REVIEW

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Studying insect photoperiodism and rhythmicity: Components, approaches and lessons

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Abstract. Components of daily and seasonal timing systems in insects are reviewed. Photoperiod indicates seasonal position reliably, but signals can be much modified by habitat, latitude and season. Several receptor features and pigment systems are known, with different daily, seasonal and general functions, including differences between circadian and seasonal reception. Clocks can serve several different purposes, functioning as daily oscillators, interval timers or through successive requirements. The molecular functioning of circadian clocks is best known, but even so there is considerable complexity and diversity and much remains to be discovered. We know relatively little about the internal states that provide information for timed responses (such as the photoperiodic "counter"), about the central controlling mechanism, or about the effectors that transmit output signals. Nevertheless, temporal responses serve a very great range of purposes in insects, and the reported complexity in all of the components of timing systems reflects complex ecological needs across daily and seasonal intervals. The variety of components and the complexity of interactions reported (even within species), as well as the diversity of such elements as photosensitive pigments, molecular clock function and potential neurotransmitters, suggests that – unlike some earlier expectations – there is no single master clock for all timing functions in insects.

Insect photoperiodism and rhythmicity have been studied by both observational or direct approaches (examination of system elements or devices, and qualities such as survival), and by inferential or indirect approaches (such as interpretation of various responses to photoperiod, modelling, and estimating fitness). Many students work with only one approach, but the power of different approaches is not equal, and knowledge at one level may not give answers at another. These difficulties tend to limit our understanding of the linkages among components.

This overview suggests several lessons for the study of photoperiodism and rhythmicity. There are multiple elements, complex integration and a diversity of clocks, showing that different processes serve different purposes. The diversity of findings also results from the fact that different investigative approaches, which depend on the question being asked and on the perspective of the investigator, can influence the outcome of the investigation. Given these complexities, I believe that the key to interpreting photoperiodic and circadian responses is their ecological value. Notwithstanding the interest of timing mechanisms or their parts and of specific responses, daily rhythms and seasonal timing are best understood through the essential context provided by the ecological demands on the actual organisms under study.

INTRODUCTION

Photoperiodism and rhythmicity exist in a wide setting, so that the complex biology of whole organisms has to be taken into account to interpret the meaning of particular timing mechanisms. For example, the components of daily clocks are diverse and complex, and photoperiodic responses for seasonal activity reflect even wider integration with a range of external environments. Therefore, useful insights are at least as likely to come from investigations properly based in biological and ecological settings as from probing at the molecular level or from hopeful manipulation of "black boxes" based on various assumptions and models. For example, I disagree with current opinions that the molecular analysis of clock mechanisms in *Drosophila* is the major way in which progress in understanding these adaptations can be made. To draw on an analogy developed in the conclusions, knowing how several engine components work tells us little about the rest of the car, and even less about its destination.

This paper therefore emphasizes the view that it is important to consider timing in its full biological and ecological context. For convenience, the term "photoperiodism" is used here to refer to seasonal responses, while daily responses are labelled as "circadian" or "rhythmic". I first provide a brief review of the multiple components of insect photoperiodism and rhythmicity, and of different approaches to studying their mechanisms, properties, and values. Some general lessons are drawn from such a review.

COMPONENTS

A scheme showing the many elements of photoperiodism and rhythmicity, and that can be used to organize discussion, is shown in Fig. 1. The *conditions* of the environment, especially photoperiod, provide signals about environmental quality. Mechanisms to receive these *inputs* involve either central responses, mediated with reference to the timing mechanisms we call *clocks*, or peripheral responses that are effected independently. A *control* centre integrates current signals and timing references, as well as *internal states* that serve to store infor-

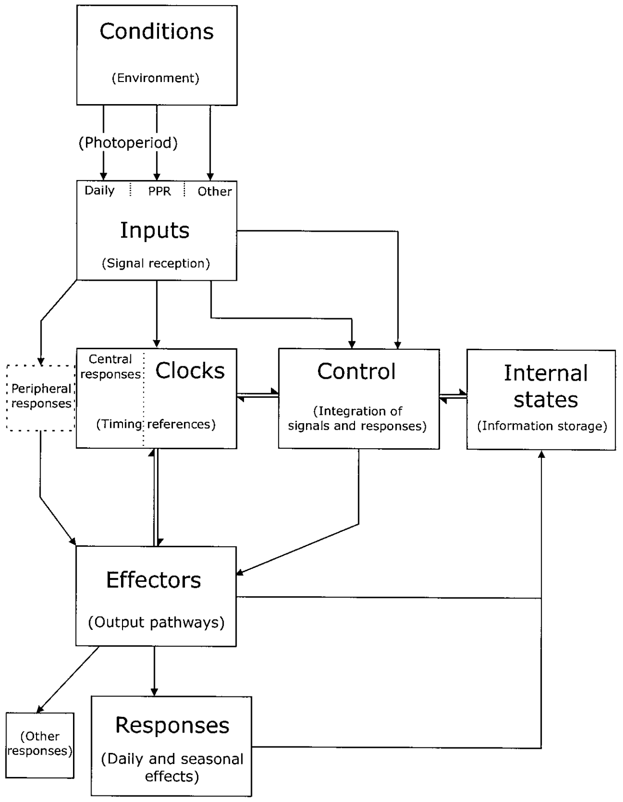

Fig. 1. Major components of photoperiodism and circadian systems.

mation (such as the number of photoperiodic cycles experienced), to produce appropriate outputs. *Effectors* that act on output from the integrator, chiefly neuroendocrine systems, produce *responses*, especially daily and seasonal effects related to photoperiodic signals.

