

Circadian Surfaces and the Diversity of Possible Roles of Circadian Organization in Photoperiodic Induction

(theoretical/biological clock/oscillator/resonance effects)

COLIN S. PITTENDRIGH

Department of Biological Sciences, Stanford University, Stanford, California 94305

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ABSTRACT It is an established fact that circadian rhythmicity is often somehow involved in the physiology of photoperiodic induction. It is shown, however, that there are three possible ways in which such rhythmicity could be involved. For the most part available data are inadequate to discriminate among these three roles, only one of which is covered by “Bünning’s Hypothesis.” Emphasis is given to a previously ignored possibility in which circadian organization is involved in photoperiodism—but not as the clock responsible for the time-measurement. The meaning of circadian surfaces and their bearing on the interpretation of a widely used experimental protocol is developed.

The switch from one seasonally appropriate metabolic strategy to another—such as from vegetative growth to flowering, or normal development to developmental arrest (diapause)—is controlled in a great variety of multicellular organisms by the relative duration of light and dark in the daily periodicity of environment: such switches are said to be *photoperiodically* controlled. This phenomenon of photoperiodism implies that in some sense the organism measures time (the duration of darkness or light), and in recent years it has become fashionable to state the central issue as the nature of the “clock” that effects the photoperiodic time measurement. The major debate for two decades has been whether the clock that effects the photoperiodic time measured is some sort of “hourglass” or a circadian oscillation of the type known to be utilized as the chronometer used in time-compensated sun orientation and other instances where organisms identify phases of the daily cycle of the external world.

The debate is currently in a confusing state in several respects, the full extent of which lies beyond the scope of this brief theoretical note that intends only: (i) to state some general aspects of the complexities not made sufficiently explicit in current discussions; (ii) to draw attention in particular to an additional complication believed to be both significant and not previously recognized; and (iii) to suggest that progress in clarifying currently confounded issues will probably best be made by the measurement of what are here called *extended circadian surfaces* (or topographies), rather than by traditional experimental protocols that constitute only one transect across such surfaces.

Convergent evolution in photoperiodic phenomena

Photoperiodism itself is by no means the only way in which selection has met the challenge of recognizing the time of year; and what formal similarities there are among those plants and animals that do utilize daylength as a noise-free cue to season

may well be no more than that—formal, and in a sense superficial—the product of a convergent evolution that obscures significant differences in the underlying concrete mechanisms involved. Indeed, there is little doubt this is in fact the case, making the search for a unified “theory” founded on facts from different organisms hazardous, at best. Thus, while it is abundantly clear that circadian rhythmicity is *somehow* involved in the photoperiodism of many plants, birds, and insects, the evidence increases (1–4) that at least in *some* insects an hourglass is also involved, or even solely responsible.

Even in those cases where circadian rhythmicity is unequivocally involved, it has more than one potential role: it could be involved without itself effecting the photoperiodic time measured; and when (and if) it does function as the clock, there is more than one way that it might do this. For the most part, the existing literature relating circadian rhythmicity to photoperiodism focuses on only one of these three possibilities, which was first developed by Bünning in 1936 (5).

Circadian organization as clock: “external coincidence”

Bünning’s classic paper went well beyond—indeed bypassed—the more general proposition that what was then called “endogenous daily rhythmicity” was *somehow* involved in photoperiodism: it stated a highly specific model of how circadian rhythmicity could effect the measurement of daylength, thus functioning as the clock responsible for the photoperiodic time measured. The essential feature of his model was that photoperiodic induction occurs only when some phase of the circadian rhythm coincides in time with a phase (light) in the external daily cycle of environmental change. Pittendrigh and Minis (6) later called this a “coincidence model.” It is useful (see below) to denote it more explicitly as an *external coincidence* model (coincidence of a phase in some internal rhythm with a phase of an *external* cycle). This idea underlies nearly all published discussion of the circadian oscillator approach to the photoperiodic time measurement. In its loosely stated form, it appears adequate to explain many of the known facts in those cases where circadian rhythmicity is demonstrably somehow involved in photoperiodism; there are some facts (especially in birds) that can, perhaps, only be explained by external coincidence. However, as Pittendrigh and Minis (4) point out, there are other facts that “external coincidence” cannot explain, especially in those cases involving a photoperiodic response curve of the type (itself common in insects) exemplified by *Pectinophora gossypiella* (4).

