Time Measurement in Insect Photoperiodism: Reversal of a Photoperiodic Effect by Chilling

Abstract. *Spectacular reversals of the photoperiodic control of diapause are obtained if females of* Nasonia vitripennis *are chilled for 4 hours in certain light-dark cycles. Experiments in which chilling is combined with short light breaks (night interruptions) show that the first peak of diapause inhibition moves in response to the chilling. This result provides an explanation for the photoperiodic reversal; it is also circumstantial evidence for the participation of circadian rhythms in photoperiodism.*

The induction and inhibition of larval diapause in the parasitic wasp *Nasonia vitripennis* is controlled by the photoperiod experienced by the female parent (*1*). Apart from this maternal aspect, *N. vitripennis* is a typical long-day species with a well-defined critical daylength of 15 to 15¼ hours per 24 hours (critical nightlength of 8¾ to 9 hours per 24). At short daylength females produce developing progeny for the first few days of imaginal life and then "switch" to the production of diapause larvae after 5 to 11 short-day cycles. At long daylength the switch occurs late in imaginal life (22 to 30 days).

In many cases of biological chronometry, a high degree of temperature compensation is observed, although below about 5°C the mechanism of the clock appears to be slowed down or stopped (*2*). This effect has also been found in *N. vitripennis*, and if a 4-hour period at 2°C is applied daily to ovipositing females in certain light-dark cycles spectacular reversals of photoperiodic effect have been recorded. For instance, in a light-dark cycle of LD 14 : 10, chilling in the dark period converted an otherwise short-day effect into a long-day one and resulted in the elimination of diapause among the larvae subsequently produced (Table 1); a similar period of chilling in the light component had no such effect. Conversely, at LD 16 : 8, chilling in the light converted the response from a long-day response to that of a short-day, whereas chilling in the dark did not. At LD 8 : 16, which is well short of the critical daylength, chilling in both components of the cycle had no marked effect upon the photoperiodic response.

Table 1. The reversal of photoperiodic effect in *Nasonia vitripennis* by a daily period of chilling.

Temperature (°C)	Chilling applied	Females (No.)	Mean days to "switch" (± S.E.)	Delay (+) or acceleration (−) in "switch" (days)
		LD cycle 14 : 10		
18	No chilling (control)	58	9.7 ± 0.35	
2	4 hours daily after lights-on	40	8.3 ± .37	− 1.4
2	4 hours daily in middle of light	18	8.6 ± .50	− 1.1
2	4 hours daily after lights-off	39	24.0*	+14.3
2	4 hours daily in middle of dark	36	23.0*	+13.3
		LD cycle 16 : 8		
18	No chilling (control)	36	23.0*	
2	4 hours daily after lights-on	16	10.6 ± .70	−12.4
2	4 hours daily in middle of light	19	12.7 ± .72	−10.3
2	4 hours daily after lights-off	20	23.0*	0.0
		LD cycle 8 : 16		
18	No chilling (control)	36	10.6 ± .69	
2	4 hours daily after lights-on	16	12.2 ± 1.09	+ 1.6
2	4 hours daily after lights-off	19	7.6 ± 0.65	− 3.0
2	4 hours daily in middle of dark	18	9.5 ± .80	− 1.1

* None of the females had "switched" after 23 to 24 cycles.

At the present time a controversy exists concerning the nature of the timing mechanism involved in insect photoperiodism. On the one hand, results from "night interruption" (*3*) experiments in *Pieris brassicae* (*4*), *Pectinophora gossypiella* (*5–7*), and a number of other species have indicated that circadian rhythms are involved. These results have led to the "co-incidence model" (*7, 8*) in which a substrate rhythm, phase-set by the main light period, and a light-induced enzyme reaction, are envisaged.

In this hypothesis diapause is presumed to occur when the light-activated phase of the enzyme does not coincide with the peak of the substrate rhythm and is inhibited when coincidence does occur. On the other hand, very similar results from night interruption experiments in the aphid *Megoura viciae* (*9, 10*) have led to an entirely different hypothesis, principally because the reactions to short light breaks were found to be the same after a main light period of 8, 13½, or 25½ hours, and a single long night was found to be fully inductive even when coupled with light periods as long as 36 hours. For these reasons Lees assumes that time measurement in *Megoura* is accomplished by reference to an "interval timer" that measures duration of the dark period with little or no reference to the light.

The effects of chilling described in this report cannot be explained on the basis of Lees's (*10*) interval-timer hypothesis, since chilling in the light appears to affect the measurement of the dark component. However, the results can be accounted for by Pittendrigh and Minis' (*7*) "co-incidence model," as the following experiment shows.

Females of *N. vitripennis* were supplied daily with two pupae of their flesh-fly host *Sarcophaga barbata* and kept at 18°C in a light-dark cycle of LD 14 : 10 for 30 days (*11*). Light interruptions of 1 hour at different positions in the 10-hour night produced two peaks of diapause inhibition, similar to those described in other insects (*4–10*).

The first peak occurred when the

light break was placed between 15 and 16 hours after the beginning of the main light component (Zeitgeber time 15 to 16), and the second when placed at Zt 21 to 23. According to Pittendrigh and Minis (*7*), the first peak represents the position of the substrate maximum (or the critical daylength). When the light break is placed later in the light-dark cycle a phase-jump occurs, until at Zt 21 to 23 the light break serves to phase-set the substrate rhythm so that its peak now coincides with the end of the main light component, and diapause is again eliminated.