Fig. 1 reinforces the fact that information is communicated among many successive and simultaneous elements, including both one-way signals and feedback pathways. It also demonstrates the fact that there are multiple inputs, because photoperiod is only one of the potential environmental conditions. Moreover, there are multiple effects, including behaviour, colour, wing morph, diapause, dispersal and other responses. These different responses could use similar or different environmental references and similar or different internal components.

Conditions

Conditions of the environment, especially photoperiod, provide information to insects about daily or seasonal time. Key features of photoperiodic signals are shown in Table 1 (for more details see especially Danks, 1987 and references cited there). The exact coincidence of photoperiod with astronomical events (top part of the table) makes it a very reliable indicator of seasonal position. However, despite their basic astronomical precision the signals can be greatly modified by habitat, latitude and season (bottom part of Table 1), changing the potential value of the information received by an insect. Moreover, other environmental conditions such as temperature provide simultaneous seasonal and daily temporal information. Interactions with photoperiod can be complex because the daily and seasonal patterns of these other conditions may not coincide with those of photoperiod, because habitat, season and latitude influence them in different ways, and because they are received by different sensors.

Although many factors therefore can modify "clean" photoperiodic signals, little formal analysis of these factors has been done in considering the function of the biological clock. The wavelength and intensity of light change according to the time of day and the habitat (Holmes & McCartney, 1976; Henderson, 1977; Roenneberg & Foster, 1997); sensitivity often differs between dawn and dusk (because of different receptors as well as different acclimation: Danks, 1987, p. 235); sinusoidal signals like the gradual onset of light and darkness through twilight, as in nature, may have different effects than square functions like the sudden lights on and lights off typical of the laboratory (cf. Bell & Walker, 1973; Byers & Unkrich, 1983; and see Volkovich & Sokolova, 2000 for thermoperiodic effects); and seasonal and daily temperatures lag photoperiods (e.g. Fig. 14 in Danks, 1987). These differences have seldom been evaluated in the context of signal reception and use by the insect.

TABLE 1. Key features of photoperiod as an environmental signal.

Feature	Correlation	Notes
Coincidence with season	Exact	Astronomical events
Predictability of seasonal change	Exact	Astronomical events
Frequency for monitoring	High	Twice per day
Availability of sensors	High	Eyes and other, especially neural, tissues are light-sensitive
Coincidence with other conditions in a given season	Moderate	(e.g. temperature)
Signal strength	Varies	Habitat dependent: sunlight more than 10^8 different in intensity from starlight but some habitats, as well as structures such as cocoons, can attenuate or even virtually eliminate the signal
Degree of seasonal change	Varies	Latitude dependent: from none at equator to complete at poles
Seasonal rate of change	Varies	From virtually none during solstices and at equator, to several minutes per day at higher latitudes during equinoxes (e.g. 6 minutes per day at latitude 60 degrees)

Inputs

How photoperiodic signals are received by insects has been investigated by seeking the location, structure, pigments, and sensitivity of supposed photoreceptors. Numerous experiments have measured responses with and without destruction, masking or transplantation of potential photoreceptors, by narrowly targeting light signals through optic fibres, and by supplementary illumination using phosphorescent paint (review by Numata et al., 1997). The wavelength and intensity of light signals, in addition to their duration, necessary to elicit a response have been tested, and detailed dissections and labelling of potential structures of interest have been carried out. Other work measures the responses of organisms that have been deprived of certain photoreceptor pigments through genetic or dietary manipulation.

One general problem of interpreting light-sensitive organelles or pigments stems from the fact that light reception by organisms serves a number of purposes. Vision (provided by the compound eyes) generally gives the acuity required for hunting or other more or less continuous activities. Compound as well as simple eyes also provide detection of the sort of rapid changes in light that might be caused by an approaching predator. Both of these functions imply very rapid measurement and integration of incident light signals to allow prompt responses. In contrast, monitoring daylengths requires only the daily distinction of light and dark, which would allow slow reception by eyes or other light-sensitive tissues. Consequently, the different fast and slow functions may or may not have the same receptive structures, light-sensitive pigments or neural pathways.

