Circadian Clock in Insect Photoperiodism

Abstract. *Night-interruption experiments with the wasp* Nasonia vitripennis *maintained in 48- and 72-hour light : dark cycles reveal two and three peaks of light sensitivity (diapause inhibition), respectively. The first peak is 19 hours after lights-on in all regimes tested; the later peaks occur at 24-hour intervals thereafter, providing evidence for the circadian nature of the photoperiodic clock in this insect.*

Some of the strongest evidence for the circadian nature of the seasonal photoperiodic "clock" comes from night-interruption experiments (*1*) in which organisms are maintained in 48- or 72-hour cycles that contain a short light component (8 to 10 hours) and a long dark period systematically interrupted by a supplementary light pulse. In plants (*2*) and birds (*3*) such experiments often reveal maximums of photoperiodic effect at roughly 24-hour intervals in the extended night. In the Biloxi variety of soybean maintained in a light : dark cycle (LD) of 8 : 64, for example, flowering was inhibited at Zeitgeber time (Zt) 16, Zt 40, and Zt 64 (*4, 5*). These results are usually interpreted as showing that a light-sensitive phase repeats itself in the extended night with circadian frequency.

Similar experiments have been performed in at least two insect species—with different results. For instance, in the codling moth *Carpocapsa pomonella* (*6*), maintained in an interrupted cycle of LD 8 : 40, maximum inhibition of