Few photoperiodically induced phenomena are all-or-none: the amount of flowering, the percent of larvae entering dia-

pause, or the amount of testicular growth in a vertebrate is often a quantitative function of both the duration of the photoperiod and the number of cycles experienced. From existing data the only safe generalization that can be made about the role of circadian rhythmicity in photoperiodism (when it is involved) is that *the amount of induction is a function of the entrained steady-state of the circadian organization*: in some steady-states induction is maximal; in others it occurs to a limited extent; in still others no induction occurs. That general statement clearly accommodates the specific model of external coincidence (the Bünning Hypothesis): it is only in some entrained steady-states that the postulated photo-inducible phase of the circadian cycle of an organism will coincide with light.

However, the generalization as stated accommodates other possible models, one of which was initially suggested by Pittendrigh (7) and has recently—apparently independently—been more fully developed by Tyshchenko and colleagues (8, 9), especially for insect photoperiodism. A useful English summary of the Tyshchenko version will be found in Danilevsky *et al.* (10).

Circadian organization as clock: "internal coincidence"

This second model, conveniently distinguished as "internal coincidence," was based initially (7) on what at the time was a proposition with limited empirical basis: namely, that it was probably misleading to speak of "the" (implying single) endogenous circadian rhythm of an organism; that multicellular systems must to some (uncertain) extent comprise a population of circadian oscillations whose mutual phase-relationships will probably have a significant effect on physiological function. It was noted that change in photoperiod would inevitably change the entrained steady-state of the multioscillator system, and that only under some photoperiods would constituent oscillations be so phased that what is here labeled *internal coincidence* would occur—only under some photoperiods would critical phase-points of two (internal) oscillations coincide, thus closing a switch and effecting photoperiodic induction.

Since 1960 the existence of many independent circadian oscillations in multicellular organisms has become much clearer. Aschoff's demonstration (11) of the separability of at least two circadian oscillations in man, each with its characteristically different frequency, is especially important. Pittendrigh (12) and Hoffmann (13) have shown that at least two oscillations are involved in the control of activity cycles of a rodent and a primate, respectively. There is, moreover, strong evidence (Pittendrigh, in preparation) in the rodent case that one of these is locked to sunset and the other to dawn. Takimoto and Hamner (14) showed some years ago that light-on and light-off steps each generate separate oscillations in the plant *Pharbitis nil*.

It is unnecessary to press the plausibility of an *internal coincidence* mechanism for the photoperiodic time measured further at this point or to explore the significance of what differences there are in Pittendrigh's and Tyshchenko's versions. The purpose is only to note that (apart from Tyshchenko and coworkers) the possibility is not generally being explored and that nothing in the published literature clearly excludes it. It is also worth noting if "internal coincidence" does underlie "photoperiodic" induction in some cases, it may well be possible to effect such induction without the use of light at all. Thus, if induction is a function of some set of entrained steady-

states of a multioscillator system, more specifically the phase relations of constituent oscillators, the inductively effective steady-states might be realizable by entraining the organism with agents (*Zeitgebers*) other than light. A systematic examination of this important possibility has apparently never been undertaken. On the other hand, several sets of data (e.g., ref. 4) indicate that induction can be effected (*without changing the duration of light*) by changing the entrained steady-state with temperature. Tests of this internal coincidence model by attempting to effect "photoperiodic" induction with *Zeitgebers* other than light seem to me to be a highly desirable undertaking in the current state of the field.

A non-clock role for circadian organization: the resonance effect

A third possible way in which circadian organization may be involved in the expression of photoperiodically induced phenomena is suggested by the now increasing evidence that physiological function is impaired, or changed, when the system is driven away from resonance with its entraining cycle. In this role circadian organization is not necessarily involved in the time measurement at all.

