Photoperiodic regulation of insect and molluscan hormones

Ciba Foundation symposium 104

1984

Pitman

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The participants at this symposium are an intriguing mix of people, with similar research problems which are being approached in very different ways. The idea behind this meeting, suggested initially by David Saunders, was to bring together people working on insect or molluscan photoperiodic clocks and on hormones to see if we could find any common ground. Several people here work in both areas, but most of us are at one or other end of the spectrum. I hope that we can find some common points of synthesis as we hear about work on circadian and photoperiodic rhythms in insects, arachnids (mites) and molluscs. Perhaps we can in the next few days become even closer in our understanding of the interface between perception of environmental cues, in this case photoperiodic changes, and the initiation or the inhibition of an endocrine-controlled event. The transduction mechanism between perception of the stimuli and a change in an endocrine-controlled event is, for me, a particular fascination. For most of the organisms that we shall be considering, the only real manifestation that we have available to indicate that the animal has perceived a photoperiodic response is some developmental or physiological event that we can monitor.

My own work tends to be on neurosecretions but I shall try to set an example, as a good chairman should, of not being preoccupied only with my own particular interests, so that we can hope to see some gelling together of the two major aspects of this symposium.

Introduction: the links between 'wet' and 'dry' physiology

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By way of introduction I should first say something about our varied definitions of the words 'photoperiodism' and 'photoperiodic'. In this symposium we shall be dealing with photoperiodism in its widest possible sense, to include rhythmic and clock-like phenomena that are entrained or regulated by the daily light cycle, as well as photoperiodism in its strictest sense—as a seasonal phenomenon regulating annual cycles of reproduction, polymorphism, etc. If we kept strictly to this latter aspect, we would have to exclude circadian phenomena. But, as we shall see, some theories about photoperiodic time-measurement are based on circadian rhythmicity, and if we are to understand anything about the wiring patterns of the 'clock' in the insect or molluscan brain, this wider treatment is essential.

Fig. 1 shows the essence of the problem of photoperiodism, as depicted in A. S. Danilevskii's book (1965) on seasonal development in insects. This figure shows the changes in photoperiod with the months of the year, at different latitudes. The seasonal changes in photoperiod proceed with mathematical accuracy, and thus provide very good ('noise-free') time cues that both plants and animals can use to govern their seasonal activity. For example, in London at a latitude of 50 °N, the photoperiod in the depth of winter is about 8 h. At the equinox the photoperiod is, not surprisingly, 12 h of dark and 12 h of light at all latitudes. At the height of summer in London we have about 16 h of light, but a biologically effective photoperiod of 17.5 to 18 h if the twilight 'zones' are included. Some people at this symposium are from Chapel Hill, North Carolina (35 °N), where day-lengths show an annual range from about 10 to 14.5 h; another participant (Pekka Lankinen) works within the Arctic circle (65 °N) where days range from 4 h in the winter to continuous illumination in midsummer. Organisms have evolved a wide variety of responses to such differences, here the most important being a longer critical day-length at higher latitudes.

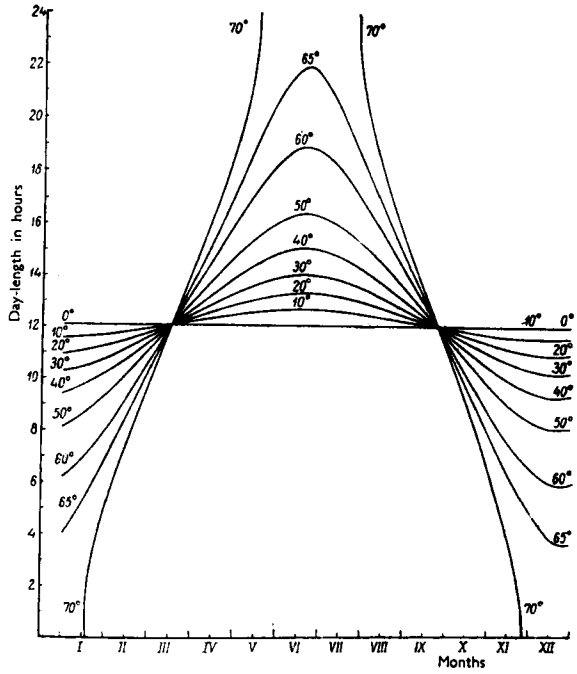

FIG. 1. The seasonal changes in day-length at different latitudes in the northern hemisphere (from Danilevskii 1965).

Fig. 2 shows the 'clock' end of the problem. It illustrates the sharp photoperiodic response curve of *Pyrrhocoris apterus*, the red or Linden bug. Such a curve is typical for a 'long-day' species with a winter diapause. It is obtained by exposing populations of the insect to different *static* photoperiods at the time when the insect is sensitive to photoperiod—in this case during the 4th and 5th (final) nymphal instars. The proportion of the insects that go into diapause at each photoperiod is then obtained. Females of *P. apterus* have an ovarian or reproductive diapause, and it is a simple matter to discover, by dissection, whether the ovaries have developed or not. If the ovaries have remained small, as at short day-length (8 to 15 h), the insects are deemed to be in diapause. If the insects become reproductive and their ovaries full of mature eggs, as at long day-length (16+ h), they are counted as being in a non-diapause state. In Fig. 2 there is a typical and remarkably precise critical day-length between 15 h and 16 h of light, below which all bugs enter a firm ovarian diapause. Day-lengths shorter than the minimum day-length that they encounter in their natural environment (~8 h) produce a weaker diapause response. The ecologically important switch mechanism between long days

