations parallels the greater genetic variation for critical photoperiod in these populations and provides pivotal corroborating evidence for our contention (Hard et al. 1992, 1993; Lair et al., 1997; Bradshaw and Holzapfel, 2000) that the higher heritability for critical photoperiod in the northern populations results from the release of additive from epistatic variance due to isolation and drift following the more recent founder events in these populations.

## 4. Circadian involvement in photoperiodic response of *W. smithii*

Various models have been formulated for the functional basis of photoperiodic time measurement; but, in essence, they reduce to two basic categories: those models that rely on a rhythmic, self-sustaining circadian pacemaker and those models that involve only a non-repeating, interval or hourglass timer. The circadian basis of photoperiodic time measurement was first proposed by Erwin Bünning in 1936. This proposition was pursued by Pittendrigh during the 1960s and remained a central concern of his for the remainder of his life. "The notion that a circadian clock is somehow involved in photoperiodic time measurement has become more and more established, but which constitutive element of the circadian system plays a crucial role is not yet known" (Takeda and Skopik, 1997). We now know that in *Drosophila* some 6–7 loci comprise the functional circadian clock (Young, 2000) and that the *period* locus is crucial to the expression of overt circadian rhythmicity. Yet, in the one photoperiodic strain of *Drosophila melanogaster* (Canton-S), Saunders (Saunders et al., 1989; Saunders, 1990) has shown that flies entirely lacking the *period* gene have arrhythmic eclosion but still possess an intact photoperiodic response curve, albeit shifted towards shorter daylengths. Mutants at the *period* locus that affect the period of overt circadian rhythmicity also affect the position but not the basic structure of the photoperiodic response curve. This very crucial set of experiments leads to two major conclusions about the relationship between circadian rhythmicity and photoperiodic time measurement in *Drosophila melanogaster* and provides an important implication about the evolution of photoperiodic time measurement in *Wyeomyia smithii*:

1. The physiological and biochemical bases for constructing a functional photoperiodic response curve are genetically independent of the circadian clock responsible for overt rhythmicity.
2. Nonetheless, the position of the photoperiodic response curve is modified by genetic variation at the *period* locus; i.e. variable expression at the *period* locus acts as an epistatic modifier of the critical photoperiod in *Drosophila melanogaster*.
3. When the epistatic modification of photoperiodic response by the *period* locus is disrupted, the critical photoperiod of *D. melanogaster* is shifted towards shorter daylengths; analogously, when epistatic interactions within populations of *W. smithii* are disrupted in the recombining generations of hybrid populations (Fig. 5), the critical photoperiod is shifted towards shorter daylengths. The implication here is that in *W. smithii*, it is the epistatic modification of the photoperiodic response curve by the circadian clock that is being disrupted in the recombining generations.

The two most indicative tests for a rhythmic basis of photoperiodic time measurement are night-interruption (Bünsow protocol) and T-experiments (resonance

experiments or Nanda–Hamner protocol) (Pittendrigh, 1981; Saunders, 1982; Takeda and Skopik, 1997; Vaz Nunes and Saunders, 1999). In *W. smithii*, the results of the two types of experiments are qualitatively similar and, for brevity, we shall consider here only the latter. T-experiments are so-called because they involve modifying T, the period of the light–dark cycle by holding the photophase at a constant short day while, in separate experiments, varying the length of a long night (Fig. 6). An L:D=10:14 cycle is a short-day photoperiod for *W. smithii* from all latitudes (Bradshaw and Lounibos, 1977) and, if photoperiodic response in *W. smithii* is effected by an hourglass or interval timer, L:D cycles of 10:16, 10:18,... 10:62 should also constitute short-day photoperiods, regardless of whether the mosquito measures the length of day or the length of night. However, if the photoperiodic clock is not an interval timer, but rather, a circadian-driven, periodic sensitivity to light, then there should be a rhythmic long-day response to increasing night length. Indeed, southern populations of *W. smithii* do show a strong rhythmic response to varying T (Fig. 6), but northern populations show only a marginally detectable rhythmic response to varying T. Circadian involvement in photoperiodic time measurement appears to decline with increasing latitude in *W. smithii*.