Went (15) was the first to discover that the efficiency of physiological function was impaired when an organism was driven by an external cycle with a period (T) different from its natural or freerunning circadian period (τ). Growth rate and total growth were maximal when T was close to τ (resonance) and fell off when T exceeded or was less than τ . These and related phenomena discovered by Hillman and Ketellaper have been reviewed before (e.g., in ref. 7) but their great importance seems, generally, to have been neglected: little has been done to explore their generality. Apart from one brief report by Pittendrigh in 1960 (ref. 7, p. 172), it is only very recently that the phenomenon has been found in animals: (a) Pittendrigh and Minis (16) find that the longevity of *Drosophila melanogaster* is impaired if the insects are driven by light cycles with periods of $T = 21$ or $T = 27$; it is maximal at $T = 24$; (b) Aschoff (personal communication) finds similar effects in the fly *Phormia terraenovae*; (c) Saunders, D. S. (1972) *Proc. Nat. Acad. Sci. USA* 69, 2738–2740, adds important further evidence: the growth rate of the larvae of *Sarcophaga argyrostoma* is a complex function of the relation between T and τ . Clearly the complex of circadian oscillations in multicellular organisms, their mutual phase-relationships, and their proximity to resonance with the environment play a major role in their overall temporal organization and, hence, the success with which physiological functions are discharged.

The potential bearing of this emerging generality on the relationship between circadian rhythmicity and photoperiodic induction is exemplified by my earlier analysis (17) of Beck's data (2) on the induction of diapause in the moth *Ostrinia nubilalis* (Fig. 1). Beck had reported the relative inductive success of a wide range of light-dark cycles in which he systematically varied both the duration of light (L) and dark (D); the period (T) of each cycle is equal to $L + D$. He found that for every value of L he used, induction was maximal when $D = 12$ hr and concluded, setting Bünning's approach aside, that the photoperiodic time measured was effected by an hourglass system measuring dark duration.

However, his data left no doubt that whatever the "hourglass" was, it was complex in the sense that while induction was always maximal at $D = 12$, the absolute value of the

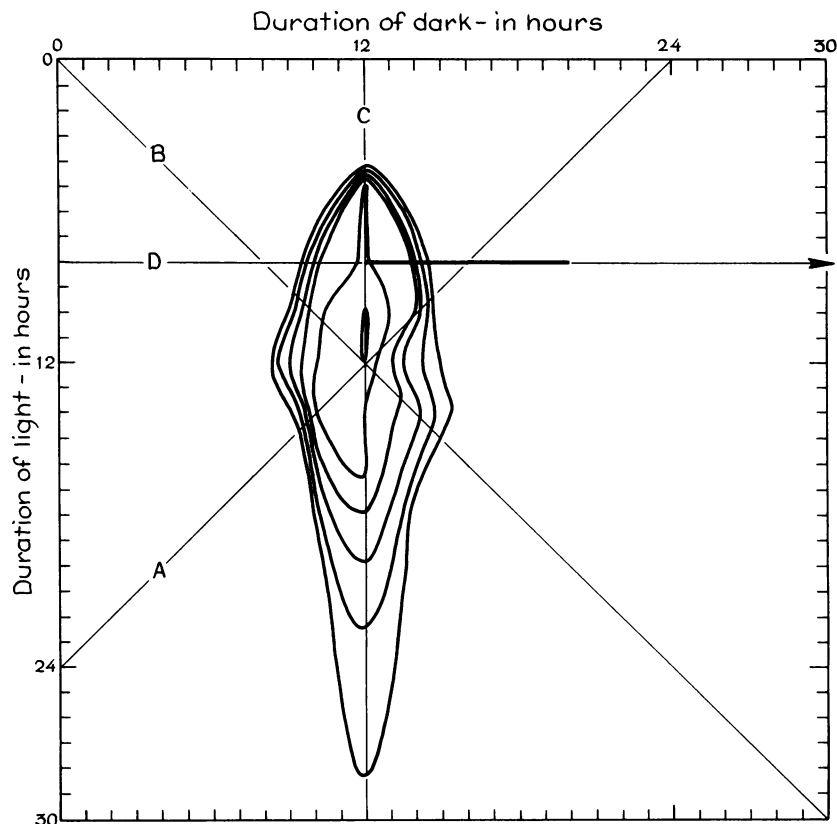


FIG. 1. *A Circadian Surface*. Based on Pittendrigh's treatment (17) of Beck's data (2) for *Ostrinia nubilalis*. Isoinduction contours (for 60, 70, 80, 90, 95, and 100% diapause) define the various L/D cycles that will induce a given percent of diapause. The contours define a surface that peaks when $T (=L+D)$ is close to τ ; it peaks when the circadian system is close to resonance with its driving cycle. The transects (A, B, C, and D) across the surface are discussed in the text. Extension of the D transect to T values up to 48, 72, and 96 hr yields the experimental protocol introduced by Nanda and Hamner (18).

maximum was a function of L . In attempting to clarify this dependence (17), I fitted smoothed curves to Beck's raw data to permit the derivation of interpolated values for L and D that would yield a given amount of induction—say 70%. These estimates yielded a series of *isoinduction lines* on cartesian coordinates consisting of L and D . Each isoinduction line is a closed loop defining a theoretically infinite number of light cycles (differing in T , L , and D) that will yield the same level of induction. Such isoinduction lines for various levels of induction do not overlap: they constitute contours that describe a *photoperiodic induction surface* which, in the case of *Ostrinia*, has two strong features.