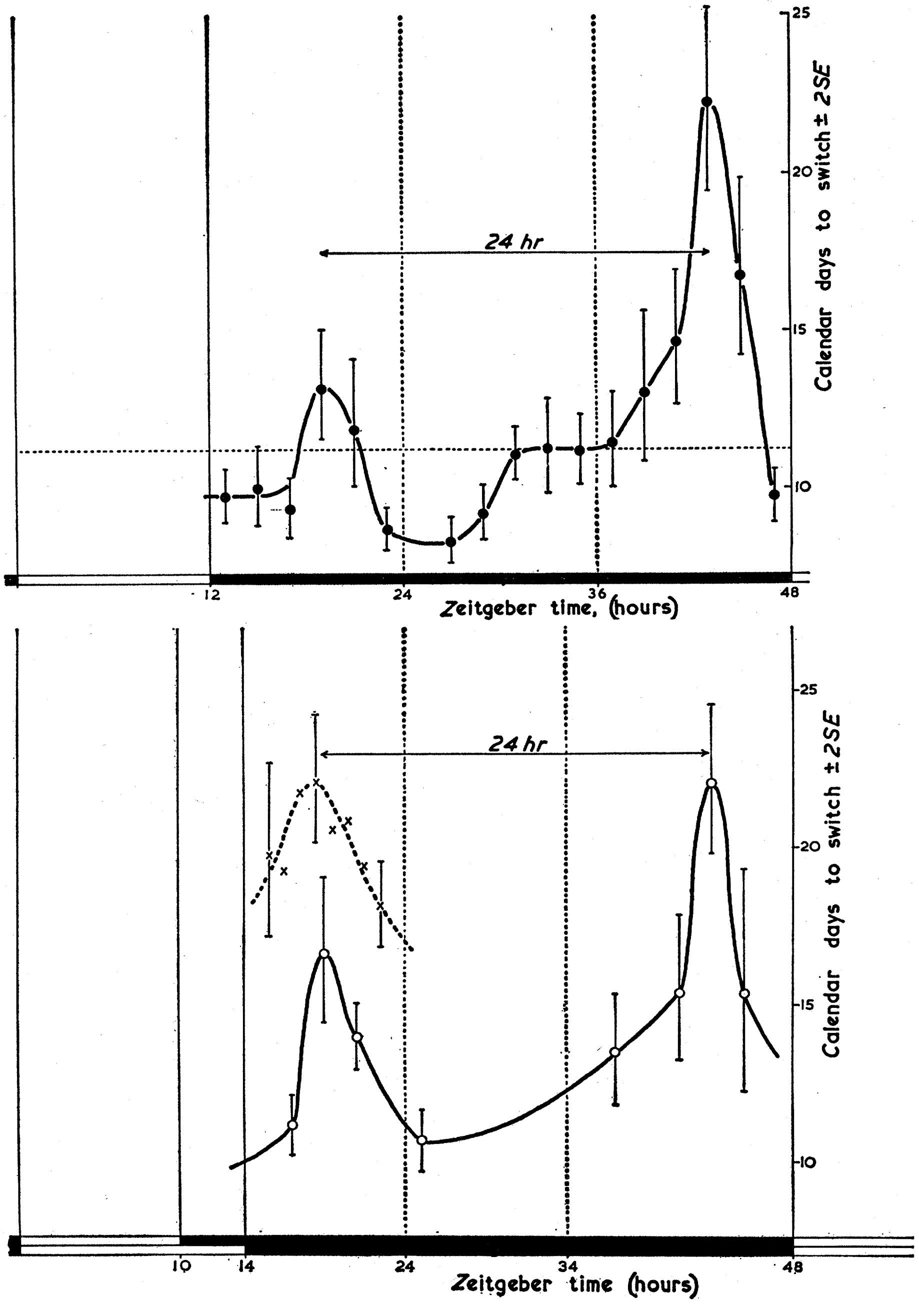

Fig. 1. Effect of light breaks (night interruption) on the inhibition of diapause production by females of *Nasonia vitripennis* in 48-hour light : dark cycles. The top panel shows 2-hour light breaks in an LD 12 : 36 cycle, the lower panel shows 1-hour light breaks in LD 14 : 34 (dashed line) and 2-hour light breaks in LD 10 : 38 (solid line). The horizontal dotted line in the top panel shows the response to an uninterrupted cycle of LD 12 : 36. The first peak of inhibition is at Zt 19 in all regimens, the second peak 24 hours later. Each point represents the mean age at the "switch" from developing to diapause larvae for about 40 females.

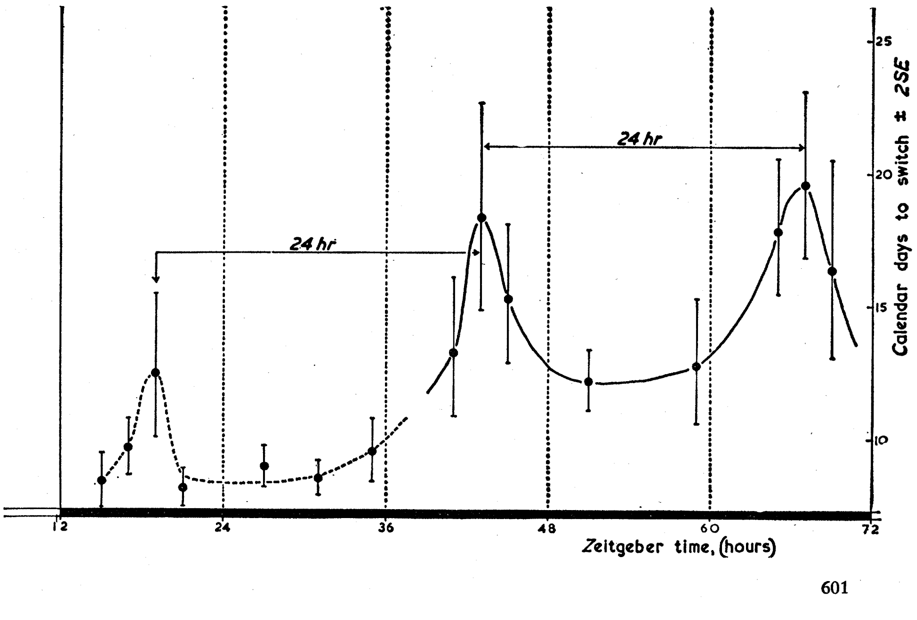

Fig. 2. Effect of 2-hour light breaks in an LD 12 : 60 cycle. Three maximums of photoperiodic effect are produced, the first at Zt 19 and the later peaks at 24-hour intervals. Each point represents the mean age at the "switch" for about 40 females.

larval diapause was found at Zt 16 and Zt 40. On the other hand, in the aphid *Megoura viciae* (*7*), at LD 8 : 64, there was only one point of long-day effect at Zt 16 but no repetition 24 or 48 hours later.

