

Circadian Rhythmicity and Photoperiodism in the Pitcher-Plant Mosquito: Can the Seasonal Timer Evolve Independently of the Circadian Clock?

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ABSTRACT: The two major rhythms of the biosphere are daily and seasonal; the two major adaptations to these rhythms are the circadian clock, mediating daily activities, and the photoperiodic timer, mediating seasonal activities. The mechanistic connection between the circadian clock and the photoperiodic timer remains unresolved. Herein, we show that the rhythmic developmental response to exotic light: dark cycles, usually used to infer a causal connection between the circadian clock and the photoperiodic timer, has evolved independently of the photoperiodic timer in the pitcher-plant mosquito *Wyeomyia smithii* across the climatic gradient of eastern North America from Florida to Canada and from the coastal plain to the mountains. We conclude that the photoperiodic timing of seasonal events can evolve independently of the daily circadian clock.

Keywords: climatic adaptation, circadian clock, evolution, photoperiodism, seasonal timing.

The two major rhythms of the biosphere are daily and seasonal, relating to the rotation of the earth about its own axis and the earth's rotation about the sun, respectively. All eukaryotes and at least some prokaryotes possess an internal, self-sustained circadian clock that regulates daily activities (Dunlap et al. 2004); a wide variety of plants and animals use the length of day (photoperiodism) to anticipate and prepare for the changing seasons (Withrow 1959; Frisch 1960; Aschoff 1965; Menaker 1971).

At northern latitudes or higher altitudes, winter arrives early, when local day length is still long; at southern latitudes or lower altitudes, winter arrives late, when local

day length is short. Therefore, northern or mountain populations switch from active development to dormancy when days are long; southern populations switch from active development to dormancy when days are short. Among temperate arthropods, the day length used to switch between direct development and dormancy or diapause (the critical photoperiod, or CPP) increases with latitude and altitude of origin (Andrewartha 1952; Danilevskii 1965; Bradshaw 1976; Taylor and Spalding 1986; Danks 1987, his table 24). It is important to note that day length is used as only an indirect or "token" cue to anticipate the arrival of winter and that it is the timing of winter's advent, not day length per se, that imposes selection on the CPP. The geographically variable seasonal environment imposes selection on the optimal time to enter diapause and, hence, on the CPP mediating the onset of diapause. In the pitcher-plant mosquito *Wyeomyia smithii*, CPPs are closely correlated with latitude and altitude, with R^2 repeatedly >0.90 (Bradshaw and Holzapfel 2001a).

In 1936, Erwin Bünning proposed that the daily circadian clock provided the necessary causal mechanism underlying the seasonal photoperiodic timer. Bünning's proposition has had enduring appeal among biologists because if true, it would mean that a single mechanism is responsible for orchestrating both daily and seasonal events in the lives of plants and animals. The major prediction from Bünning's proposition is that the photoperiodic timer should exhibit a rhythmic, circadian sensitivity to light that persists in constant darkness without the need to be reset every 24 h by an external stimulus. The experiment most widely used to implicate some involvement of the daily circadian clock in the seasonal photoperiodic timer is the Nanda-Hamner protocol (NH; fig. 1). In NH, organisms are exposed to a short day followed by a long night of varying duration in separate experiments, creating a total cycle length (T) of light plus dark ($T = L + D$) of one to several days; typically, $T = 24-72$ h. The proposition is that if photoperiodic response is determined by the circadian clock, then the underlying sensitivity to light should cycle between light insensitivity and light sensitivity during the long dark periods. If the dark period ends at a light-insensitive time of the rhythm, a short-day response