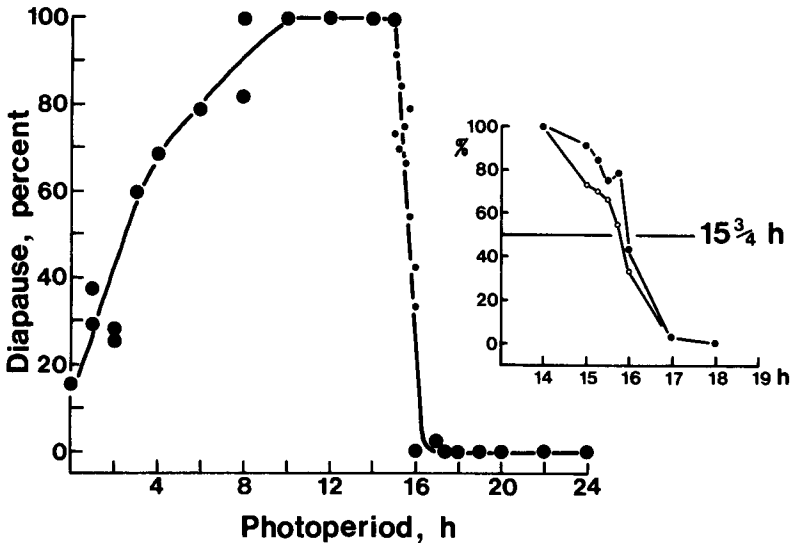

FIG. 2. Photoperiodic responses of the linden bug, *Pyrrhocoris apterus*: induction of ovarian diapause as a function of day-length. Large and small data points show results from different experiments. *Inset:* Data for bugs maintained for 21 days (closed circles) or 28 days (open circles) at photoperiods close to the critical value (15.75 h per 24). Total number of bugs = 470 (from Saunders 1983).

and short days is clearly a product of natural selection and implies that the organism is able to measure either day-length, or night-length, or some more complex combination of the two.

Fig. 3 shows what I consider to be the minimal requirements for the photoperiodic regulation of diapause. The light : dark cycle is perceived by a photoreceptor, which tells the animal whether it is day or night. This information is fed through to a clock, whose job is to do the measuring: i.e. it differentiates between a long day and a short day or, commonly, between a long night and a short night. A decision is then made at some stage in the organism's development, which diverts the 'information' about night-length, via hormonal mechanisms, down two pathways: development or diapause. Yet this is not the whole story because the sensitive period for photoperiodism often occurs much earlier than the appearance of the hormonal effectors. For example, in flesh flies (*Sarcophaga spp.*) the diapause occurs in the pupal instar, but the insect is sensitive to photoperiod during its embryonic and early larval stages; i.e. photoperiodic sensitivity comes to an end long before pupal diapause supervenes in the insect's life-cycle. During the sensitive period, one long day or one long night is not sufficient: the animal needs to accumulate and to integrate a *number* of long or short photoperiods. The

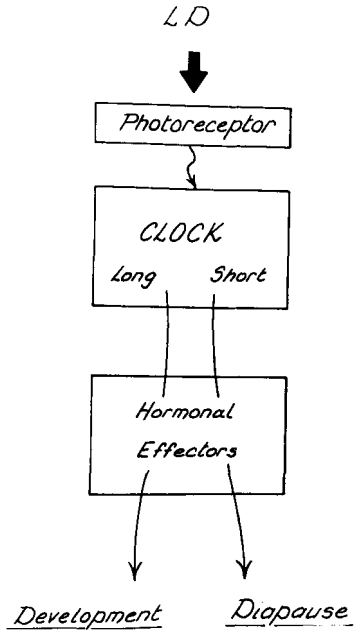

FIG. 3. Schematic representation of the minimal chain of events involved in photoperiodic regulation of the hormones controlling diapause or development.

whole problem seems to involve the transduction of environmental information (long or short day or night) by the 'clock', the accumulation and storage of this information in the central nervous system (about which little is known), and the ultimate transmission of this stored information into the hormonal system which decides whether the animal goes into diapause or not. At this symposium we shall have people talking about all levels: photoreceptors; clocks, and how they differentiate between short and long days; the transmission of information from the clock to the endocrine effectors; and the ultimate hormonal regulation of diapause induction, maintenance and termination.

The clock and the hormonal approaches have been quite separate until now. People working on the clock 'end' of photoperiodism have tended, because of the enormous difficulties of the subject, to regard the insect as a 'black box' whose net response to light is assessable experimentally. This has encouraged model building to explain how time-measurement is achieved, and we shall be hearing, for example, about circadian models, hour-glass models, internal and external coincidence, and other formal approaches to the problem. At the other end of the scale, endocrinologists have tended to accept photoperiodism as merely an unexplained discrimination between long

and short days, and to postulate, for example, that long-days facilitate release or synthesis of the hormones necessary for development. Searching questions about how long and short days are differentiated by the insect have tended not to be considered by this group. Michael Menaker has called the clock-work aspects of the subject '*dry physiology*' and the hormones '*wet physiology*'. Our job here is to draw the two approaches and the two groups of investigators together. Over the next few days I am confident that progress will be made in this direction.