*Wyeomyia smithii* is not alone in exhibiting this pattern. We have identified three studies in the literature that examined latitudinal variation in response to T-experiments (Fig. 7). All of them, including Lepidoptera, Coleoptera, and Acari show the same pattern as *W. smithii*: Northern populations show a reduced long-day response to varying night lengths. Dispersal into the North-Temperate Zone by a taxonomically diverse array of arthropods has resulted in a decrease in the rhythmic component of photoperiodic time measurement. We propose that one or more of three evolutionary processes underlies this pattern of phenotypic evolution common to mites, moths, beetles, and mosquitoes:

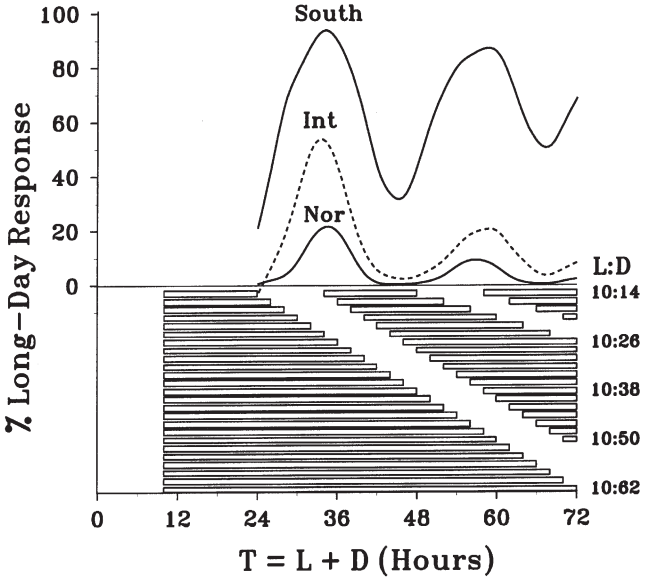


Fig. 6. Developmental (long-day) response of *W. smithii* to T-experiments with a 10-h photophase and varying scotophase to create T=24−72 h. The rhythmic long-day response indicates an underlying rhythmic sensitivity to light and implicates circadian involvement in photoperiodic time measurement. The curves represent cubic spline fits to two replicates from each of two northern populations (bottom curve, ME and ON in Fig. 1), two intermediate populations (middle curve, NJ in Fig. 1), and two Gulf Coast populations (top curve, AL and eFL in Fig. 1). Plotted from Bradshaw et al. (1998) from data in Wegis et al. (1997).

1. Circadian rhythms are strongly coupled to southern but not northern photoperiodic response. If rhythmic long-day responses to T-experiments are the manifestation of an underlying, causal circadian pacemaker, then these results indicate that the coupling of the circadian pacemaker to photoperiodic time measurement declines as latitude and the critical photoperiod increase. Since northern populations show a robust photoperiodic response curve, these results also indicate that as the influence of the rhythmic component of photoperiodic time measurement declines, its function is then subsumed by an interval or hourglass timer.
2. Photoperiodism among Insecta and Acari follows common developmental and physiological pathways. Circadian rhythmicity is ubiquitous to eukaryotes and involves negative feedback loops, some of whose elements are shared by mammals and fruit flies (Dunlap, 2000; Scully and Kay, 2000; Shearman et al., 2000; Young, 2000). It is therefore quite possible that arthropods possess a common genetic architecture for photoperiodic response and that the various manifestations of arthropod photoperiodism represent modifications of a common physiological bauplan. This scenario does not require or exclude circadian involvement in photoperiodic time measurement.
3. The seasonal environment, in addition to selecting for the overt use of daylength as a cue for programming seasonal growth, development, reproduction, dormancy, and migration may also impose selection on the underlying formal properties of photoperiodic time measurement. Thus, the seasonal environment itself may place functional constraints on particular physiological and developmental pathways. Pittendrigh's amplitude hypothesis (Pittendrigh et al., 1991; Pittendrigh and Takamura 1989, 1993) provides one such scenario. There may be others.

## 5. Synthesis

Our experimental results show that the adaptive evolution of *Wyeomyia smithii* over the climatic gradient of North America has resulted in longer critical photoperi-