In many insects, receptors for the photoperiodic signals that induce diapause or other seasonal effects are in the brain itself (e.g. Williams, 1963, 1969; Claret, 1966a, b; Kono, 1973; Seugé & Vieth, 1976; Bowen et al., 1984; Hasegawa & Shimizu, 1987; and see below), apparently confined to a specific area or set of cells (e.g. anterodorsal: Steel & Lees, 1977). Such localized reception does not distinguish, of course, between discrete organelles and a more diffused receptivity, although several presumptive receptors have been identified (review by Numata et al., 1997). Compound eyes are used to perceive photoperiodic signals in several other species (Ferenz, 1975; Numata & Hidaka, 1983; Numata, 1985; Shiga & Numata, 1996, 1997; Numata et al., 1997; Nakamura & Hodkova, 1998; Morita & Numata, 1999). Even so, not all ommatidia of the eye are involved. For example, central ommatidia are chiefly responsible for receiving photoperiodic signals in the bug *Riptortus clavatus* (Morita & Numata, 1997). Neither the ocelli nor the optic lobes themselves are normally involved in photoperiodic reception (e.g. Saunders & Cymborowski, 1996; Numata et al., 1997).

The photoreceptors involved in *circadian* rhythms are in either the brain or the compound eyes, depending on the species (review by Nakamura & Hodkova, 1998; Shiga et al., 1999), as for photoperiodic reception. In the cricket *Gryllus bimaculatus*, only the dorsocaudal ommatidia of the compound eye are involved (Tomioka & Yukizane, 1997). In only a few species have photoreceptors for both photoperiodic responses and circadian entrainment been localized: both may be in the brain (Williams & Adkisson, 1964; Truman, 1972; Cymborowski et al., 1994; Saunders & Cymborowski, 1996) or both in the eyes (Nakamura & Hodkova, 1998). Even so, the wider differences reviewed above, the further localization of receptors described in some species, and differences in specific responses to photoperiod suggest that even circadian and photoperiodic receptors within the same structure may be different from each other and, perhaps, from one species to the next.

There is also evidence for redundancy of receptors in at least some species, so that injury to one site or partial burying or concealment of the body (or different photic environments) do not blind the animal to photoperiod. For example, both compound eyes and extraretinal receptors entrain circadian rhythms in the cricket *Dianemobius* (Shiga et al., 1999). The telson, compound eyes and median ocelli are all sensitive in the horseshoe crab *Limulus* (Renninger et al., 1997). In some species one receptor is dominant but effects remain even when that receptor is removed (Morita & Numata, 1999).

The pigment responsible for vision in insects, and hence associated especially with the retina of the compound eye as well as with the retinula of the ocellus (e.g. Pollock & Benzer, 1998), is rhodopsin, consisting of a chromophore, retinal (a vitamin-A metabolite), coupled with a protein, opsin. Different wavelength sensitivities are given by different opsin molecules associated with the chromophore, and many different opsins have been identified, even in a single species (e.g. Briscoe, 1998).

Pigments supposed to mediate photoperiodic reception have been investigated especially by histological means, and by determining the sensitivity of response to different wavelengths. For example, in the mites studied by Veerman (2001, Table 1), results at different wavelengths could best be explained by differences in the receptors for photoperiod and circadian rhythms respectively. Molecules that mediate photoperiodic responses appear to consist of a chromophore also derived from carotenoids (provitamins) or vitamin A, which would be coupled to a protein such as an opsin. The evidence, from the effect of diet restrictions and from action spectra, was reviewed by Veerman (2001). Shimizu et al. (2001) suggest that an opsin present in the brain of larval *Bombyx mori* is the photoreceptive pigment.

In contrast, the primary receptor for circadian responses is cryptochrome, as suggested by evidence for plants (e.g. Cashmore, 1997, 1998; Somers et al., 1998) and more recently for mammals, *Drosophila* (dCRY), and other organisms (e.g. Emery et al., 1998; Stanewsky et al., 1998; Cashmore et al., 1999; Ceriani et al., 1999; Egan et al., 1999; Ishikawa et al., 1999; Selby & Sancar, 1999; Hall, 2000; Sancar, 2000; Ivanchenko et al., 2001). Cryptochrome has a chromophore based on vitamin B_2, with (in *Drosophila*) pterin light-harvesting and flavin catalytic cofactors, and is sensitive to blue and UV-A light.

The fact that there are two chromophores with different spectral absorption makes for difficulties in drawing conclusions based on action spectra (cf. Sancar, 2000).

Notwithstanding the role of cryptochrome in entraining circadian rhythmicity, multiple photoreceptors transmit information about photoperiod to the circadian clock in *Drosophila* (Helfrich-Förster et al., 2001), with compensation if some of the pathways are damaged. Thus eye-based rhodopsins, ancillary photoreceptors, and cryptochromes all provide light input to the clock controlling daily behaviour (Helfrich-Förster et al., 2001). Moreover, their functions are not simply additive. Cryptochrome primarily entrains the evening peak in *Drosophila*, while the other routes entrain mainly the morning peak. (In this and other species, therefore, morning and evening peaks of activity appear to be controlled by different oscillators: e.g. Rietveld et al., 1999; Alpatov et al., 1999a; Helfrich-Förster, 2001). Multiple photoreception for circadian timing is also known in animals such as reptiles and birds (e.g. Menaker et al., 1997), and even in unicellular organisms (e.g. Roenneberg, 1996).

Findings for photosensitive pigments, despite common ground, suggest that the means by which photoperiod is received are diverse, both among and within species and between the major functions of circadian entrainment, photoperiodic reception, and vision. The adaptive value of such multiple pathways for photoreception may lie partly in the fact that different wavelengths predominate at different times of day or in different habitats (see Conditions above).