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Unity and diversity in insect photoperiodism

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Abstract. The universal occurrence of photoperiodism in insects is due to convergent evolution. The neuroendocrine system, playing the central role in the photoperiodic response, gives diversified terminal expressions of adaptive significance. The response curve reflects the distributions of individual threshold photoperiods in three different ranges corresponding to the minimum number of dark and light hours that are presumably required for the time-measuring function and the genetic time-scale (critical photoperiod). Variations of the photoperiodic response curve can be derived from graded shifts of these distributions. There are, however, some unusual response curves that do not fit this model. Resonance tests suggest two different types for the photoperiodic clock mechanism: the hour-glass and the circadian oscillator. For the latter, the possibility cannot be ruled out that the observed periodicity is due to the interference by the circadian organization and not to the oscillatory nature of the timer itself. In any case, the long-night effect is similarly reversed by light breaks, and various modes of the response to night interruption may be ascribed to variations in the duration and light sensitivity of the two separate stages in the dark reaction. The possible existence of pre-adaptation to seasonal photoperiodism is suggested by the response of hybrids between the temperate and tropical forms of ground crickets.

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This review is a cursory glance at unity and diversity in insect photoperiodism, an approach that is a feasible, though perhaps biased, way of drawing a quick sketch of the many diversified photoperiodic phenomena in insects. The large body of published work on insect photoperiodism, accumulated since the first experiments over 50 years ago, has been thoroughly reviewed recently (Beck 1980, Saunders 1982, Tyshchenko 1977), providing ample material for discussion on any special problem in this field. I have relied on these excellent reviews for background information, and have omitted detailed references for rather general statements.

Multiple origins of photoperiodism

Photoperiodism as a means of seasonal adaptation is one of the most remarkable examples of convergent evolution, being universal in both animals and plants. Moreover, the ways in which various organisms respond to photoperiod are so similar that methods invented by botanists have been effectively used to analyse formal properties of the clock machinery in insects and mites. This similarity is probably due to adaptational and functional necessities rather than to the direct common ancestry of the photoperiodic clocks themselves.

The same may be true even in closely related species. The two species of ground cricket in northern Japan, *Pteronemobius mikado* and *Pteronemobius nigrofasciatus*, regulate nymphal growth by responses of the short-day type, and they regulate egg diapause by responses of the long-day type (Masaki 1978). These species are more closely related to tropical species than to each other: the former to *Pteronemobius taprobanensis* and the latter to *Pteronemobius fascipes*. I examined these tropical forms and found only slight, if any, sensitivity to photoperiod: they extend north to the subtropical islands of Japan where they show responses of the long-day type to control nymphal growth, in contrast to the responses of the short-day type shown by the temperate species.

As crickets are believed to be of tropical origin, this geographic pattern suggests that the photoperiodic responses of the two temperate species have evolved independently of each other. Similar examples can be found in many other groups of species. The reason for such convergence is obvious. Day-length is the most accurate seasonal cue ever available for any organism that is both sensitive to light and capable of measuring time.

Convergence of insects in photoperiodism might have been enhanced also by their physiological endowments. In any photoperiodic response, the insect must perform at least the following sequence of functions: reception of photoperiodic signals; measurement of light or dark time; transduction and summation of the photoperiodic information; control of neural and endocrine outputs; and manifestation of the terminal, or final, responses. Only the neuroendocrine system is capable of taking the central part in this sequence. Moreover, the photoperiodic receptor itself is extra-retinal and located in the brain, with the possible exception of *Pterostichus nigrita* (Ferenz 1975). Kono et al (1983) have recently discovered organelles that are suspected to be light-sensitive in giant glial cells and in perineurium cells of the brain of *Pieris rapae*.

Diversity in terminal expression of photoperiodism

Since the neuroendocrine system controls and integrates various developmental and metabolic phenomena, it can express photoperiodic influences in various ways: diapause responses (induction, termination, preprogramming of intensity, selection of alternative stages for diapause); growth responses (rate of growth, number of moults, timing of adult differentiation); morphological responses (seasonal forms, wing forms, other less dramatic structural or colour variations); behavioural responses (tactic movements, flight and migration, aggregation, cocooning, selection of oviposition sites); and sex-determining responses (mode of reproduction, sex ratio).

Only a few examples of previously little-known responses will be cited here. *Aedes togoi* (Nagasaki population) undergoes diapause at either one of two widely separated stages of development, depending on the larval photoperiod within the short-day range: at the egg stage in LD 12:12 (12 h light:12 h dark) and at the fourth larval instar in LD 10:14 (Mogi 1981). In *Chelonus inanitus* the ratio of females to males among the progeny increases from 0.66 in LD 14:10 to 1.11 in LD 12:12 (Rechav 1978), while in *Polistes chinensis* the production of male progeny by foundresses is promoted by short day-lengths (Suzuki 1981).