I now report the results of similar experiments with the parasitic wasp *Nasonia vitripennis*, a long-day species in which the photoperiod acting upon the mother controls the onset of larval diapause (*8*). These experiments demonstrate a well-defined circadian rhythmicity in response to the light pulses.

Newly emerged females of *N. vitripennis* were enclosed in small (5 by 1.2 cm) glass tubes with a male and three to five 3-day-old pupae of their fleshfly host *Sarcophaga barbata*. Groups of 40 of such tubes were placed in glass Kilner jars over a saturated solution of $NaNO_3$ to maintain the relative humidity within the jars at about 70 percent; the Kilner jars were then placed on different light : dark cycles at 18°C (*9*). Groups of females were exposed to LD 10 : 14, LD 12 : 12, or LD 14 : 10 for four cycles and then transferred to the appropriate 48- or 72-hour cycle. This procedure was adopted so that the photoperiodic rhythm, if present, would be entrained to the 24-hour light : dark cycle before being "released" into the longer regimens with "weaker" signals. The parasitized pupae of *S. barbata* were replaced by fresh pupae only when the lights were on, that is, every 48 or 72 hours. They were then kept at 25°C for 10 days and opened to ascertain whether the parasites within were diapause or developing larvae.

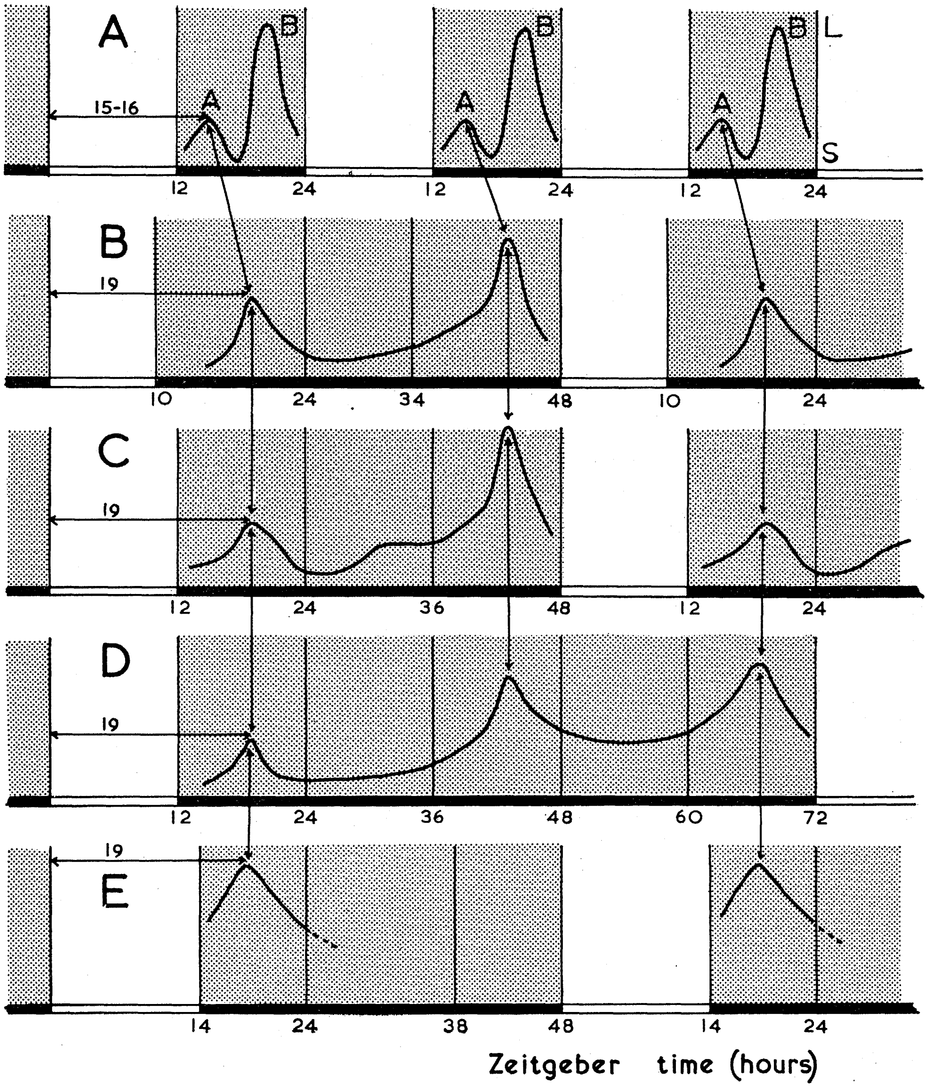

Fig. 3. Schematic drawing of the effect of light breaks (night interruption) in *N. vitripennis*, showing the relation between the peaks of diapause inhibition obtained in 48- and 72-hour cycles with the two peaks (*A* and *B*) obtained in each night of a 24-hour cycle (*11*). (A) One-hour light breaks in LD 12 : 12 producing two peaks, one (*A*) 15 to 16 hours after lights-on, and a second (*B*) 15 to 16 hours before lights-off. (B–E) Effect of light breaks in LD 10 : 38, LD 12 : 36, LD 12 : 60, and LD 14 : 34. The first peak in all of these regimens is 19 hours after lights-on and is considered to be equivalent to peak A in a 24-hour cycle. Later peaks occur at circadian intervals thereafter. *L*, long-day effects; *S*, short-day effects.

In a 24-hour periodic environment, females of *N. vitripennis* respond to the effects of photoperiod by "switching" to the production of diapause larvae after a number of light : dark cycles; short-day effects result in a rapid switch (after 8 to 11 days), and long-day effects result in a delayed switch (after 20 days or more). At 18°C, short-day conditions produce about 65 to 75 percent of diapause in the progeny and long-day conditions less than 5 percent (*8*). In the present experiments results were calculated as the number of calendar days to the switch so that they are comparable to earlier results for this species (*8*).