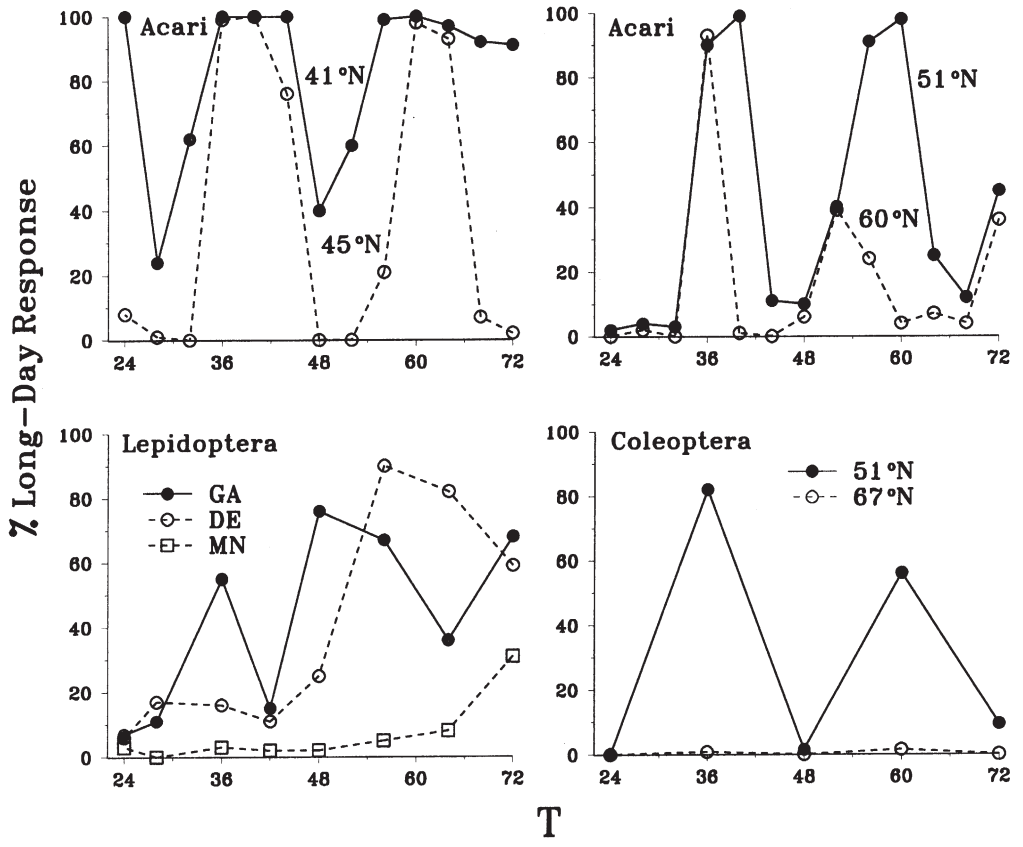


Fig. 7. Long-day response to T-experiments with a short-day photophase and varying scotophase in European Acari (Vaz Nunes et al., 1990), North American Lepidoptera (Takeda and Skopik, 1985), and European Coleoptera (Thiele, 1977). For the Lepidoptera, GA–DE–MN (Georgia, Delaware, Minnesota) represents a south–north cline.

ods, greater genetic variation for photoperiodic response, and decreasing coupling to an underlying circadian pacemaker in the more recent, northern genetic lineages than in the more ancient, southern genetic lineages. Concomitantly, there is a decline in epistatic differences in photoperiodic response among the more recently evolved populations than among more ancestral populations or between ancient and recent lineages. Breakdown of epistatic interactions in the recombining, hybrid generations results in a consistent, significant bias towards shorter critical photoperiods. To explain these results and observations with *W. smithii* and the common arthropod pattern in response to T-experiments, we propose the following evolutionary process (Fig. 8):

Firstly, we propose that the photoperiodic response curve is functionally a genetically variable hourglass or interval timer. This genetic variability provides adaptability but, at any given locality, can result in the critical photoperiod of the local population tracking a series of early or a series of late winters. This tracking of autocorrelated seasons renders the population mal-adapted in times of short-term reversals. Variation of habitat quality in space can, under certain circumstances, maintain genetic variability through multiple niche polymorphism; but, variation of habitat quality in time always selects for convergence on the long-term optimum (Hedrick et al., 1976; Hedrick, 1986). Autocorrelation of habitat quality in time can delay reaching this long-term optimum (Taylor, 1990). Since the onset of winter tends to be positively correlated between adjacent years, this environmental autocorrelation can displace a genetically variable photoperiodic response from an adaptive peak (Fig. 9). The tendency of fitness to 'wander' about this peak could be stabilized by a reduction in genetic variability or by buffering that genetic variability against environmental variability. We propose that this buffering is provided by coupling photoperiodic response to the physiologically highly stable circadian oscillator. Relative to the change in photoperiodic response with rapid change in climate, the canalizing of critical photoperiod via epistatic coupling to a circadian pacemaker takes longer evolutionary time because selection in the vicinity of the long-term optimum is weak (Fig. 9). But, in large populations, even weak selection can be highly effective over a longer time. This proposition follows from the low heritability [Fig. 3(B)] and strong circadian coupling (Fig. 6) of photoperiodic time measurement in the more ancient, southern lineages.