Most terminal expressions of photoperiodic response are closely linked to the neuroendocrine mechanism of diapause and, together with biochemical adjustments not listed above, form the diapause syndrome. In *Diatraea grandiosella*, for example, the sequence of complicated and highly adaptive behaviour before diapause can be elicited by administering a mimic of juvenile hormone (Chippendale 1978). In *Oncopeltus fasciatus*, a switch from diapause and migratory flight to mating and oviposition is invoked by environmental factors, including photoperiod, and this switch corresponds well with the changing titre of juvenile hormone (Rankin 1978). The photoperiodic control of seasonal polyphenism and ovarian diapause in *Polygonia c-aureum* are both mediated by the median neurosecretory cells in the pars intercerebralis (Fukuda & Endo 1966, Endo 1972). However, photoperiodic responses are not always included in the diapause syndrome. For example, many univoltine species of cricket obligatorily enter diapause only at the egg stage, and yet they control nymphal growth in response to photoperiod (Masaki 1978).

The neuroendocrine system coordinates responses to various endogenous and exogenous stimuli. Possibly through this process, or in some other way, most of the photoperiodic expressions listed above are subject to modification by environmental factors such as temperature, food, population density, etc. In the complex chain of events that culminates in various terminal expressions, the photoperiod-receptive site and pigments and the formal properties

of photoperiodic time-measurement have been the subjects of pioneering studies. The direct endocrine control of some terminal expressions has been elucidated to a certain extent. However, the link between these events, i.e. the transduction of the photoperiodic cue into the neuroendocrine output, is still missing, though some suggestions have been made. For example, Saunders (1982) related the different patterns of accumulation and release of secretory granules by the median neurosecretory cells in short-day and long-day larvae of *Pieris rapae* (Kono 1975) to the photoperiodic information storage.

Variability of photoperiodic response curves

Various expressions of photoperiodism may show similar or dissimilar response curves, the most common of which are long-day types such as those found in diapause induction of many species that are active in summer and dormant in winter (Fig. 1). Mirror-images of those responses are short-day types, of which only several examples are known.

At first sight, there seem to be several different types of response curve (Fig. 1, upper panel). However, most photoperiodic response curves can be viewed as cumulative distributions of individual thresholds in three different

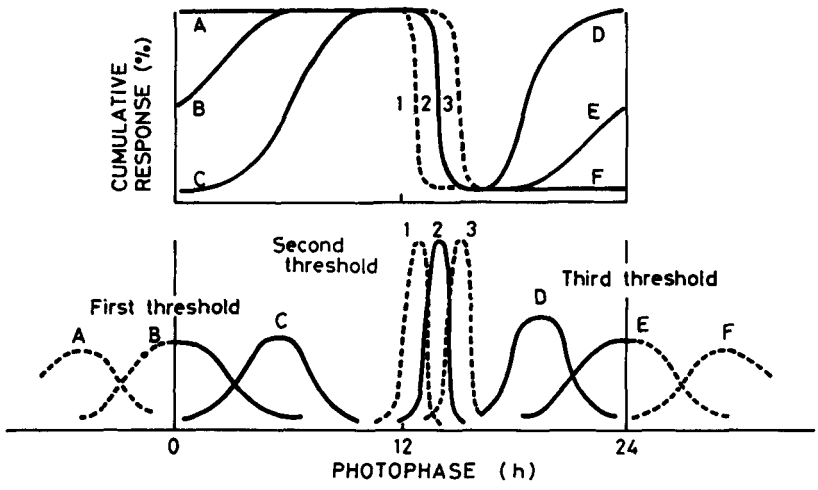

FIG. 1. Upper panel: Photoperiodic responses of long-day type plotted as cumulative percentage responses. Curves except for BD and CD are all known to occur. Broken lines show shifts in critical photoperiod. Lower panel: Corresponding distributions of individual thresholds. Broken curves at <0 h and >24 h represent non-existent hypothetical distributions just to show that graded shifts in threshold distribution produce various photoperiodic response curves.

ranges of photoperiod (lower panel). The first and third thresholds in Fig. 1 might be related to the minimum number, respectively, of light and dark hours required for photoperiodic time-measurement. The second threshold is the so-called critical photoperiod—a genetic time-scale dividing 'long' from 'short' photoperiods. This is less variable than the others, owing to natural selection, for the accurate timing of diapause, etc. Various photoperiodic response curves are derived by shifting these threshold distributions. There are, however, some strange response curves that do not fit the three-threshold model (Fig. 2). Presumably, the left-hand curves in Fig. 2 are the results of an absence or a relaxation of natural selection for the critical photoperiod.