When this experiment was repeated with the addition of a 4-hour period at 2°C at the beginning of the main light period, the first peak of inhibition, although depressed, was observed to have moved further into the "night" by 2 hours (to Zt 17 to 18) (Fig. 1) (*12*). This is assumed to be because the substrate rhythm is now forced to oscillate within the 20 hours available at suitable temperature (Zt 4 to 24), but its shape remains symmetrical. In other words, the substrate rhythm now has a period of 20 hours and reaches its peak at Zt 17 to 18 instead of at Zt 15 to 16.

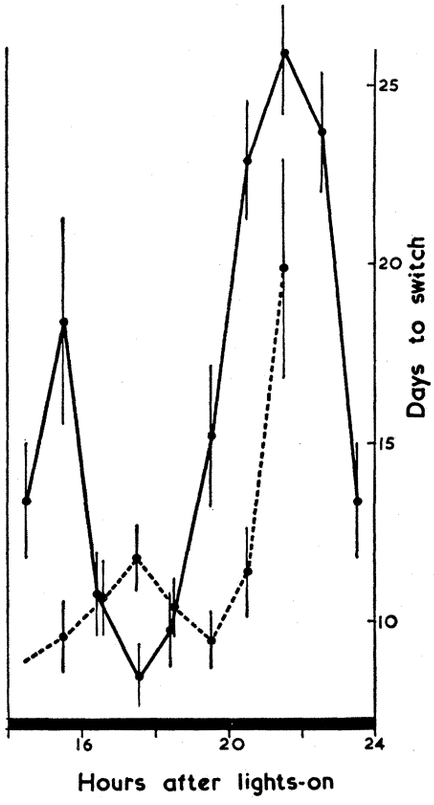

Fig. 1. Effect of 1-hour light breaks (night interruptions) on the inhibition of diapause production by females of *Nasonia vitripennis* in a light-dark cycle of LD 14 : 10 (data given are for the 10-hour dark period). The first peak of inhibition moved to the right by 2 hours when chilling was applied for the first 4 hours of the light component. Each point represents the mean age at the "switch" from developing to diapause larvae for about 20 females; vertical lines, plus or minus twice the standard error. Solid line, in LD 14:10 without chilling; dashed line, in LD 14:10 with chilling at 2°C for 4 hours after lights-on.

This result is interesting for two reasons. (i) It provides an explanation for the results summarized in Table 1. For instance, if a similar period of chilling had occurred at the beginning of the main light period in an LD cycle of 16 : 8, the 2-hour shift in the substrate maximum to the right would have resulted in the conversion of a long daylength to a short one. (ii) This result provides circumstantial evidence for the participation of a circadian rhythm in insect photoperiodism, since chilling in the light affects the position of the first peak in a way that can be predicted from Pittendrigh and Minis' (*7*) "co-incidence model." If an interval timer, which measures the duration of the dark component only, was involved, chilling in the light would not have had such an effect.

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References and Notes

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2. E. Bünning, *The Physiological Clock* (Springer-Verlag, Berlin, 1964), p. 48; J. Harker, *The Physiology of Diurnal Rhythms* (Cambridge Univ. Press, Cambridge, 1964), p. 51.
3. In "night interruption" experiments, a short light break (usually 1 to 2 hours, or as short 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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11. These experiments were conducted in Gallenkamp cooled incubators fitted with Philips 8W striplights controlled by Londex time switches.
12. The mean ages at the "switch" for the two groups at Zt 16 are significantly different ($t = 6.187$, $P < .001$); so are those for the two groups at Zt 18 ($t = 3.755$, $P < .001$).
13. I thank Mrs. M. H. Downie for technical assistance, and the Science Research Council for financial support.

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Synaptic Loci on Parietal Cortical Neurons: Terminations of Corpus Callosum Fibers

Abstract. *Dendritic spines disappear when the synaptic terminals impinging on them are destroyed. Terminal synaptic sites of axonal projections can be mapped by interrupting the afferent system and then comparing the density of dendrite spines in the suspected recipient area with controls. The corpus callosum was sectioned in five rabbits at birth. When the rabbits were 30 days old, the loss of dendrite spines was limited to the oblique branches of apical dendrites in the parietal cortex.*

It has been well established by means of both light microscopy (*1–3*) and electron microscopy (*4–6*) that the dendritic spine or thorn is an essential part of the postsynaptic membrane of most neurons. Gray showed that the spine receives a special class of presynaptic terminals [Gray, synaptic type I (*4*)], while Colonnier's study indicates that spines are resorbed if the presynaptic terminals are lost (*7*). We have reported (*8*) that after interruption of the visual afferent system (enucleation, lateral geniculate lesion), there is moderate to marked loss of spines along the central third of the apical shafts of pyramids in the visual cortex. The present report deals with results of interruption of another cortical afferent system, the callosal projection.

The corpus callosum was sectioned in five newborn rabbits in the following manner. Under light ether anesthesia, a curved cutting needle threaded with 2-0 silk was forced through the skull just to the right of the midline between the eyes and swept deep and posteriorly in such a way as to emerge just anterior to the posterior fontanelle. The course taken by the suture material was parallel to and to the right of the sagittal suture and below the corpus callosum. The suture was then