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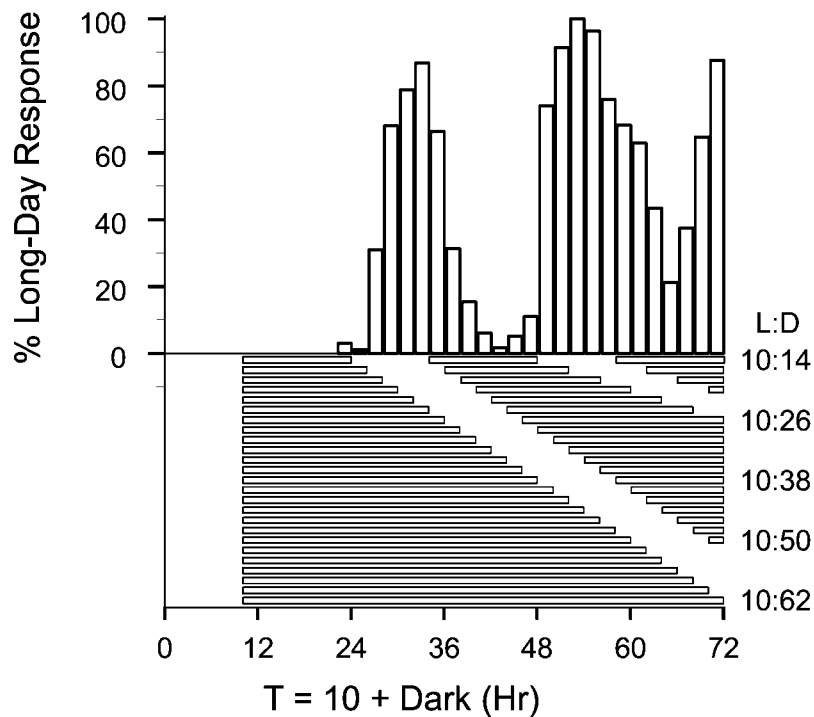


Figure 1: Rhythmic long-day response (percentage development) of *Wyeomyia smithii* to the Nanda-Hammer protocol (Nanda and Hamner 1958) when provided with a 10-h day and variable night length to create $T = \text{light} + \text{dark} = 10 + \text{dark}$ from 24 to 72 h in 25 separate experiments. The curve shows the long-day response of a southern (30°N) population. Note that the perception of a rhythm depends on both its period and its amplitude; without amplitude, there is no rhythm. The amplitude ($\log[\text{area under the curve}]$) of this rhythmic response varies with geography; the period (peak-to-peak interval) does not vary with geography (Bradshaw et al. 2003).

should ensue. If the dark period ends at a light-sensitive time of the rhythm, a long-day response should ensue. Consequently, as T is increased from 24 to 72 h and the underlying circadian-driven sensitivity to light cycles between light-sensitive and light-insensitive phases, there should be a rhythmic long-day response when percentage long-day response (development) is plotted as a function of T . Such a rhythmic response (fig. 1) has been observed among plants and animals and has been used to infer a circadian basis for photoperiodic time measurement (Pittendrigh 1981; Takeda and Skopik 1997; Vaz Nunes and Saunders 1999; Tauber and Kyriacou 2001; Goldman et al. 2004). However, at least in arthropods, the inference that this rhythmic response to NH (an expression of underlying circadian rhythmicity) constitutes the mechanism underlying adaptive evolution of the seasonal timer (photoperiodism) has come under increasing scrutiny (Bradshaw and Holzapfel 2001*b*; Veerman 2001; Saunders 2002, pp. 479–481; Bradshaw et al. 2003; Danks 2003, 2005).

The perception of any rhythm depends on two properties, period and amplitude. The period of a rhythm is the interval over which it repeats itself; we define the pe-

riod of a rhythm as the interval between successive peaks. The period of the rhythmic long-day response to NH is not significantly correlated with CPP among widespread populations of either mites in Europe (Vaz Nunes et al. 1990) or mosquitoes in North America (Bradshaw et al. 2003). Consequently, we now focus on amplitude. The amplitude of a rhythm is the magnitude of the peaks; we define the amplitude of a rhythm as $\log(\text{area under the curve})$. A number of studies have shown an apparent decline in the amplitude of the rhythmic response to NH in northern as compared to southern arthropods (Thiele 1977; Takeda and Skopik 1985; Vaz Nunes et al. 1990; Pittendrigh and Takamura 1993; Wegis et al. 1997; Bradshaw et al. 2003). None of these studies regressed amplitude on altitude or included altitude in a multiple regression with latitude. More importantly, none of these studies regressed CPP (the seasonal trait under selection) on the amplitude of the rhythmic response to NH (the output from the daily circadian clock and putative underlying mechanism).