The photoperiodic response is also characterized by the temporal pattern,

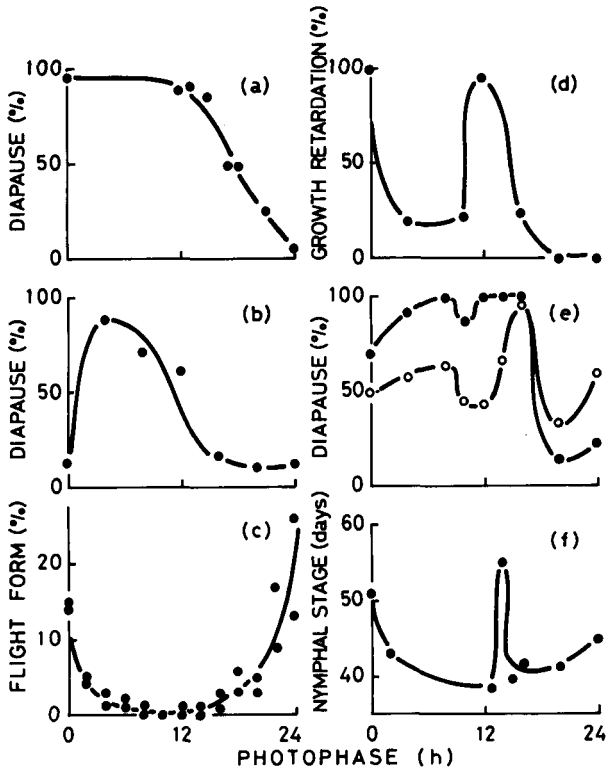

FIG. 2. Unusual examples of photoperiodic response: (a) *Choristoneura fumiferana* (Harvey 1957); (b) *Tetranychus telarius* (Bengston 1965); (c) *Callosobruchus maculatus* (Utida 1969); (d) *Agrotis ypsilon* (Goryshin & Akhmedov 1971); (e) *Bombyx mori* larvae kept as eggs at 25 °C (closed circles) or 16 °C (open circles) in constant dark (DD) (Sumimoto 1974); (f) *Pteronemobius nigrofasciatus* (R. Igarashi & S. Masaki, unpublished results).

as defined by the arrangement of the sensitive and responsive stages in the life-cycle. Once again, insects show diversity in this respect. The time-interval between the two stages is variable from species to species and, in extreme cases, the photoperiodic information is transmitted from one generation to the next. Further complication occurs when different ranges of photoperiod are required in successive stages to elicit the response. The temporal pattern represents the genetic programme of the 'storage' and 'release' of photoperiodic information, which is one of the important unknown aspects of insect photoperiodism.

Diversity in response to resonance tests

As time-measurement is an essential function in photoperiodism, the question naturally arises of whether it is performed by the circadian system or by other kinds of biological clock. Resonance tests, either of the Nanda-Hamner or the Bünsow protocol (see Saunders 1982), provide a feasible first approach to this problem. Several species, representing five different insect orders and Acarina, have been studied in this way, but no consistent phylogenetic pattern of distribution of the two clock types has emerged.

Moreover, two different geographic populations of a single species, *Pterostichus nigrita*, give positive and negative responses to resonance tests, respectively (Thiele 1977). The same photoperiodic clock of *Sarcophaga argyrostoma* shows a clear circadian periodicity at 20 or 22 °C but not at 16 °C (Saunders 1982). A similar temperature effect has been observed in *Drosophila auraria* (Pittendrigh 1981). These examples throw doubt on the reality of a distinction between the circadian and the hour-glass types of photoperiodic clock, and Pittendrigh (1981) has attempted to explain the absence or presence of circadian resonance by a sophisticated multiple oscillator model.

I myself have come across both the hour-glass and the circadian responses of the photoperiodic clock. *Plodia interpunctella* seems to measure the photoperiodic time from dusk. When a constant duration of light, ranging from 4 to 24 h, was combined with a variable duration of darkness, the percentage diapause similarly varied as a function of the number of dark hours, sharply increasing as the dark period exceeded the critical night-length of 13.5 h (S. Kikukawa & S. Masaki, unpublished results). When the dark period was extended to 90 h, there was no resonance of the diapause response, at least at 25 °C (Takeda & Masaki 1976). On the other hand, the wing-form response of *Pteronemobius fascipes* showed a beautiful circadian periodicity in light regimes of the Bünsow protocol, with cycles of LD 12:36 or 12:84.

How is a circadian component involved?

In the cricket's wing-form response, the 'free-running' rhythm of the photoperiodic clock was shorter than 24 h, as indicated by the response phases advanced on the third and fourth days in constant darkness compared with those on the first and second days (S. Masaki & R. Igarashi, unpublished results). This was in accordance with the free-running rhythm of locomotor activity (Y. Watari & S. Masaki, unpublished results).

As deuterium oxide (heavy water, D_2O) is known to lengthen the circadian period in a wide variety of organisms, we also examined its influence on the cricket's critical photoperiod. Neither 10% nor 20% D_2O gave any significant result. The critical photoperiod was between 12 and 12.5 h, being similar to that in the control groups (Fig. 3). This did not, however, disprove the involvement of a circadian component in the photoperiodic clock, because D_2O at these concentrations did not affect the free-running rhythm of locomotor activity (S. Masaki & Y. Watari, unpublished results).