In the first experiment groups of 40 females of *N. vitripennis* were exposed to LD 12 : 12 for 4 days and then transferred to 48-hour cycles each with a 12-hour main light component and a 2-hour supplementary light pulse placed at different positions in the dark (Fig. 1). Maximum sensitivity to light (producing long-day effects) was observed when the supplementary light periods fell at Zt 19 and at Zt 43. A maximum short-day effect was obtained when the pulses fell between Zt 22 and 28. In other words, the two peaks of diapause inhibition were 24 hours apart, and the greatest short-day effect was observed when the pulse was placed in a position where the lights would have come on had the insects been maintained in a 24-hour regimen (that is, at the beginning of the "subjective day"). Light pulses falling in the second half of the subjective day (Zt 30 to 36) produced an effect indistinguishable from the uninterrupted control (LD 12 : 36).

In a similar experiment with a light : dark cycle of LD 12 : 60, three peaks of diapause inhibition were observed (Fig. 2). The first was at Zt 19, the second at Zt 43, and the third was after another 24-hour period at Zt 67. Light pulses falling between these peaks—during each "subjective day" (Zt 23 to 36 and Zt 48 to 60)—produced short-day effects.

In both of these experiments the peaks of diapause inhibition appeared at identical positions in the night and occurred at 24-hour intervals. The experiment using a 72-hour cycle is particularly good evidence for the involvement of the circadian system in photoperiodism and is the first to show clearly three peaks of light sensitivity in an insect system. In *Megoura viciae* (*7*) there was no evidence of a circadian rhythm, and in *Carpocapsa pomonella* maintained in an LD 8 : 64 cycle there was evidence of a complex timing system that involved two conceptually different clocks: an hour-glass timer associated with "dusk" and a 24-hour rhythm associated with "dawn" (*10*). The 72-hour experiment also removes the objection that each peak represents the result of a direct interaction between the pulse and the main light component. In other words, the middle peak is too far removed from either the preceding or following light periods to be caused by a direct interaction; the nearest effective light signals are those at the other peaks, both 24 hours away.

Since it was possible that the apparent 24-hour rhythm was generated in some way by the choice of light : dark cycles, which both included a 12-hour main component, two further experiments were carried out with 48-hour cycles that contained a 10-hour and a 14-hour main light component, respectively (Fig. 1). In these experiments the females were exposed to LD 10 : 14 and LD 14 : 10 for four cycles before being released into the longer regimens. Both of these experiments revealed maximums of photoperiodic effect at Zt 19 and, in the LD 10 : 38 experiment, a second maximum at Zt 43. This showed that the positions of the peaks were unaffected by the duration of the main light component. It is not known why the amplitude of the first peak is greater in the experiment with the 14-hour main light component.