Clocks

The role of a "biological clock" has been demonstrated for daily activities and also assumed for seasonal responses that depend on daylength (cf. Hastings & Follett, 2001), because both need timing references of the sort that can be derived from daily changes in the durations of light and darkness. However, daily and seasonal needs are different. Typical daily requirements are for an internal circadian rhythm, used for programming diel activities, that is set by light-dark cues. Therefore, the clock gives information about a particular time of the day or cycle. Seasonal responses also take information from daylength or nightlength (and so theoretically could use the circadian rhythm as their timing reference), but typically they accumulate information from such daily cues over the longer term (cf. the counter, noted under Internal states below) and use it to adjust seasonal development. Therefore, they measure duration or accumulation rather than a specific time point, and so might act in the same way as an hourglass. Although long-term endogenous rhythms of response are known, which appear to rely on oscillators – like the daily clock but with a longer period – they have been described so far in relatively few species: for annual development (Danks, 1987, pp. 187–190; Kipyatkov, 1994; Dautell & Knülle, 1997; Nisimura & Numata, 2001); for tidal, lunar and circaseptan periodicities (e.g. Neumann, 1976, 1995; Ernst, 1995; Mikulecky & Bounias, 1997; Meyer-Rochow & Brown, 1998; McMeechan et al., 2000); and for annual antifreeze synthesis (suspected by Meier & Zettel, 1997).

Activities over short time frames (seconds to hours) are not governed by a circadian clock, but by an hourglass, called an "interval timer" in the work on short-term vertebrate timing and memory that has provided most of the general theory (e.g. review by Hinton & Meck, 1997). An example available for insects is that *Trichogramma* wasps adjust the number of eggs laid on each host according to the time it takes the female wasp to walk across the host, a function of host size (Schmidt & Smith, 1987, 1989). Unlike circadian clocks, this interval timer is not temperature-compensated (Schmidt & Pak, 1991). A third sort of timer is based on the so-called domino effect (Rensing et al., 2001) because it is determined by a required sequence of successive events. The fact that there are different kinds of clocks means that although we now know a great deal about the molecular workings of the daily clock this knowledge has not been very useful in interpreting other responses, especially seasonal ones.

Mechanisms

The circadian clock mechanism of *Drosophila melanogaster* has been explained in broad outline. The core oscillator consists of delayed feedback loops based on transcription of key proteins (e.g. Dunlap, 1999; Glossop et al., 1999; Williams & Sehgal, 2001). Post-transcriptional and regulatory mechanisms add appropriately timed biochemical constraints that increase the precision of time-keeping and entrainment (Edery, 1999). A simplified summary of this system as currently understood is as follows. Transcription of the *Drosophila* Clock gene (*dClk*) leads to production of dCLK protein, which dimerizes with Cycle (CYC) protein. This dimer activates *period* (*per*) and *timeless* (*tim*) genes, through E box promoter elements, to produce their respective PER and TIM proteins. These proteins can heterodimerize too, but doubletime (*dbt*) delays the formation of the dimer by causing PER to be degraded in the absence of TIM, which itself is degraded by light. The action of light on TIM (probably mediated by cryptochrome) synchronizes the cycle with photoperiod, because when – at a particular time of the cycle – both PER and TIM reach high levels they form a relatively stable dimer, which enters the nucleus from the cytoplasm. There PER-TIM inhibits transcription of the *per* and *tim* genes (apparently by binding to dCLK-CYC), so that PER and TIM production stops. The existing PER and TIM proteins degenerate and *dClk* is reactivated, completing the cycle. However, other genes contribute to the clock: Vrille (*vri*) generates cyclic products which also feed back on PER-TIM. Cryptochrome (CRY) expression (see above) is light-regulated and shows daily cycles; it interacts with TIM and is regulated by clock feedback (cf. Brody, 2002). Therefore cryptochrome is both a photoreceptor and part of the clock. Such a role is feasible because (unlike other photoreceptors) cryptochrome is in the cell nucleus. The clock is therefore complex (cf. Hong & Saunders, 1998), and undoubtedly by no means all of its molecular functioning is yet clear.

The extensive literature on the circadian clock of *Drosophila* includes also work to identify promoters, dimerization domains, degradation pathways, and the chromosomal sites of *per* and other genes. Other work considers the mechanism of temperature compensation, other genes linked to particular circadian activities (e.g. gated eclosion), and so on. There are also many papers that more or less hopefully describe various other molecular components of development as having some connection with circadian systems, although most of them are clearly downstream of the clock.