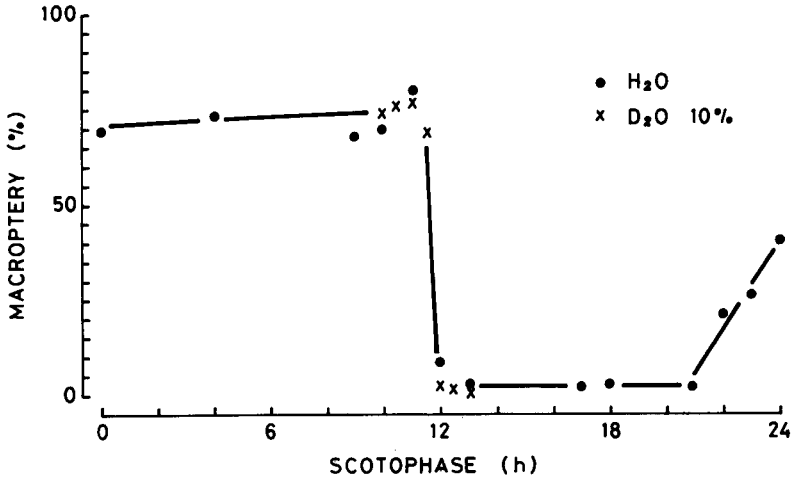

FIG. 3. Effect of deuterium oxide (D_2O) on the photoperiodic wing-form determination in *Pteronemobius fascipes* fed on dry insect feed. Crosses: 10% D_2O given as water supply. Circles: H_2O control.

Night-interruption experiments provided a further opportunity to compare the cricket's photoperiodic clock and its rhythm of activity. When an interruption in early night was followed by a dark period shorter than the critical length, the long-winged form predominated (see Fig. 5, c-1, below). Probably, the interrupting pulse had reset the clock. On the other hand, the activity rhythm was phase-set by the main light:dark transition, and no

phase-shift by night interruption was observed (Y. Watari & S. Masaki, unpublished results).

The photoperiodic clock of the cricket certainly shows a circadian resonance, and yet its identity is still in doubt. The related temperate species, *Pteronemobius nigrofasciatus*, poses a related question. This cricket has three different photoperiodic responses: egg diapause, wing form and nymphal development. When the cricket was subjected to light : dark schedules of the Bünsow protocol, the first two responses—diapause and macroptery—showed clear circadian periodicity, but the nymphal development, expressed in terms of the mean development time, did not. Nevertheless, the statistical variance of development time increased and decreased in a circadian fashion (Fig. 4).

The circadian increase in the variance might be a reflection of some unfavourable disturbing effect of out-of-phase light signals on the circadian organization but not on the time-measuring system itself. There is a general

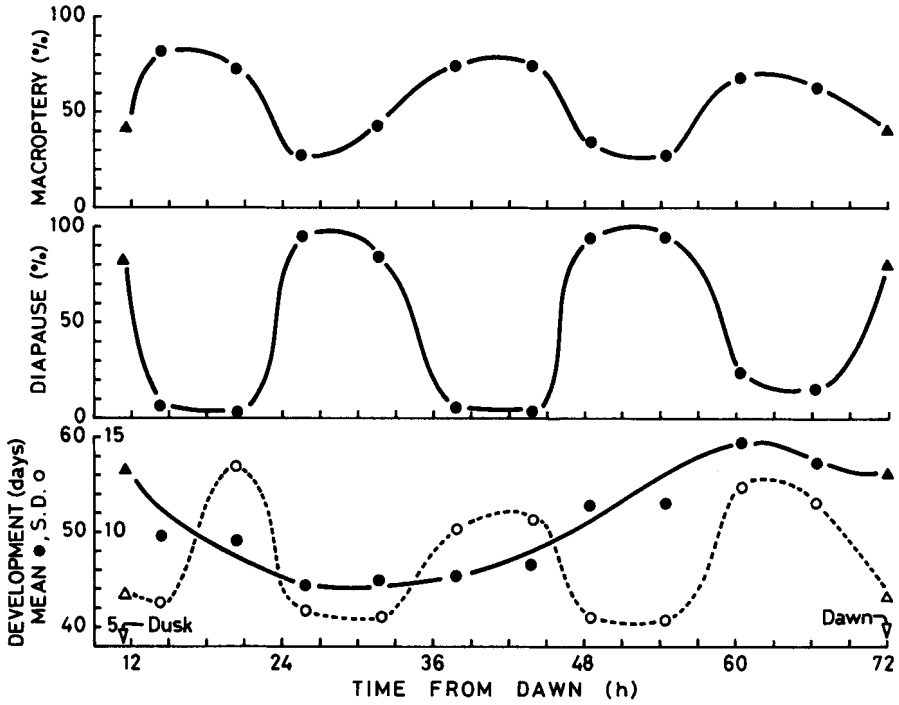

FIG. 4. Resonance responses to a Bünsow protocol (LD 11.5 : 60.5, 1.4h interrupting light pulse, 26°C) in *Pteronemobius nigrofasciatus*. Horizontal axis indicates the time of onset of interrupting light pulse (S. Masaki & R. Igarashi, unpublished results). Solid line, mean development time; dotted line, variance in development time (1 SD).

tendency for the variance in development rate to increase when insects are exposed to various kinds of environmental stresses. An abnormal light cycle is one such stress.

If we extend this interpretation to the other two responses which, at first sight, so strongly support the direct involvement of a circadian component, a quite different picture emerges. The photoperiodic clock itself may be an hour-glass, and an extended night would induce egg diapause and short-wing development. However, the effector functions would be impeded when the circadian organization of the general physiology is disturbed by light cycles far from modulo τ (the free-running period). Circadian periodicity would then appear in the terminal expression, but it might be a manifestation of the circadian organization itself and not of the kinetics of photoperiodic time-measurement. There is as yet no conclusive evidence to exclude either the circadian or the hour-glass hypothesis. The 'hour-glass timer–oscillator-counter' model recently proposed by Vaz Nunes & Veerman (1982) seems to give a new line of approach to photoperiodic time-measurement.

