ever, it seems most likely that human activity has already significantly perturbed the atmospheric weather system. The effect of particulate matter pollution should be most severe in the highly populated and industrialized Northern Hemisphere. Because of the rapid diffusion of CO_2 molecules within the atmosphere, both hemispheres will be subject to warming due to the atmospheric (greenhouse) effect as the CO_2 content of the atmosphere builds up from the combustion of fossil fuels. Because of the differential effects of the two major sources of atmospheric pollution, the CO_2 greenhouse effect warming trend should first become evident in the Southern Hemisphere. The socioeconomic and political consequences of climate change are profound. We need an early warning system such as would be provided by a more intensive international world weather watch, particularly at high northern and southern latitudes.

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Calibrating Duckweeds: Light, Clocks, Metabolism, Flowering

Special characteristics of Lemnaceae may offer unique insights into plant development.

William S. Hillman

Experimental organisms with special, seemingly atypical characteristics often facilitate advances in biology. *Drosophila* in cytogenetics, *Neurospora* and *D. pneumoniae* in biochemical genetics, and the *Avena* coleoptile in plant growth regulation are classic examples. In contrast, work on sexual reproduction in higher plants—a process crucial to agriculture, horticulture, and forestry—has exploited unusual systems to a lesser degree. This is partly because of the importance of working with economically valuable forms, but it also reflects reluctance to use atypical material. Nevertheless the Lemnaceae, small floating plants commonly called duckweeds, seem particularly suited for research on flowering and related processes. This article begins with a summary of the general biological context and then describes some experiments on the Lemnaceae, concluding with an extended account of current work on a basic control mechanism.

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Photoperiodism: Phytochrome, Timing, and Florigen

Much of our knowledge about flowering and other aspects of plant growth derives from the discovery more than 50 years ago of photoperiodism (*1*), the control of development by the timing of light and darkness. Photoperiodism is now widely known among animals as well as plants. Thus, reproduction in many species can be controlled by manipulations of the light-dark regime, such as exposure to daily light periods longer or shorter than some critical value, or interruption of the daily dark period by a short "light break" (*2*). In plants, the effects of light breaks are mediated by phytochrome, a blue-green protein that is activated by low energies of red light (about 660 nm) and inactivated by far-red light (about 730 nm). In some systems, activation and inactivation are repeatedly reversible, so that phytochrome can act like an on and off switch. Many plant processes other than flowering, including seed germination and leaf growth, can be controlled in this way. Phytochrome appears to be associated with membranes, and may act by modulating the flux of various ions (*3*).

Photoperiodic responses often involve remarkably precise timing: some plants

discriminate between daily dark periods differing in length by 15 minutes or less. Work on photoperiodic timing has not, so far, revealed a mechanism, but the problem is an important source of interest and information on endogenous circadian rhythms, the "biological clocks" now recognized as important in areas as diverse as bird navigation and tumor therapy (*4*).

Another consequence of work on photoperiodism in plants is the observation that the flowering state induced by appropriate day lengths can be transmitted to other plants by grafting—often even to different species with different photoperiodic requirements for flowering. However, the hypothesis that such results indicate the existence of a specific flowering hormone, "florigen," common to many plants remains undemonstrated, since the results of attempts to isolate such a substance or to transmit it otherwise than by grafting are equivocal at best. While the known plant hormones, notably the gibberellins, play a major role in flowering, the "florigen" phenomenon is not understood (*2, 5*).

Thus the real advances summarized above have not explained either photoperiodism itself or the hormonal control of flowering. But they suggest that greater understanding of plant development would reward additional efforts that use new approaches.

Duckweeds in Nature and in Culture

Species of *Spirodela*, *Lemna*, *Wolffiella*, and *Wolffia* grow in temperate and tropical zones in fresh or somewhat saline, often highly polluted, water. The individual plant bodies, termed "fronds," are rarely more than 3 millimeters thick and range from 1 millimeter (*Wolffia* sp.) to 1.5 centimeters (*S. polyrhiza*) in length or diameter. They are the smallest of the angiosperms, monocotyledons in the aroid line; the flowers are reduced to a single pistil and one or two stamens (Fig. 1). Except in a few *Lemna* species, flowering is infrequent in nature and almost unknown in some areas, but all species have vigorous vegetative reproduction. Simultaneous "communal" flowering of several genera in particular ponds, but not in others nearby, has occasionally been observed. Plants overwinter or survive dry seasons in the form of ordinary fronds, specialized dormant bodies (turions), or as seeds (*6, 7*).