There are no comparable detailed molecular models for the seasonal or photoperiodic "clock", although at least some overlap at the molecular level has been claimed (Košt'ál & Shimada, 2001), and rhythmic timing may differ between diapause and non-diapause individuals (Watari, 2002). Many genes are upregulated, notably heat-shock proteins, or downregulated during diapause (review by Denlinger, 2002), but there is hardly any information about what they do, and many of them have roles unrelated to clocks, for example defence against fungi (Daibo et al., 2001). In the absence of detailed molecular findings about the photoperiodic "clock", various more general and entirely theoretical models have been developed. Confidence in these models is tempered by the fact that they arose successively as results from different species were obtained that did not fit earlier models. The models are based, for example, on the coincidence of light or darkness with hypothesized oscillators (reviews by Vaz Nunes & Saunders, 1999; Hardie & Vaz Nunes, 2001). A recent version of the double circadian oscillator model (Vaz Nunes, 1998; Hardie & Vaz Nunes, 2001) supposes that two oscillators, a long-night system and a short-night system, each have their own light sensitivity, period and damping rate. Each mechanism measures night length. The long-night system reports a long night when, during the descending phase of the oscillation (i.e. late in the cycle), either lights on occurs or there is no light. The short-night system reports a short night when, after a threshold interval during the ascending phase of the oscillation (i.e. early but not extremely early in the cycle), lights on occurs. The insect is assumed to accumulate these values (long minus short) and compare this sum – the counter, see below – with its induction threshold. Various experimental results can be explained with such a model by varying the damping rates or other parameters.

Consistent with a preoccupation among scientists with "biological clocks" and the results of experiments that show cyclic response, many such models claim circadian properties for the photoperiodic response in insects. However, Veerman (2001) assembled several lines of evidence to show that an hour-glass model that measures only a single interval is sufficient to explain all photoperiodic responses. "Circadian" findings in some species would then be attributed to circadian systems used for wider reference rather than as part of the photoperiodic clock itself, or to disturbances to the circadian system caused by unnatural regimes (e.g. Wei et al., 2001). Moreover, as Saunders (2001a) has pointed out, even if photoperiodic induction is a function of the circadian system, the circadian clockwork does not explain how the various components of seasonal responses work (compare the elements in Fig. 1).

Interval timers in general could work through the decay or accumulation of a product in reference to a threshold, and unlike circadian clocks they would not then be expected to show temperature-compensation. More complex models developed for the short-term interval timers that govern mammal behaviour rely on the accumulation of gated pulses from a very fast random oscillator (review by Hinton & Meck, 1997).

Diversity

Clock-related mechanisms are diverse. For example, even at a molecular level the system described for *Drosophila melanogaster* is not universal. Despite many commonalities, somewhat different systems have been reported in other species, including *Antheraea pernyi* and other silkmoths (Sauman & Hashimi, 1999; Takeda et al., 1999) and *Musca domestica* (Sauman & Hashimi, 1999; cf. Piccin et al., 2000). In *Drosophila*, the clock may not work the same in all tissues (e.g. Saunders, 2001a).

Moreover, apparently in insects there is not necessarily a single central circadian clock as favoured by most earlier theorists – multiple, peripheral, and even fully autonomous clocks have been demonstrated (see especially Cheng & Hardin, 1998; Vafopoulou & Steel, 1998; Krishnan et al., 1999; Giebultowicz, 2000; Giebultowicz et al., 2000, 2001). Therefore, there is not necessarily a hierarchy of clock controls, and clocks may or may not be linked to a central system or to each other, though all are likely to be entrained to the same external light-dark cycles. A given circadian rhythm may be co-ordinated by more than one clock. For example, cockroaches and crickets have bilateral clocks located in the optic lobes that normally act in an integrated way (through neuronal communication) to govern daily locomotor rhythms. Moreover, the clocks are redundant, so that if one of them is removed the other provides sufficient control to retain the rhythm (Tomioka et al., 2001).

The clock used for seasonal photoperiodic responses appears to be different from the circadian clock, based especially on the fact that the clocks have different receptor pigments and locations (see above), even if information from the circadian system were to be used by the seasonal clock as a daily timing reference or in some allied way.

This potential diversity of clocks should not be surprising. The key to understanding such timing systems is their value to the organism, and different needs can be met in different ways, including some that do not depend on a central master clock. Therefore it is unlikely that timing devices are of one universal type, even though major parts of the circadian clock mechanism, notably the key cycling proteins such as PER, are highly conserved at the molecular level (cf. Ishida et al., 1999; Liu et al., 1999). Even so, the cryptochromes involved in circadian photoreception seem to have evolved at least twice (Cashmore et al., 1999), and clocks in different organisms have different components (Devlin & Kay, 2001). Even in mammals there are hints of unexpected diversity. The pacemaker in the suprachiasmatic nucleus (SCN) in the brain (and which is also involved in photoperiodic responses: Schwartz et al., 2001) was believed responsible for all circadian features (e.g. Ralph & Hurd, 1995). Recent work suggests instead that retinal and some other peripheral circadian activity is not governed exclusively from the SCN (Basalobre et al., 1998; Hall, 2000; Hastings & Maywood, 2000).

Internal states

Appropriate temporal responses require internal storage of information. Such requirements are considered here, although they might actually be part of the system of control that integrates input and output (see below).