Similarity in the response to night interruption

Irrespective of whether oscillatory or hour-glass, all the tested cases of photoperiodic response are highly sensitive to night interruption, and the long-night effect is reversed by light breaks. There are two peaks of such an effect, one occurring soon after dusk (peak A) and the other before dawn (peak B) (Saunders 1982). The response pattern is considerably variable among different species, as indicated by the relative sizes of the two peaks. In extreme cases, either A or B alone persists. Even the same species may show different patterns of response depending on the experimental conditions (Fig. 5).

Presumably, peaks A and B represent two distinct light-sensitive stages equivalent to stages 1 and 3 recognized for the *Megoura viciae* clock (Lees 1973). When the two stages (A and B) are separated by a period of light insensitivity, the response is bimodal (Fig. 5, a-1 and b-1). However, if the dark period after night interruption exceeds the critical length for diapause induction, the short-day effect is exerted and peak A does not appear (Fig. 5, a-2). When stage A is very closely followed by stage B, or the intervening light-insensitive stage is very short, only one wide plateau of response may occur (Fig. 5, c-1). Even in such a case, stages A and B can be distinguished by using a brief (10 min) interrupting light that is perceived only in stage B (Fig. 5, c-2). Conversely, in *Mamestra brassicae*, a 1 h interruption gives peak B alone, but when a 3 h pulse is used peak A also appears (b-1, b-2).

Various response curves to night interruption can thus be derived from

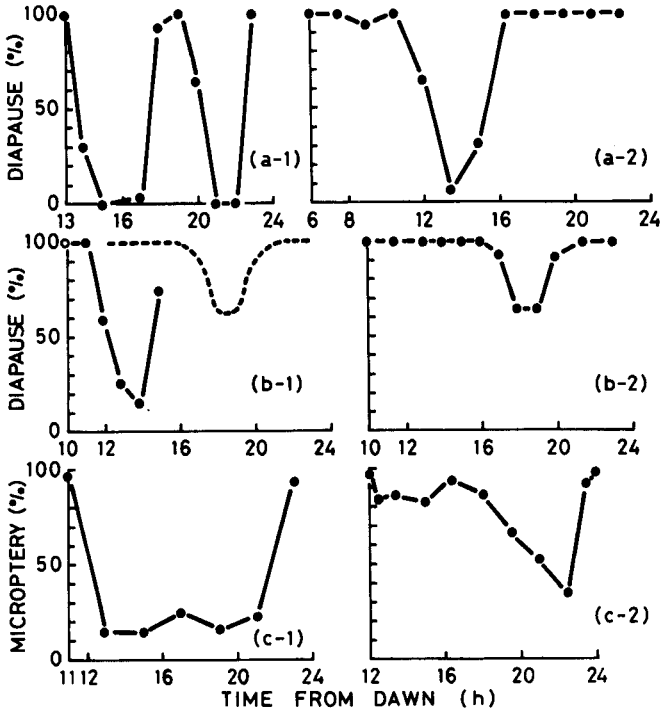

FIG. 5. Night-interruption effects under different experimental conditions. Data points are plotted against the time of onset of interrupting light pulse. (a) *Hyphantria cunea* (1) LD 13:11, 1 h interruption; (2) LD 6:18, 1.5 h interruption (S. Masaki & M. Takeda, unpublished results). (b) *Mamestra brassicae* (1) LD 10:14, 3-h pulse-scanned from hour 11 to 15 (solid line); (2) LD 10:14, 1-h pulse-scanned throughout the scotophase. This result is replotted as the broken line in (1), for comparison (Furunishi et al 1982). (c) *Pteronemobius fascipes* (1) LD 11:13, 1 h interruption; (2) LD 12:12, 10 min interruption (Y. Watari & S. Masaki, unpublished results).

variations in the duration and the light sensitivity of the two stages in the course of dark-time measurement. The common feature in the response suggests that the same principle of time-measurement is operating. Although some recent models of photoperiodic clock are based on hypothetically quantified reactions in light and darkness, students of photoperiodism have been rather reluctant to follow the kinetics closely since the last intensive study by Lees (1973). One of the reasons for this is probably the difficulty in the formal analysis owing to interference by the circadian system. In such analysis, the light and dark components of the cycle must be varied independently of each other. This is possible only in a typical hour-glass example such as the *Megoura* clock. In other cases, the circadian inter-

ference would hamper the expression of the time-measuring response as the light : dark cycle deviates from a 24 h duration.

Some evolutionary problems

Photoperiodism comprises complicated physiological processes even in each of its component functions. This highly complex system of adaptation can hardly be formed by only a single step of genetic change. As in many other cases of major evolutionary change, photoperiodism would have been established through progressive modification of pre-existing physiological endowments.