In a 24-hour light : dark cycle two peaks of diapause inhibition are observed in *N. vitripennis* (*11*) and other insects (*12*) when the night is scanned by light interruptions. In these insects the first peak (A) is always a certain number of hours after lights-on ("dawn") and the second peak (B) always the same number of hours before lights-off (dusk). These observations form the basis of the coincidence model for the photoperiodic clock (*13*). This model is essentially a rhythm of sensitivity to light which is itself phase-set by the light : dark cycle, and has the same properties as overt circadian rhythms such as pupal eclosion in *Drosophila* or oviposition in the moth *Pectinophora gossypiella* (*13, 14*). The crux of this model is the dual action of light: (i) as an entraining agent and (ii) as an inducer when the light coincides with the photoperiodically inducible phase. According to Pittendrigh (*14*), pulses of light placed at both A and B act as entraining agents, but only one (at either A or B) coincides with the inducible phase.

In the present experiments the first maximum of photoperiodic effect had the same relationship to the beginning of the main light component in each of the regimens tested. For this reason it must be equivalent to a peak A in a 24-hour cycle (Fig. 3). The later peaks must therefore represent this phase of the rhythm repeating itself in the night with circadian frequency. In *N. vitripennis* maintained in 24-hour night interruption experiments, peak A occurs 15 to 16 hours after lights-on (*11*). The fact that it is 19 hours after lights-on in 48- and 72-hour cycles must indicate that the photoperiodic rhythm assumes a different phase relationship with a 48-hour or 72-hour Zeitgeber. The last peak in the night is not considered to be equivalent to peak B because it is 15 hours before lights-off in the LD 10 : 38 cycle and 17 hours before lights-off in the LD 12 : 36 and LD 12 : 60 cycles.

In none of the experiments was there any indication of two peaks of diapause inhibition in each "subjective night." This was so even in the LD 14 : 34 experiment in which the first 10 hours of the night were scanned by 1-hour pulses, and the two peaks, if present, would be expected to be at their most divergent (Fig. 1). It appears, therefore, that peak B or its equivalent does not appear in 48- or 72-hour cycles. Although these experiments are consequently difficult to interpret in terms of the coincidence model, they do provide strong evidence for the circadian nature of the seasonal photoperiodic clock.

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References and Notes

1. In night-interruption experiments, a short light break (usually 1 to 2 hours, or as little as a few minutes) is introduced into the dark component of the cycle and repeated as a daily signal. Different experimental groups receive light regimes with the interruption in a different position in the night.
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3. W. M. Hamner, in *Circadian Clocks*, J. Aschoff, Ed. (North-Holland, Amsterdam, 1965), p. 379.
4. The Zeitgeber is the forcing oscillation (that is, the light : dark cycle) which entrains a biological rhythm. Zeitgeber time (Zt) is measured in hours after the beginning of the main light component.
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9. These experiments were conducted in Gallenkamp cooled incubators fitted with Philips 8W striplights controlled by Londex time switches.
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Terrestrial and Aquatic Orientation in the Starhead Topminnow, Fundulus Notti

Abstract. *Starhead topminnows from various shores of a small woodland pond were displaced to unfamiliar surroundings, and their orientation was tested in aquatic and terrestrial arenas. These fish used a sun compass to move in a direction which, at the location of their capture, would have returned them to the land-water interface. The fish accomplished directional terrestrial locomotion by using the position of the sun to align its body for each jump. On heavily overcast days many fish were unable to orient their bodies in a consistent direction from jump to jump; this inability to orient resulted in random rather than linear movement. There was considerable individual variation in terrestrial locomotor ability.*

Terrestrial locomotion and orientation of fishes have been investigated in tidewater *Fundulus* (*1*) and in *Bathygobius soporator* (*2*). *Bathygobius soporator* use local landmarks for orientation and do not rely on sun cues; however, the cues used for orientation by *Fundulus* were not understood. The mosquito fish, *Gambusia affinis* (*3*), and several species of amphibians (*4*,