Secondly, during the founder events concomitant with range expansion (such as following a glacial retreat in

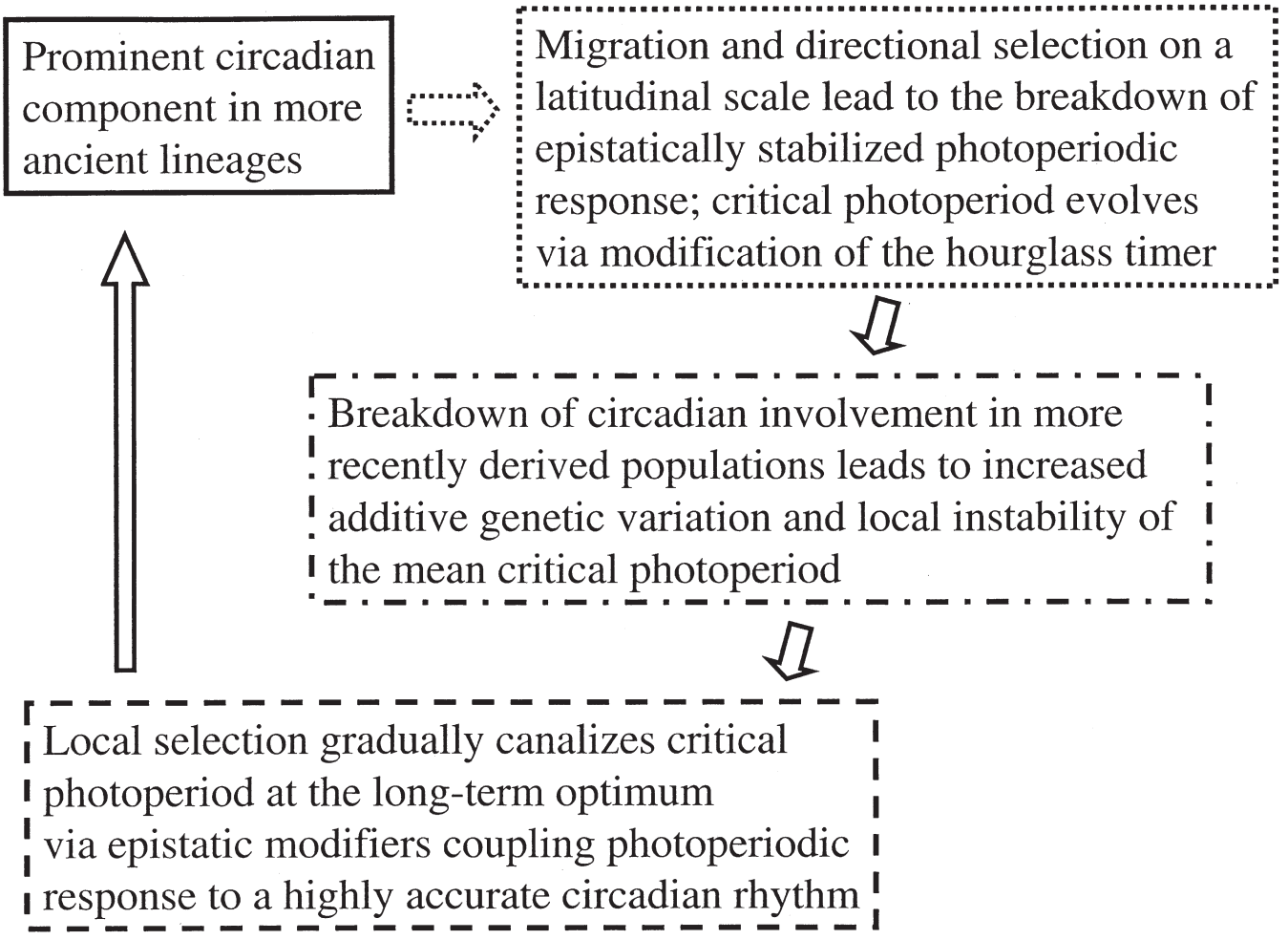


Fig. 8. Proposed genetic processes involved in the breakup and re-establishment of epistatic coupling between the circadian and photoperiodic (hourglass timer) clocks during and following rapid range expansion into north-temperate regions.