Tyshchenko (1977) presented seven hypothetical steps in the evolution of photoperiodism: (1) direct response to light; (2) appearance of exogenous circadian rhythms; (3) establishment of endogenous circadian rhythms; (4) integration of circadian rhythms; (5) photoperiodic regulation of physiological functions; (6) acquisition of photoperiodic memory function; and (7) transmission of photoperiodic information through generations. He assumes that quantitative responses such as continuous control of growth appear at step 5, and that qualitative responses such as discrete diapause or morph determination appear at step 6.

At step 4, various oscillators should have definite phase-relations to the driving oscillator or to one another. Environmental modification of such phase-relationships would have a favourable or an unfavourable impact on various aspects of physiology. The establishment of step 4 would therefore tend to invoke natural selection, leading to step 5. Photoperiodism should then be an unavoidable evolutionary consequence of circadian organization, if we accept the multiple oscillator theory (Pittendrigh 1981).

Another possible course of evolution is suggested by the results of crossing experiments between the temperate strain Nara (N) of *Pteronemobius nigrofasciatus* and the tropical strain Bali (B) of *Pteronemobius fascipes*. The temperate form programmes its egg diapause in response to the maternal photoperiod while the tropical form virtually lacks egg diapause. When they were crossed, the incidence of egg diapause was affected by both parental genotypes, but the critical photoperiod—defined as the mid-point in the critical range of the response curve—seemed to be a maternal character. In F1 (N♀ × B♂) or backcross (N × NB) progeny, the critical photoperiod was close to the temperate duration of 14 h. In backcross (BN × N) eggs, the critical photoperiod was shorter, between 12 and 13 h (A. Nagase, unpublished; see p 21).

Although the Bali stock does not show any diapause response, it seems to have genes that affect the critical photoperiod. It might have a photoperiodic

clock with a short critical photoperiod that is used for some other purpose. This stock showed, in fact, a slight wing-form response to photoperiod, and produced some long-winged adults only in photoperiods of 13 h and longer (S. Masaki & A. Nagase, unpublished results). Therefore, the critical photoperiod can exist before an effector mechanism (egg diapause) has been established. From this I am tempted to suppose further that an hour-glass, such as the hatching timer of *Metrioptera hime* (Arai 1979), might also be homologous to the photoperiodic clock and might be pre-adapted to seasonal photoperiodism. If such a timer occurs universally, it would provide a basis for convergent evolution of photoperiodism in insects.

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DISCUSSION

Gilbert: In your experiments with deuterium oxide, is the D_2O fed to the crickets or injected?

Masaki: It is fed. We feed the crickets on a dry diet and give them a water supply in a small bottle.

Pittendrigh: But surely the crickets can generate a lot of metabolic water from their dry food, and the heavy water will be only a small proportion of their total water?

Masaki: Even if the crickets produce a lot of metabolic water, they cannot survive on it alone. If the water supply is removed, the crickets will die within one or two days, so they apparently do drink the water.

Pittendrigh: Was it correct that you found no effect of D_2O on either the rhythm or the time-measurement?

Masaki: We found no influence of 10 and 20% D_2O either on the free-running rhythm of locomotor activity or on the critical photoperiod for wing-form determination. We intend to examine the effect of a higher concentration of D_2O on the locomotor activity. If there is any effect, then we can start again to study its influence on the critical photoperiod.

Denlinger: D_2O has a very impressive effect on *Sarcophaga* (Rockey & Denlinger 1983). When we incorporate 20% D_2O into the larval diet we can completely obliterate the diapause response. This effect does not appear in constant darkness, so we are certain that we are not interfering with the animal's capacity for diapause. We envisage that the light-sensitive phase of the circadian oscillation is being shifted. With D_2O we eliminate the perception of short days, and hence no diapause appears.

Masaki: We would expect D_2O to increase the critical night-length, at least in the case of external coincidence, which is suggested by Dr Saunders for *Sarcophaga argyrostoma* (Saunders 1979). Your observation seems to suggest another effect. If the sensitive period is shifted, by D_2O, to the later stage of development, the number of short-day cycles seen by the maggots decreases and, accordingly, the incidence of diapause is lowered. This possibility can be tested by extending the shifted sensitive stage by a low temperature.

Beck (1980) also did an experiment with D_2O and the corn borer *Ostrinia nubilalis*. I plotted his data and found no clear influence on the critical photoperiod. What I saw was a general decrease in the incidence of diapause by D_2O.

Sokolove: Have you done any experiments on thermoperiod and temperature cycles to see their effect on behavioural rhythm?

Masaki: I have not done any thermoperiodic experiments on behaviour. However, in the ground cricket, the wing-form response is not affected by thermoperiod (R. Igarashi, unpublished work). In the Indian meal moth *Plodia interpunctella* we have found a very clear thermoperiodic response (Masaki & Kikukawa 1981).

Sokolove: One could, in principle, use thermoperiod to drive a behavioural rhythm, and then use light-pulses to probe various phases of the activity cycle to see whether there is any relationship between the behavioural 'clock' and the sensitive period of the 'photoperiodic clock'.

Masaki: It seems worthwhile to try such experiments. One way to discriminate between circadian interference and photoperiodic time-measurement is to give temperature and light cycles in various phase-relationships.

Mordue: If the tropical and subtropical species of cricket that do not respond to photophase are moved to a temperate environment, do they start responding; do they have the mechanism for measuring photoperiod?

Masaki: I have not attempted a large-scale temperature experiment. A