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0014-4754/87/080933-03$1.50 + 0.20/0

Insect photoperiodism: the linden bug, *Pyrrhocoris apterus*, a species that measures daylength rather than nightlength

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Summary. Independent variation of the dark and light components of the daily photocycle has shown that the linden bug, *Pyrrhocoris apterus,* unlike other species, 'measures' daylength rather than nightlength. Greatly extended dark periods coupled with a short photophase (a Nanda-Hamner protocol) shows peaks and troughs of diapause at about 16-h intervals, an extremely short period for a circadian clock. If circadian oscillations are involved in photoperiodic time measurement in this species, a photoinducible phase might lie in the early rather than the late subjective night.
Key words. Diapause; photoperiodism; daylength measurement; circadian system.

Photoperiodic regulation of diapause or seasonal morphs is very widespread in the insects, particularly among those in the temperate zones[1]. In those species that have been adequately investigated, it is concluded that the duration of the scotophase or dark (D) component of the daily photocycle is more important than the duration of the photophase or light (L)[2–5]. In the European corn borer, *Ostrinia nubilalis,* for example, Beck[6] showed that larval diapause induction was maximal with D between 10 and 14 h, but when D was held constant at these values, the duration of L could be varied from 5 to 18 h. In the green vetch aphid, *Megoura viciae,* the range of L giving a high incidence of the autumnal egg-laying morph (the ovipara), when coupled to 12 h of D, was 12 to 36 h, whereas, with a constant 8 h of light, the duration of darkness reached a sharp critical nightlength at 9.5 h[7]. These observations suggest very strongly that the principal element of the photoperiodic clock begins at the light-off signal and the clock measures nightlength rather than daylength.

These conclusions are further supported by the results of 'resonance' or Nanda-Hamner experiments in which L is kept short but the length of the dark component is greatly extended to give LD cycles up to 3 or 4 days in duration. Experiments of this type may reveal two apparently different forms of nightlength measurement: hourglass or oscillatory. In *M. viciae,* for example, a dark period hourglass is indicated because the incidence of ovipara production rises sharply after 9.5 h of darkness and then remains consistently high[7]. In other species, such as the flesh-fly *Sarcophaga argyrostoma*[8] and the red spider mite, *Tetranychus urticae*[9] diapause incidence rises and falls with a circadian periodicity as D is extended, and the circadian oscillator(s) involved are clearly phase-set from the beginning of darkness.

The experiments reported here were carried out with the linden bug, *Pyrrhocoris apterus,* which overwinters in the adult instar with an ovarian diapause if the last nymphal instar and the young adults are exposed to short days[10,11]. In the central Bohemian strain (Prague, 50° N) used in this investigation, the 'critical' daylength for diapause induction occurs in a LD cycle of about 15.75 h of light and 8.25 h of darkness[11]. Groups of about 40–50 5th instar nymphs were maintained in glass Kilner jars with dry lime seed *(Tilia cordata)* and water, and exposed to a variety of experimental light regimes at 25 °C. Three weeks later, when the bugs had developed to the adult instar, the females were dissected in saline to ascertain the status of their ovaries, according to the criteria described by Hodek[12]. By this time, non-diapausing bugs had either laid eggs (Hodek's stage 6), contained chorionated eggs (stage 5), or showed various stages of vitellogenesis (stages 3 or 4). Diapausing bugs, on the other hand, possessed ovaries with undifferentiated oocytes (stage 1) or small oocytes lacking yolk (stage 2). The proportion of diapause in the group was expressed as a percentage of bugs in stages 1 and 2.

Bugs were exposed to light cycles with the photophase (L) held constant (L = 12, 15, 16 or 17 h) and the scotophase (D) varied, or with D held constant (D = 7, 8, 9 or 12 h) and L varied (fig. 1). In each experiment the incidence of diapause was compared with the critical day or nightlength obtained from 24-h cycles[11].