North America or Europe), isolation and drift disrupt the epistatic coupling between the hourglass timer and the circadian pacemaker, exposing genetic variability previously invisible to natural selection. Critical photoperiod then evolves via modification of the hourglass timer involving mainly the additive and dominance effects of genes. This proposition follows from (A) the high heritability of critical photoperiod in conjunction with the low heterozygosity of protein-coding loci in northern populations [Fig. 3(B) vs Fig. 2], (B) the lower expression of a circadian component of photoperiodic time measurement in northern populations (Fig. 6), and (C) the consistent differences in critical photoperiod due to epistasis within the southern populations or between northern and southern populations but not within the more recently-derived, northern populations themselves (Table 1).

Thirdly, as mean critical photoperiod approaches the local, long-term optimum (Fig. 9), strong directional selection (steep slope on the fitness curve) changes to weaker stabilizing selection (shallow slope on the fitness curve). Because the stabilizing selection about the local optimum is weak, re-establishment of epistatic coupling to the circadian pacemaker requires longer evolutionary time than the relatively stronger directional selection imposed by rapid range expansion. This proposition follows from the general lack of genetic differences due to epistasis in the more recently-established, northern populations despite their large geographic separation and large differences in mean phenotype (e.g. NJ×ON, Table 1).

We are quick to point out that the above proposition was derived to explain, *a posteriori*, the assemblage of our experimental results with *W. smithii*. We also make this proposition without reference to any specific heuristic model of photoperiodism (Takeda and Skopik, 1997). We are confident that, by modification of constants, the evolution of photoperiodic response in *W. smithii* could be modeled as a damped oscillator using the Saunders–Lewis (Lewis and Saunders, 1987; Saunders and Lewis, 1987a,b) construct, as Saunders (personal communication, 2000) has suggested. However, this exercise

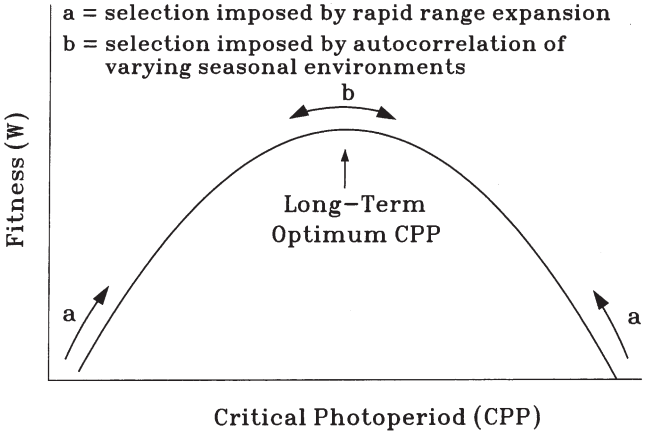


Fig. 9. Relationship between fitness and critical photoperiod (a) when strong directional selection is imposed by a new seasonal environment following rapid latitudinal range expansion or (b) in long-established populations where autocorrelation in varying seasonal environments tends to displace critical photoperiod from the long-term optimum for that latitude. Note that the strength of selection is represented by the slope of the curve, so that selection is relatively weak in the vicinity of the long-term optimum and increases at greater phenotypic divergence from this optimum.

would get us no closer to understanding the specific processes underlying the adaptive evolution of photoperiodic time measurement in *W. smithii*. Rather, we have chosen to propose an empirical, mechanistic process that not only explains our data with *W. smithii*, but also is consistent with the observations that the longer critical photoperiods in north-temperate populations represent a declining involvement of the circadian pacemaker as seen by the decreasing long-day response to T-experiments by arthropods in general (Figs. 6 and 7).

Circadian rhythms are ubiquitous to eukaryotes and provide a highly accurate, temperature-compensated clock for regulating the daily activities of protists, plants, and animals. Photoperiodism is widespread in higher plants, vertebrates, and arthropods and permits these organisms to anticipate and prepare for future seasonal changes in their environment. Over 60 years ago, Bünning proposed that circadian and photoperiodic clocks were causally connected. This proposition remains intriguing: Why not co-opt a pre-existing, highly reliable, and environmentally-buffered circadian mechanism for keeping track of periodic seasonal change as well as daily time. Indeed, investigating the causal connection between circadian and photoperiodic clocks has been the focus of many research efforts for decades. But, despite the tremendous advances that have been made in understanding circadian rhythmicity at the molecular level, virtually nothing is known about how or whether any of the downstream 'clock-controlled genes' connect with photoperiodic time measurement. Even less is known about how this connection changes with seasonal adaptation of photoperiodic response through evolutionary time. It is our goal and desire that our top–down approach to the evolution of photoperiodic time measurement will meet and mesh with the bottom–up approach that is being developed so fruitfully for circadian clocks.

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