Internal state is used by other systems as a reference to permit or prevent certain actions or developments. For example, the "photoperiodic counter" (e.g. Saunders, 1982) is assumed to sum information and use that sum to determine whether or not diapause will be induced. However, both status and experience can provide information of seasonal value. *State-dependent measures* such as instar, size, energy reserves or other indices of condition (which are not themselves based on time, although they may be a function of the time needed to reach a given size or nutritional status), provide information about individual quality such as stored energy, and "quality" may be easier to monitor than time itself. For example, control of development by a minimum size requirement for metamorphosis is well known (e.g. Blakley, 1981). *Time-dependent measures* based on cumulative temporal events such as the number of photoperiods seen or the elapsed duration of a given activity such as flight or diapause integrate information about time directly. Such counting of days or intervals is effective if daylength or other temporal information is reliable and the actual time of the season is critical. Nevertheless, the structure of the "counter" used to accumulate photoperiodic information is chiefly theoretical (review by Takeda & Skopik, 1997), and has been referred to by such terms as "diapause titre", "required day number", and "packet of photoperiodic information". One major conclusion emerges from the various studies – that the photoperiodic counter is complex. Thus the "inductive values" of different signals can be modelled in different ways; continuous darkness can have a different value than a long night; both short and long photoperiods may be counted; counters may be temperature-compensated or not; and so on (Saunders, 1982; Hardie, 1990; Vaz Nunes, 1990; Vaz Nunes & Hardie, 1987, 1993, 1994, 1999, 2000a, b; Hardie & Vaz Nunes, 2001).

Finally, both status and experience can work together to control seasonal development. Larvae of the blow fly *Calliphora vicina* that are very small (and thus energy-deficient) avoid diapause even when their experience of short photoperiods is enough to induce diapause in their larger siblings (Saunders, 1997).

In summary, as with the photoperiodic clock itself, we have no detailed knowledge of how internal states are recorded or integrated into the photoperiodic response.

Control

Again, we know next to nothing about how inputs such as daily timing references and stored information about elapsed time are integrated to control the seasonal development of an individual. Consequently, hardly any discussion of the important control link is possible. Of course, it is possible to suggest likely components such as feedback loops, which have been shown to control many physiological processes, and some models have been developed that emphasize the counter (see above).

We also know that a very large number of inputs can be integrated. Unlike circadian rhythms where photoperiod is likely to be the major and even the only Zeitgeber, many other factors can provide seasonal information. For example, the incidence and duration of diapause depend not only on current and accumulated photoperiodic cues, but also on temperature, food, moisture, density and mating (e.g. Table 2 in Danks, 1994a). This diversity of inputs suggests that the control centre does much more than count photoperiods. Indeed, Zaslavski (1988, 1996) developed a model that included a "commanding" or "managing" mechanism that integrates photoperiodic and other inputs at the endocrine level.

Effectors

Control centres are presumed to generate or inhibit the signals that govern responses such as emergence and diapause. Typically, such developmental responses are effected by transmission along neuronal axons (to specific targets including endocrine releasers), and by release of hormones into the haemolymph (to reach more general targets). Many neuroendocrine pathways for development have been discovered by experiments involving precise techniques such as dissection and histological examination (including the use of vital dyes), ligation, surgical excision and transplantation, hormonal assays, and treatments with hormones and anti-hormones. These experiments show that the same few major hormones normally influence diapause-related development as well as ordinary development (overview by Danks, 1987) – temporal programmes control their production and release. Obviously, accumulation or degradation of such substances could be one way in which information is stored (see Internal states above). The control system sends signals to other organelles or peripheral tissues. Detailed histological and other work in a few species has elucidated several of the relevant neural pathways, including both stimulatory and inhibitory avenues (e.g. Shiga & Numata, 2001).

Messengers hypothesized to control responses downstream of the circadian clock or for longer-term photoperiodic responses include biogenic amines (e.g. serotonin, dopamine) and a small number of neuropeptides (e.g. Takeda et al., 1985; Takeda & Skopik, 1997). Several different neuroendocrine factors are found in neurons expressing PER protein, and so might be messengers

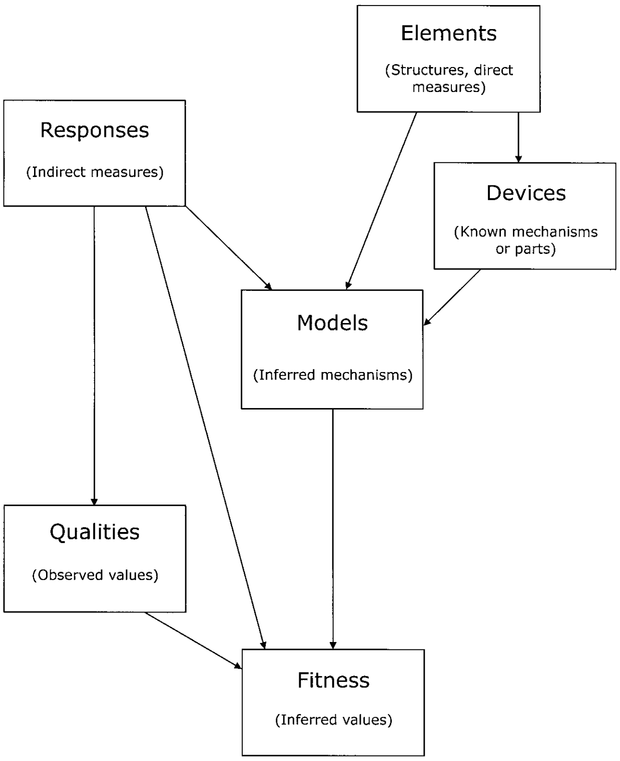

Fig. 2. Synopsis of approaches to studying insect photoperiodism and rhythmicity.