To determine the degree of covariation between CPP and amplitude of the rhythmic response to NH in natural

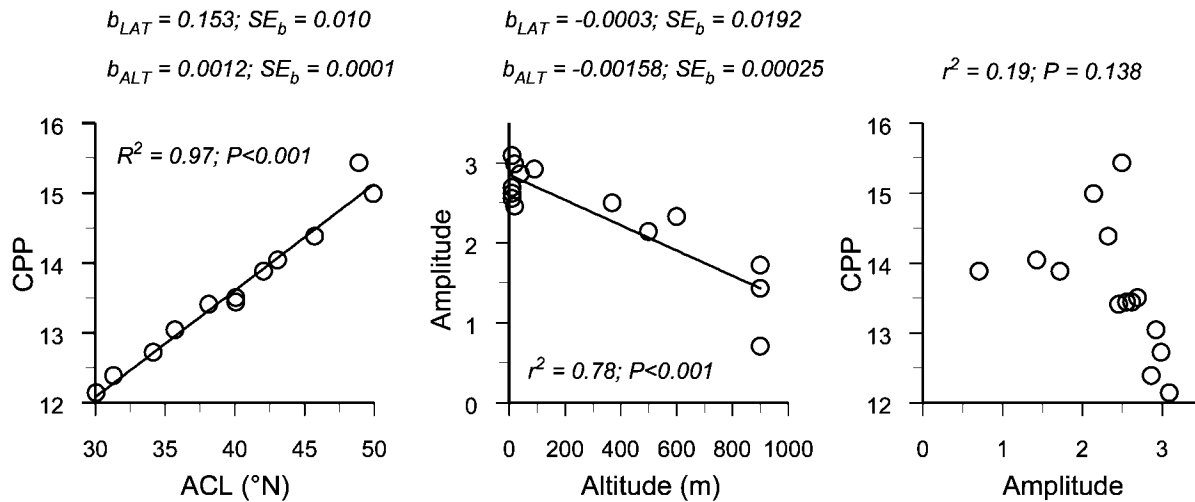


Figure 2: Critical photoperiod (CPP) and amplitude of the rhythmic response to the Nanda-Hamner protocol over latitudinal and altitudinal climatic gradients in eastern North America. *Left*, CPP is tightly correlated with altitude-corrected latitude: $ACL = \text{latitude} + \text{altitude} (b_{alt}/b_{lat})$, where b_{alt} and b_{lat} are the coefficients from regression of CPP on altitude and latitude, respectively. *Middle*, amplitude of the rhythmic response to the Nanda-Hamner protocol is negatively correlated with altitude but not latitude of origin. *Right*, lack of significant correlation between CPP (the trait undergoing selection) and amplitude of the rhythmic response to the Nanda-Hamner protocol (representative of the circadian clock).

populations, we use data from a previous study (Bradshaw et al. 2003) that included 14 North American populations of the pitcher-plant mosquito *W. smithii*, ranging from the Gulf of Mexico to Canada (30°–50°N) and from the eastern coastal plain to the Appalachian and Pocono Mountains (10–900 m elevation). There were at least two populations from each of five geographic regions: Gulf coast (30°–31°N), coastal North Carolina (34°–35°N), mountainous North Carolina (35°N), mid-Atlantic coast (38°–40°N), and far north (46°N). To reduce maternal effects, populations were reared for three generations in the laboratory before the start of experiments. Critical photoperiods are taken from Bradshaw et al. (2003, their table 1), as are the amplitudes of the rhythmic response to NH (their fig. 4). Both the CPPs and the responses to NH were determined at a constant temperature of 23°C. The CPPs and responses to NH were each run as a single block; within each block, all L:D treatments were started on the same date, and all populations receiving a given L:D cycle experienced that cycle in the same photoperiod cabinet, where dishes were rotated within the cabinet three times a week.