(i) The single peaked surface is not conical; it has a long ridge along the $D = 12$ -hr coordinate. This is only another way of representing what Beck himself had stressed: no matter what the value of L , the most effective value of D is 12 hr.

(ii) However, the surface does make graphically clear what the generalization is that underlies the dependence of $D = 12$ on the value of L : the surface peaks at a value of T close to 24 ($D + 12 + L = 12$); in other words, when T is close to τ and the system is close to resonance. Thus, the surface I derived from Beck's data is not only a surface defined by isoinduction contours; it is also a *circadian surface* that informs us that *no matter how the measurement of $D = 12$ is made*, the success with which induction is effected is maximal when the circadian system is at, or close to, resonance with its driving cycle.

The detailed topography of the *Ostrinia* surface warns us that no matter what other role (clock) circadian organization *may* play in photoperiodic induction, we must reckon with the fact that its proximity to resonance is likely to modulate significantly the expression of whatever pathways the time-measurement itself initiates. This role of circadian organization in photoperiodism is nowhere reckoned within current discussions of the problem.

The potential utility of measuring extended circadian topographies

In Fig. 1 the transects (A, B, C, and D) across the surface illustrate several commonly used experimental protocols, whose outcome is always discussed in the unidimensional terms of the line indicated.

Transects with a positive slope of 45° represent a series of cycles of constant T and changing L/D ratio. The transect A itself yields the standard photoperiodic response curve for $T = 24$ hr. Transects B with a negative slope of 45° represent a series of cycles in which L/D is constant, but T increases. This defines experiments of the type performed by Went (15) and Pittendrigh and Minis (16), showing the dependence of some physiological performance (growth rate, longevity) on proximity to circadian resonance. The C transect, *in this insect*, illustrates the dependence on proximity to resonance of the

effectiveness with which the measurement of a 12-hr night length is subsequently utilized.

Transects D perpendicular to the L axis are of the greatest potential interest in clarifying the complex of possible relationships between circadian rhythmicity and photoperiodic induction. The heavy section of D corresponds with the series of T 's ($20 < 26$), with fixed $L = 8$, used by Pittendrigh and Minis (4) to test the *external coincidence* model in the moth *Pectinophora gossypiella*. As they noted, the systematic decrease in the induction of diapause from T_{20} to T_{26} was not compatible with the model. It is, on the other hand, just what we should expect if further study of *Pectinophora* were to yield a circadian surface of the type exemplified by *Ostrinia*.

It is, however, the extension of D transects beyond 24 hr of darkness that merits greater attention. That is the protocol, introduced initially by Nanda and Hamner (18), that is the most widely used tool in asking whether or not circadian rhythmicity is involved in photoperiodic induction. The classical positive result is the finding that when T is systematically lengthened (holding L constant) the amount of induction rises and falls, as a function of T , with inductive maxima occurring at values of T that are about $n\tau$ and inductive minima at values of T that are about $n\tau + \tau/2$. This result has always been interpreted, not just as implicating circadian phenomena somehow in the total physiology of induction, but specifically in the detailed terms of Bünning's original hypothesis, here called "external coincidence." However, one cannot simply treat such protocols as the search (with light) for the recurrence of a photoinducible phase of a circadian rhythm: the light used in the experiments drives, or entrains, the circadian system and we cannot set aside the complication that the performance of the system will be a function of its proximity to resonance, no matter how the photoperiodic time measurement is made. Since circadian organization will be most nearly normal when driven by cycles with $T = n\tau$, and least normal when driven by $T = n\tau + \tau/2$, the results of such experiments are by no means unequivocal in their meaning: all they justify is the restricted general conclusion made earlier in this paper that photoperiodic induction is a function of the entrained steady-state of the circadian system (when, in fact, it is involved).