The usefulness of these plants as experimental organisms derives, first, from the ease with which axenic cultures can be maintained. This makes possible investigations under defined conditions free of the interactions with environmental microorganisms and soil properties usually present in work with whole plants. Another advantage is in the use of clonal material, minimizing genetic variability. The rapid vegetative growth can provide the biochemist with essentially unlimited supplies of material grown under specified conditions. The Lemnaceae also have an additional advantage over other small plants, such as *Arabidopsis*, that are easily raised in axenic culture but have a more usual growth form. The small bulk and floating habit of duckweeds mean that compounds in the medium are at most a few cell layers away from any part of the plant, and less buffered from it by root and translocation mechanisms (*7*). These properties allow for complex experiments in which a great many aspects of the system can be controlled, or at least taken into account. Except in this sense, however, the physiological characteristics of duckweeds seem within the range commonly encountered among higher plants.

For example, with sucrose as a carbohydrate source, cultures grow rapidly with light too dim or infrequent to allow significant chlorophyll formation. However, such heterotrophic growth is still light-dependent, at least in several species of *Lemna*. These can grow in absolute darkness with additional supplements of amino acids and yeast extract, but only very slowly (*8*). Yet even without these supplements, rapid heterotrophic growth can be maintained indefinitely by a few minutes of dim red light every day or so. This effect is prevented if the red is followed immediately by far-red, indicating that the "nonphotosynthetic light requirement" is for the occasional presence of active (Pfr) phytochrome (*9*). In the context of higher plants in general, this is then an extreme case of photomorphogenesis—the role of phytochrome in leaf and stem development. It is extreme, however, only in the sense that such a requirement is evident in most plants only in stages (such as seedlings) having sufficient reserves to grow without photosynthesis, like *Lemna* supplied with sucrose. Precisely what, in biochemical terms, is supplied by phytochrome in these conditions remains unknown; neither the *Lemna* system nor a strain of *Spirodela* that grows in absolute darkness with sucrose as the only organic supplement (*6, 7, 10*) has been studied in this regard.

Another example of how the special characteristics of Lemnaceae cultures bring general questions into sharper focus is related to iron availability. Although many plants have difficulty taking up iron that is not in organic complexes, materials leached from the roots or already in soils usually obscure this effect (*11*). Newly inoculated *Lemna* cultures with sucrose as the sole organic material grow slowly and appear iron-deficient if the medium has been sterilized by filtration, but grow normally if it has been autoclaved. This is probably a response to a sucrose breakdown product with chelating properties. As growth continues, however, even sterile-filtered medium eventually supports normal growth; the presence in old cultures of compounds leached or released from dying fronds may be responsible (*12*).

Copper, Water, Ammonium, Aspirin

The use of axenic, defined conditions and the close contact of the medium with most parts of the plant are almost certainly responsible for the effects on duckweed flowering of substances—such as copper, water, ammonium, and aspirin—that are not normally important in other plants. Such effects nevertheless interact with perfectly usual photoperiodic requirements. Thus Kandeler, in the first successful experiments on duckweed flowering, found that two *L. gibba* strains responded as long-day plants, with the requirement for high levels of far-red light typical of many, but flowered rapidly only in medium from old cultures (*13*).

A related interaction discovered some time later in my own laboratory is that of cupric ion and long photoperiods. At relatively low concentrations of copper, achieved either by adding complexing agents or by purification of all components of the medium, *L. gibba* strain G3 responds as a typical long-day plant, while *L. perpusilla* strain 6746 is a typical short-day plant and flowers only under day lengths shorter than 14 to 16 hours. However, with 1 to 5 μM cupric ion, a concentration at the threshold of general toxicity, the responses change. Even continuous light, normally the most effective long-day treatment, now fails to cause *L. gibba* flowering or to inhibit *L. perpusilla*, which continues to flower. Copper thus promotes flowering in one species and inhibits it in another, bringing about in both of them a condition similar to that caused by short days. Hence the simplest interpretation might be that copper acts not directly on flowering, but on the ability to perceive, or respond to, long days (*14*). Recent work by Takimoto and Tanaka, confirming the

copper effect on *L. perpusilla*, has shown that similar promotions of flowering in long days are also exerted by several other sulfhydryl inhibitors (*15*).