The results show that when D was held constant (right hand panels) diapause was consistently high when L was less than about 15 h, but consistently low when L was more than 16 h. This compares well with the known critical photophase of 15.75 h/24. On the other hand, when L was held constant at

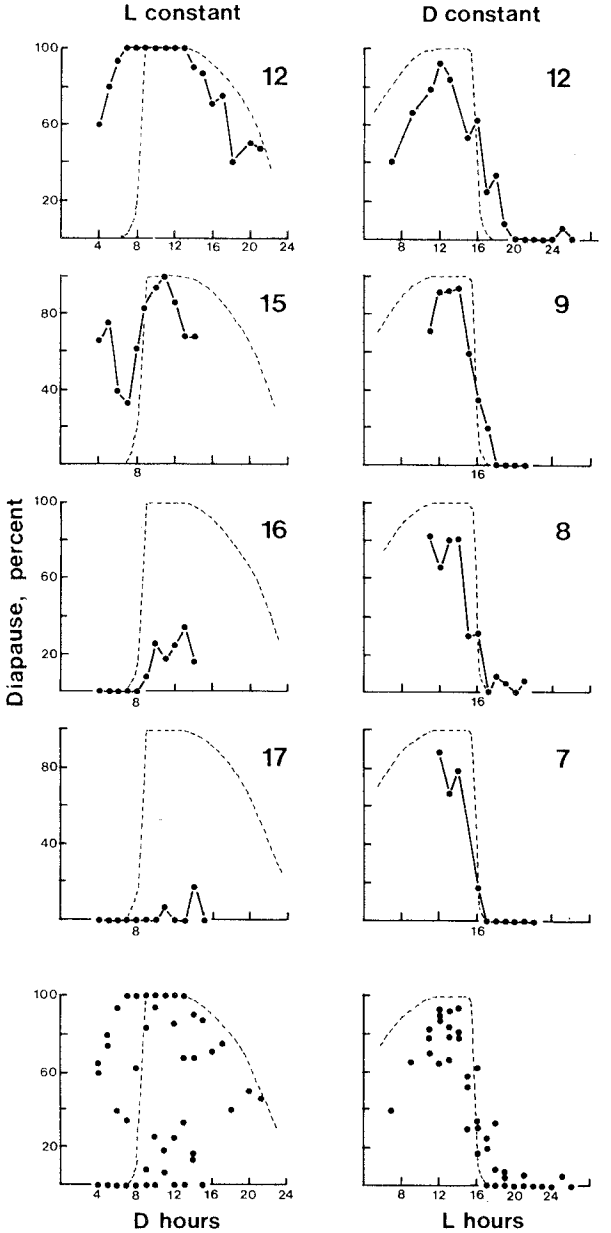

Figure 1. The incidence of ovarian diapause in females of the linden bug, *Pyrrhocoris apterus*, exposed to independently varied light (L) and dark (D) periods of the 'daily' photocycle. Left-hand panels: L held constant at 12, 15, 16 or 17 h, and D varied. Right-hand panels: D held constant at 12, 9, 8 or 7 h and L varied. Lower panels: Data for L-constant and D-constant experiments combined. In each panel the dotted curve shows the photoperiodic response curve for *P. apterus* in a 24-h cycle (from Saunders[11]).

17 or 16 h (just above the critical daylength) diapause incidence was low (30% or less) even when D exceeded the apparent critical nightlength (8.25 h/24). When L was held at 15 h (just below the critical) or at 12 h, diapause was high, almost regardless of scotophase duration. Only at L = 15 h was there an indication that diapause incidence was influenced by scotophase duration as in 24-h cycles.

Although these results indicate that both L and D are of consequence, the 24-h photoperiodic response curve was mimicked more closely by the duration of the photophase. This is strong evidence that daylength measurement is more important than nightlength measurement in *P. apterus*.