Critical photoperiod is positively correlated with both latitude and altitude (fig. 2, *left*). All populations, regardless of latitude or altitude of origin, exhibit robust photoperiodic response curves (fig. 3). These results reiterate the close relationship between CPP and the local growing season as represented by both latitude and altitude. However, the amplitude of the rhythmic response to NH is

correlated with altitude but not latitude (fig. 2, *middle*). The correlation of the rhythmic response to NH with latitude alone is not significant ($r^2 = 0.009; P = .745$). The correlation of amplitude with altitude alone is highly significant ($r^2 = 0.782; P < .001$), and the addition of latitude to this correlation increases r^2 by less than 0.001. These results mean that the amplitude of the rhythmic response to NH is correlated exclusively with altitude and not with latitude.

Across all populations, critical photoperiod is not significantly correlated with the amplitude of the rhythmic response to NH (fig. 2, *right*). It is difficult to propose that circadian rhythmicity has been a causal, necessary factor in the evolution of CPP when CPP is not correlated with either the period (Bradshaw et al. 2003) or the amplitude (fig. 2, *right*) of the rhythmic response to NH across natural populations. Each population exhibits a robust photoperiodic response curve, regardless of its CPP or the amplitude of its rhythmic response to NH (fig. 3). We have no reason to question that the rhythmic response to the Nanda-Hamner protocol represents an overt manifestation of the circadian clock. We therefore propose that *W. smithii* provides an example of Saunders's (2002, p. 481) third scenario, namely "that Nanda-Hamner periodicity is an expression of *basic* circadian rhythmicity, but PPTM [photoperiodic time measurement] is a separate mechanism" (emphasis Saunders's). From these observations, we conclude that in *W. smithii*, CPP has evolved independently of the rhythmic response to NH, and, hence, pho-

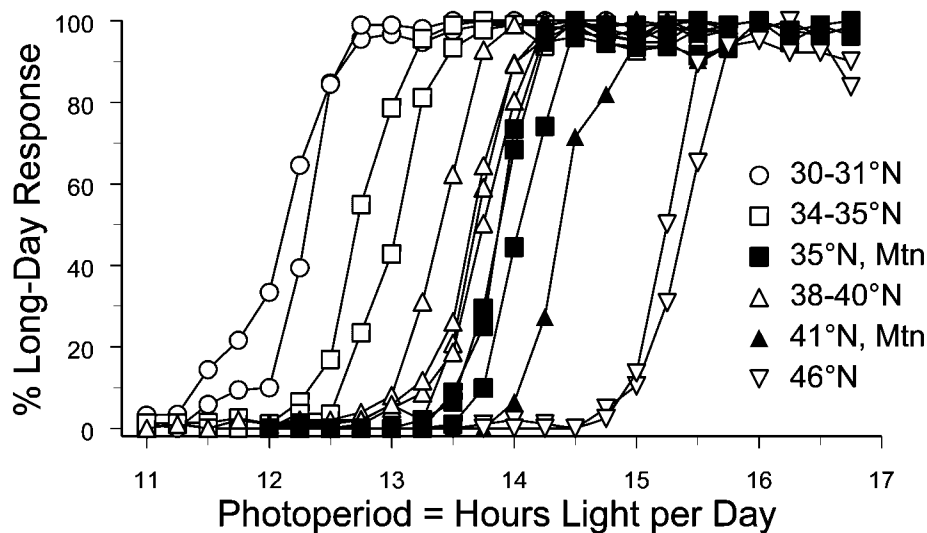


Figure 3: Photoperiodic response curves of populations of *Wyeomyia smithii* from the Gulf of Mexico to Canada and from the coastal plain to the mountains in eastern North America. Solid squares denote populations from the southern Appalachians in North Carolina; solid triangles denote populations from the Pocono Mountains in Pennsylvania.

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