(Takeda et al., 1999). They include pigment-dispersing factor (PDF) (much studied in *Drosophila*, see Williams & Sehgal, 2001) and melatonin (which is also the effector hormone from the mammalian suprachiasmatic nucleus). However, elucidating the effectors is hindered by the fact that there are hundreds of kinds of such transmitters in insects. Although some are specific for a single function, other neurotransmitters and hormones may have several roles, so that even key transmitters are not necessarily confined to tissues involved in biological timing. Moreover, different species appear to use different factors (Takeda et al., 1999).

Responses

Many different responses are affected by photoperiod, including internal as well as externally visible rhythms, over daily to much longer time frames, and involving a variety of different systems, as exemplified in Table 2. Because so many systems are affected, each species possesses multiple, and even simultaneous, responses under some form of photoperiodic influence. Notwithstanding the theoretical touchstone of "the biological clock", it is by no means certain that such a range of divergent elements, subject to so many different selective pressures and proximate demands, could be co-ordinated by a single, localized time-keeping mechanism.

APPROACHES

Information about insect photoperiodism and rhythmicity can be gathered by more than one approach, as summarized in Table 3. *Observational approaches* record information, such as the structure of photoreceptors, directly; *inferential approaches* rely on indirect measures such as formal properties of the response.

Observational approaches focus on *elements* of photoperiodic systems that include the actual structures involved in reception, such as photoreceptor organelles and pigments. *Devices* are workings of the photoperiodic mechanism or its parts that can be directly observed, such as neurosecretory releases. *Qualities* are measurable features of individuals that are influenced by photoperiodic responses, such as mortality (or survival), and fecundity. However, many observations of this sort are not sufficiently complete to explain function or value.

Inferential approaches include the study of *responses* such as receptor sensitivity, voltinism, phenology, percentage diapause and properties of the photoperiodic response, which generate summaries (such as classifications of diapause) or statistics. These measures usually cannot be related directly to specific functioning, although comparative responses (such as those based on geographic differences) are especially helpful to interpret their adaptive value. *Models* incorporate theories consistent with responses or observations that suggest how mechanisms such as the clock or counter, or its parts such as particular loops or oscillators, work. Finally, *fitness* can be estimated from parameters supposed to reflect the adaptive value of particular responses, as inferred from seasonal conditions, trade-offs, and other selective options.

Of course, these approaches and categories are not absolute. They are inter-related and even overlap (Fig. 2). For example, well documented information about molecular loops has been combined with less certain information to generate a model of clock function. The mathematical theory for modelling clock function is well advanced (e.g. Glass & Mackey, 1988). Moreover, many students of photoperiodism and rhythmicity work with only one of the categories shown in Fig. 2, the power of the different approaches is not equal, and knowledge of one level does not necessarily give answers at another. In other words, we cannot yet make all of the links implied in Fig. 2.

LESSONS

Two sorts of lessons emerge from this brief review. First, the responses have extraordinary variety, as shown by their multiple components and complex integration, and by the diversity of clocks and other adaptations. Second, in doing work on these adaptations it is necessary to distinguish between different processes, approaches, and potential adaptive values, thereby avoiding a tendency to link many different phenomena just because they occur together or because they incorporate some form of timing element.

Multiple components

Responses to photoperiod consist of multiple components (cf. Fig. 1). For example, receptors provide inputs to monitor multiple environmental conditions over a variety of time scales (see Inputs above); multiple genes are involved in various elements of circadian timing and

TABLE 2. The range of insect responses governed by systems involving photoperiodic elements. For reference to some examples, see Danks (1987, 1994b), Denlinger et al. (2001).

Circadian rhythms
Eclosion (egg, larva, pupa)
Walking
Flight
Feeding
Defecation
Grooming
Mating
Ancillary mating behaviour (e.g. pheromone release/ calling, stridulation, swarming, bioluminescence, sperm release)
Oviposition
Internal rhythms (e.g. alimentary tract, neuronal output)
Light sensitivity
Seasonal patterns
Voltinism
Diapause induction (egg, larva, pupa, adult)
End of diapause
Multiple diapauses
Circannual eclosion rhythms
Sex ratio
Dispersal
Wing morph
Colour morph
Size morph
Reproductive morph
Growth rate
Individual quality (e.g. fecundity, size, energy storage)
Number of instars
Cold hardiness
Variability of response

diapause as well as in their downstream processes. Therefore, studies of restricted focus have limited explanatory power.

Complex integration

Both circadian and photoperiodic systems integrate many elements, including current signals, stored information, timing mechanisms and so on, for the appropriate control of activity and development. Identifying this integrated control at the anatomical, physiological, biochemical and molecular level (as opposed to some of its individual parts) has generally proved impossible. It is therefore especially useful to try to understand control in terms of the ecological coherence or adaptive value of the overall response.