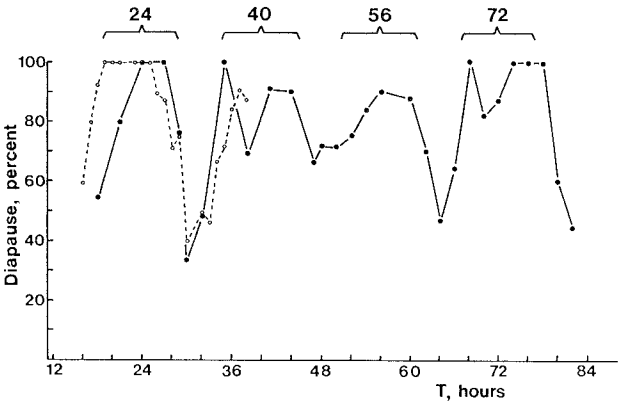

Figure 2. The incidence of diapause in photocycles containing either 8 h (●——●) or 12 h (○– – –○) of light (L) and a greatly extended period of darkness (a Nanda-Hamner experiment). T = the period of the light-dark cycle (L + D). Peaks (and troughs) of diapause incidence occur at roughly 16-h intervals as T is extended.

Two Nanda-Hamner experiments, one complete (L = 8 h, D 6–74 h) and one partial (L = 12 h, D 4–26 h) were carried out to determine whether the clock in *P. apterus* has an hourglass or circadian basis. The results (fig. 2) show a fluctuating response which certainly cannot be interpreted in terms of a simple hourglass. A periodicity can be discerned with peaks of high diapause centring at T 24, 40, 56 and 72, about 16 h apart, and troughs of low diapause at T 30, 47, 64 adn 80, again 16 or 17 h apart. An interpeak interval of 16 h is, of course, extremely short for a free-running circadian oscillation, although intervals as short as 17.5–18 h have been recorded for a Russian strain of *Tetranychus urticae*[13], about 20 h for a Dutch strain of the same mite[14], and about 22 h in the cabbage butterfly *Pieris brassicae*[15]. Free-running eclosion rhythms as short as 18.8 h have also been recorded in northern strains of *Drosophila littoralis*[16]. Therefore, if the present Nanda-Hamner results indicate that the photoperiodic clock in *P. apterus* has a circadian basis, the constituent oscillators must have a remarkably short free-running period.

If we conclude that the circadian system is involved in diapause regulation in the linden bug, the oscillator(s) cannot 'damp out' in long light periods, or be reset to a characteristic phase at the light to dark transition, as in the *Drosophila pseudoobscura* case[17] that acts as a model for the external coincidence type of clock. The present data indicate that the timing system must operate through the light period of the cycle and that the phase illuminated by the end of the photophase, rather than the beginning, may be of importance in discriminating between short and long days. A photoinducible phase lying in the early subjective night rather than the late subjective night has also been postulated for the photoperiodic clock governing antifreeze protein production in the beetle, *Dendroides canadensis*[18]. We must therefore expect variations in this feature in the insects, as well as among vertebrates[19].

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0014-4754/87/080935-03$1.50 + 0.20/0

Plant-sucking bugs can remove the contents of cells without mechanical damage[1]

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Summary. A mirid and a coreid, feeding on a variety of plant tissues, evacuated the contents of cells up to 3.5 mm from the furthest penetration of their mouthparts. A pectinase occurred in the salivary glands of the mirid and an invertase in those of the coreid, but not vice versa. Cells in the mirid lesions were apparently emptied while the walls retained their shape, whereas coreid lesions showed an immediate inward collapse of cell walls and engorgement of intercellular spaces.
Key words. Heteroptera; plant bugs; plant lesions; saliva; *Helopeltis; Amblypelta;* pectinase; cell walls.

Plant-sucking insects in the Heteroptera are reputed to pierce the cells they suck or vessels they tap. Those that make multicellular lesions and/or feed on seeds have been described as lacerating tissues by vigorously thrusting their piercing/sucking, stylet-like mouthparts back and forth[2–5]; those that feed on vascular tissue have been shown to penetrate individual phloem cells[6]. The investigation reported here, however, has revealed that some Heteroptera drain parenchymal cells en masse without penetrating them.