The work of Halaban in my laboratory also invites additional attention (*16*). Halaban found that flowering in *L. perpusilla* can be specifically inhibited by brief incubations in distilled water during the daily dark period. The time at which such incubations are maximally effective roughly parallels the time of maximum effectiveness for light breaks. The inhibition is probably due to the leaching out at a critical time of some substance or substances necessary for induction (*16*), and more recent work by Doss (*17*) suggests that inhibition of protein synthesis is involved. The "water inhibition" depends on critical levels of other components of the medium, notably sucrose and ammonium ion (*16*). Even in the absence of water treatments, the effects of ammonium ion are complex, since this ion inhibits flowering strongly and relatively specifically in some media (*18*) but not in others (*19*), possibly as a result of permeability changes (*16*). Although the water inhibition itself might at first seem unlikely to bear on the physiology of nonaquatic plants, leaching of the leaves by misting techniques is known to affect flowering in several of them. Which suggests, again, that the *Lemna* system simply provides a more precise and controllable means of studying common mechanisms.

Two other valuable lines of investigation deserve at least mention here. The first deals with interactions between photoperiodism (in the strict sense of control by the timing of relatively low light intensities) and effects of high energies of light. Observations by Kandeler and coworkers (*20*) and by Posner (*21*), the latter working on both wild-type *L. perpusilla* and an aberrant strain induced by x-rays, suggest that high light energies may act photosynthetically, but not simply in terms of substrate supply, as is often supposed. Second, Cleland (*22*) has used *Lemna* to assay for "florigen" in the phloem sap of the cocklebur (*Xanthium*) and finds strong flower promotion attributable to salicylic acid. Aspirin is also effective (*23*). The role of these substances in the normal flowering of either *Xanthium* or *Lemna* is obscure, but no others studied have yet been able to cause *L. gibba* flowering under strict short days.

At least one duckweed exhibits a phenomenon analogous to juvenility—the inability of seedlings (here, seedling strains resembling the parent) to flower before a substantial amount of growth has occurred (*24*). With this further support for the assumption that the developmental physiology of the Lemnaceae is representative of more usual plants, the remainder of this article deals with flowering in *L. perpusilla* strain 6746 as a model of the general problem of photoperiodism.

Duckweeds and Fruitflies: Clocks with Common Properties

Lemna perpusilla strain 6746 flowers rapidly on a sucrose and mineral medium with a few minutes of dim red light every 24 hours. It thus seemed to be ideal material for testing the hypothesis that photoperiodic timing depends on the content of phytochrome, and on the relative proportions of its two forms, Pr and Pfr. *Lemna* grown in the conditions described contains essentially no chlorophyll, the presence of which interferes massively with phytochrome determinations by in vivo spectrophotometry. Unfortunately, the protochlorophyll level is still sufficient to confuse matters, at least in my laboratory, but work started in this connection (*25*) provides new approaches to the timing mechanism.

With the goal of minimizing chlorophyll formation by minimizing light exposure, attempts were made to imitate the effects of regular photoperiodic treatments with "skeleton" photoperiodic schedules. To understand this term, consider the *light* : dark schedule *8* : 16, stated in hours. In the same notation, schedules such as *1* : 6 : *1* : 16 can be called skeletons of the *8* : 16. The "main light period" now consists largely of darkness, but the 1-hour light exposures mark its beginning and end, thus maintaining the proportions of the original schedule, at least on paper. The short light exposures might be reduced even further, to 0.25 hour each (giving, for example, *0.25* : 7.5 : *0.25* : 16) or to a few minutes. The question is whether flowering responds in the same way to skeleton as to normal photoperiodic schedules. Depending on the lengths involved, the answer comes in three parts: a clear-cut yes, no, and maybe.

First, with main light periods shorter than roughly 8 hours, skeleton and regular schedules have the same effects. Thus *8* : 16 and *0.25* : 7.5 : *0.25* : 16 are essentially indistinguishable, as are *4* : 20 and *0.25* : 3.5 : *0.25* : 20. Second as might be expected, when the main light periods are longer than roughly 16 hours, attempts to imitate them in this