Diverse clocks

There are many commonalities in the operation of biological clocks in different organisms (such as the usual presence of the period protein PER), but there is no one "biological clock". Rather, information already available suggests that there are at least two main types, photoperiodic (= seasonal) and circadian (= daily), based on different receptive pigments, in central and peripheral locations, with independent, integrated or redundant function, and with more or less marked differences in molecular operation. Each clock or set of clocks is thereby attuned to particular adaptive functions that differ within and among organisms. Such diversity is not unexpected too because selection is indifferent to exactly how functional requisites are met. Therefore similar environmental challenges could be overcome in several different ways.

Different processes

Different processes serve different purposes, notwithstanding the fact that they share common themes. For example, visual acuity, detection of sudden light changes, and monitoring of day and night all require photosensitivity, but these requirements are different in detail and in purpose. Daily and photoperiodic clocks both respond to day-night cycles but serve ends at very different temporal scales. Instar, morph, food storage, diapause and cold hardiness all coincide in order for an individual to survive cold winters, but involve different physiological systems. Therefore such *different* elements, whether or not they coincide in time, may or may not be linked or depend on common information or mechanisms. Commonalities are probably less prevalent than is often believed. For example, different seasonal responses in some species (although not in many others) appear to be programmed independently by photoperiod (e.g. wing morph and diapause: Harada & Numata, 1993; cold hardiness and diapause: Watanabe & Tanaka, 1997, 1999), and different tissues can have independent circadian clocks (e.g. Giebultowitz et al., 2001).

It is therefore misleading to sweep different components under the same umbrella (such as a single daily clock), without more detailed investigation of individual components. The "black box" of the biological clock may have been opened, but it contains only some of the parts to a larger jigsaw puzzle.

Different approaches

Certain areas and approaches in the study of photoperiodism and rhythmicity have generally been emphasized while others were neglected. However, the approach that

Table 3. Summary of approaches to the study of insect photoperiodism and rhythmicity.

Category	Approach			
	Observational		Inferential	
	Item	Examples	Item	Examples
Measures	Elements	Light-sensitive structures	Responses	Formal properties, percentage diapause
Mechanisms	Devices	Hormonal secretion	Models	Clocks, counters
Values	Qualities	Mortality, fecundity	Fitness	Estimators of fitness

is taken towards any investigation (cf. Table 3) should depend on the question being asked, because it will influence the outcome. For example, the observed percentages of diapause can be interpreted under various natural photoperiods in the context of environmental fitness, under various non-24-hour photoperiods in the context of clock function and phase setting, or under particular assumptions used to make the function and output of a model tractable as dictated largely by the objective of the modelling.

It is worth emphasizing that each approach has limitations and investigators should try not to over-reach the information gathered by their own approach. For example, models continue to proliferate without the biological data required to test them.

Values

In my view, the key orientation for interpreting daily and seasonal responses still is their ecological value, which has explanatory power even when all the detailed mechanisms have not been worked out. Such an ecological context shows why various patterns in time and space have been selected. Daily and seasonal patterns of feeding, inactivity, and so on (e.g. Danks & Oliver, 1972a; Danks, 1987; Kukal, 1991; Alpatov et al., 1999b; Bogacheva, 1999), and geographical differences in diapause (Masaki, 1996) are especially well known. For example, daily rhythms are weakened at high latitudes, allowing adaptive direct responses to favourable conditions (Danks & Oliver, 1972b; Lankinen & Riihimaa, 1997). Such ecological values provide context to unravel the many differences among daily and seasonal systems. It is these very differences that hinder the understanding of circadian rhythmicity and photoperiodism, because most students seek only the commonalities of mechanism and not the differences, which are clear when different taxa are compared (e.g. Helfrich-Förster et al., 1998) and are especially visible through differences in ecological effect.

CONCLUSIONS

Multiple elements with complex integration, including diverse clocks and their parts, are involved in photoperiodism and rhythmicity, and studying these adaptations needs an appreciation of differences among and within organisms, of different approaches, and of different ecological values. I conclude from these lessons that the current excitement about molecular mechanisms (e.g. Truman, 2001; Hastings & Follett, 2001; Denlinger, 2002) may be distorting attention away from other subjects that emphasize the different ecological values of different responses. Certainly we now have better probes and tools, but no less diversity or fewer problems. Indeed, most of the attention paid to genes is moot without knowing precisely what the genes do. As Bradshaw & Holzapfel (2001) noted, we need top-down as well as bottom-up approaches. Moreover, perhaps there is too much focus on "timing mechanisms" in general. Timing is an important element, but not necessarily the "central problem in photoperiodism" (as claimed by Saunders, 2001b) – many other components are included in Fig. 1. Perhaps we should even avoid the term "clocks" in the context of seasonal systems and confine its use to circadian rhythms.

My main conclusion can be re-emphasized with an analogy. Although considerable fascination attaches to how the spark plugs or other components of the engine in a car work (just like processes such as biological clocks in the developmental processes of an organism), and we can gain insight by examining the wheels and motion of the car (cf. specific responses), analysis of the driver's destinations and motivation (cf. ecological values) gives the essential context for understanding what is happening.

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