The Nanda-Hamner type of result (an extended transect D), together with the known circadian surface for *Ostrinia*, suggests that when circadian organization is involved in photoperiodic induction we should expect—for some models of the clock—multiple peaks on an extended circadian surface. Saunders, in this laboratory, has sought and found such complex extended circadian topographies in the insects *Sarcophaga argyrostoma* and *Nasonia vitripennis* (see the following paper). The details of such topographies will be instructive in unconfounding the diverse roles circadian organization can, in principle, play in the expression of photoperiodically induced phenomena. Topographic differences to be expected on the basis of the three possible roles outlined here will be pursued in a later paper.

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1. Lees, A. D. (1971) in *Biochronometry*, ed. Menaker, M. (National Academy of Sciences, Washington, D.C.), pp. 372-380.
2. Beck, S. D. (1962) "Photoperiodic induction of diapause in an insect," *Biol. Bull.* **122**, 1-12.
3. Pittendrigh, C. S., Eichhorn, J. H., Minis, D. H. & Bruce, V. G. (1970) "Circadian Systems VI: The photoperiodic time measurement in *Pectinophora gossypiella*," *Proc. Nat. Acad. Sci., USA* **66**, 758-764.
4. Pittendrigh, C. S. & Minis, D. H. (1971) in *Biochronometry*, ed. Menaker, M. (National Academy of Sciences, Washington, D.C.), pp. 212-250.
5. Bünning, E. (1936) "Die endonome Tagesrhythmik als Grundlage der photoperiodischen Reaktion," *Ber. Deut. Bot. Ges.* **54**, 590-607.
6. Pittendrigh, C. S. & Minis, D. H. (1964) "The entrainment of circadian oscillations by light and their role as photoperiodic clocks," *Amer. Natur.* **98**, 261-294.
7. Pittendrigh, C. S. (1960) "Circadian rhythms and the circadian organization of living systems," in *Cold Spring Harbor Symposia on Quantitative Biology*, ed. Chovnick, A. (Long Island Biological Ass., Cold Spring Harbor, L.I., N.Y.), Vol. 25, pp. 159-184.
8. Tyshchenko, V. P. (1966) "Two-oscillatory model of the physiological mechanism of insect photoperiodic reaction," *Zh. Obshch. Biol.* **27**, 209-222 (in Russian).
9. Tyshchenko, V. P., Goryshin, N. I. & Azarjan, A. G. (1972) "The role of circadian processes in insect photoperiodism," *Zh. Obshch. Biol.* **33**, 21-31 (in Russian).
10. Danilevsky, A. S., Goryshin, N. I. & Tyshchenko, V. P. (1970) in *Annual Review of Entomology*, eds. Smith, R. F. & Mittler, T. E. (Annual Reviews, Inc., Palo Alto, Calif.), Vol. 15, pp. 201-244.
11. Aschoff, J. (1969) "Desynchronization and resynchronization of human circadian rhythms," *Aerosp. Med.* **40**, 844-849.
12. Pittendrigh, C. S. (1967) "Circadian Rhythms, space research and manned space flight," in *Life Sciences and Space Research V* (North-Holland Publishing Co., Amsterdam), pp. 122-134.
13. Hoffmann, K. (1969) "Circadiane Periodik bei *Tupaja (Tupaia glis)* in konstanten Bedingungen," *Zool. Anz. Suppl.* **33**, 171-177.
14. Takimoto, A. & Hamner, K. C. (1964) "Effect of temperature and preconditioning on photoperiodic response of *Pharbitis nil*," *Plant Physiol.* **39**, 1024-1030.
15. Went, F. W. (1960) in *Cold Spring Harbor Symposia on Quantitative Biology*, ed. Chovnick, A. (Long Island Biological Ass., Cold Spring Harbor, L.I., N.Y.), Vol. 25, pp. 221-230.
16. Pittendrigh, C. S. & Minis, D. H. (1972) "Circadian Systems: Longevity as a function of circadian resonance in *Drosophila melanogaster*," *Proc. Nat. Acad. Sci. USA* **69**, 1537-1539.
17. Pittendrigh, C. S. (1966) "The circadian oscillation in *Drosophila pseudoobscura* pupae: A model for the photoperiodic clock," *Z. Pflanzphysiol.* **54**, 275-307.
18. Nanda, K. K. & Hamner, K. C. (1958) "Studies on the nature of the endogenous rhythm affecting photoperiodic response of Biloxi soybean," *Bot. Gaz. (Chicago)* **120**, 14-25.