In Papua New Guinea, a mirid, *Helopeltis clavifer* (Walker), feeding on cocoa pods and shoots and on sweet potato stems, and a coreid, *Amblypelta* sp., feeding on cassava and sweet potato stems, were found to cause melanized lesions, roughly spherical to more elongate depending on the tissue, extending up to 3.5 mm beyond the feeding puncture. Those of *H. clavifer* on cocoa mostly occurred in parenchyma close to or immediately below the epidermis and were visible externally; those of Amblypelta on cassava and of either insect on sweet potato stems were sometimes externally visible but more often lay mostly or entirely between and/or below the vascular tissue and were not then externally visible, although when such an occluded lesion occurred a few centimeters from the tip of a shoot, it would often wither and die.

The insects, once committed to feeding, allowed a lens to be brought almost into contact with the head, and it proved possible to observe closely the movements of the head capsule and labium during feeding. Often, very little such movement was observed, however, and for the reasons outlined below it seemed possible that typically the distance penetrated by the insect's stylets fell far short of the outermost margins of the lesion that subsequently formed.

Penetration of a substrate by phytophagous Heteroptera is by a reciprocating, 'sawing' action of the four stylets, which form a coherent 'stylet bundle'. As the insects work the bundle as a whole into the substrate, corresponding reciprocating movements of the head capsule are visible. The labium, in which the stylet bundle is housed, does not enter the substrate; its segments 'elbow' backwards as the stylets penetrate, and from the angle of the elbow and its conformation relative to the labrum, it is possible to estimate limiting values for how far the bundle as a whole has been inserted[4], as indicated in figure 1.

Such calculation implies a constant length of stylet bundle (i.e. to the opening of the salivary and food canals) hence a possible source of inaccuracy would be the difference between the maximum protraction and retraction of the stylets relative to the head capsule. This distance is reputed to be relatively small, due primarily to the anatomy of the bases of the stylets and of their actuating muscles[7]. In ad hoc experiments, the stylet bundles of the live insects were gently slipped out of the labium and the stylet tips observed under a microscope while keeping the bundle at right angles to the body. In these circumstances the insects were found to perform violent movements of the stylets, identifiable as an attempted retraction cycle[8], and the maximum distance between any two stylet tips was always less than 0.1 mm in *Amblypelta,* the larger of the species under observation, and possibly no more than 0.02 mm in *Helopeltis.* Any attempt to increase this relative displacement of the stylets by direct manipulation in living or recently dead insects resulted in breakage of the stylets or their attachment.

From these observations, it was concluded that, based on measurements of an individual insect, a geometrical estimate of the maximum exsertion of the stylets during the time the insect was observed feeding could be made as illustrated in figure 1, subject to an *underestimate* of at the very most 0.15 mm for *Amblypelta* and 0.05 mm for *Helopeltis.* Fortunately in relation to the purpose of such estimations, errors considerably greater than these would not have invalidated the conclusion that the lesions formed by the insects usually extended some mm beyond the furthest possible penetration by their stylets.

Thus, when *Helopeltis* fed on previously unattacked cocoa pods, the stylets were typically estimated to penetrate more or less normal to the surface and no further than 0.3 mm deep, yet a hemispherical lesion developed thereafter with a diameter at the surface of between 3 and 4 mm. Deeper stylet penetrations of pods were sometimes observed, most often into those already scarred by previous lesions, but occluded, spherical lesions could then be found subsequently, a few millimeters below the surface of the pod. On cocoa stems, *Helopeltis* again made insertions of less than 0.3 mm, as a result of which lesions developed up to 7 mm axial to the stem and about 3 mm wide.

Amblypelta, when feeding on young sweet potato stems and cassava tips a few mm in diameter, was typically estimated to penetrate up to 0.6 mm, and subsequent sectioning of the stem revealed a lesion in the live pith parenchyma at about that depth, but extending a further 3–4 mm in both directions up and down the stem.