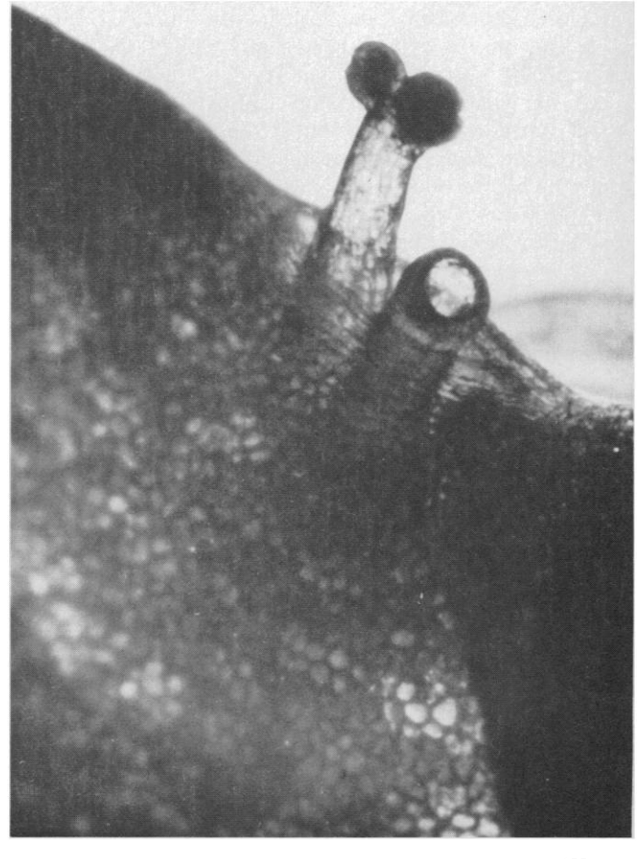

Fig. 1. Flowering *Lemna perpusilla* strain 6746. The magnification is ×80. [Photo by R. Marin, Brookhaven National Laboratory Photographic Services]

manner fail completely. That is, the skeleton schedule *1* : 17 : *1* : 5 does not at all imitate *19* : 5. Instead, it has the effect of *7* : 17, as if the longest of its two dark periods determined the sense in which the schedule was "read" by the plant.

In all these experiments, the plants are taken from stock cultures grown in continuous light; in the two cases described, it makes no difference whether they are first subjected to the long or to the short dark period of the skeleton schedule. In the third case, however, which is that of skeletons in which both portions are relatively close to 12 hours in length, the order of presentation does affect the results.

The basic schedule *11* : 13 causes rapid flowering within the 6 to 9 days of a typical experiment. A corresponding skeleton, *1* : 9 : *1* : 13, however, repeated over the same time, does so only if the plants receive the 13-hour dark period *first* after continuous light. If the 9-hour dark period is first, flowering is slow or absent within the experimental period. Apparently such skeleton schedules are ambiguous, and are read by the photoperiodic mechanism in a different way, depending on the length of the first dark period after continuous light. Further work, conducted by interpolating a single, variable-length dark period between continuous light and the start of six to nine repetitions of a given ambiguous skeleton, showed that such effects are circadian functions of the length of the interpolated dark period. That is, if a particular ambiguous skeleton causes rapid flowering when the interpolated dark period in the experimental procedure is 8 hours, it does so also when that dark period is 32 (8 + 24) or 56 (8 + 48) hours, but has the opposite effect—low flowering—when the initial dark period is 20 (8 + 12) or 44 hours (*25, 26*).

Such results have several significant consequences. First, of technical importance later, the establishment of photoperiodic control with light pulses requires schedules other than the simple skeletons described. Second, by far the simplest explanation for the data is an endogenous circadian rhythm that participates in photoperiodic timing. In fact, the *Lemna* data were first observed concurrently with studies by Pittendrigh (*27*) on the pupal eclosion rhythm of *Drosophila* under skeleton photoperiods (it is Pittendrigh's term), but with each investigator unaware of work by the other. Pittendrigh devised a model predicting the response to skeleton photoperiods of a hypothetical photoperiodic organism having a timer with properties similar to

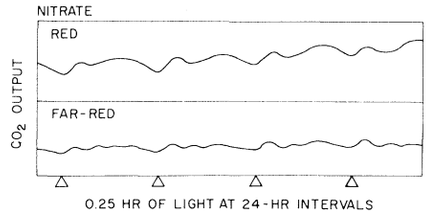

Fig. 2. Carbon dioxide output (arbitrary units, determined at 0.5-hour intervals) of *L. perpusilla* cultures on a nitrate medium under 0.25 hour of red or far-red light per day. [Redrawn from figure 2 in (*32*); days 4, 5, and 6 of experiment 6/1/73/113].

those of *Drosophila* eclosion, a typical circadian system. The *Lemna* results fit the model well enough to elicit the conclusion that they provided, in their complexity, "the most impressive single set of data supporting" the view that photoperiodic timing, in at least one organism, involves a circadian oscillation (*27*).

Lemma is the only plant so far subjected to this kind of analysis because of the difficulty of growing others nonphotosynthetically, but analogous responses to skeleton photoperiods occur in the photoperiodic fly *Sarcophaga* (*28*). Although the formal similarities between the responses of a plant and two insects might suggest that photoperiodic and rhythmic timing in all organisms involve the same basic mechanism, the arguments against this view, which cannot be summarized here, seem more persuasive (*29*). Nevertheless, it remains reasonable to suppose that understanding of any one response will have some general relevance.

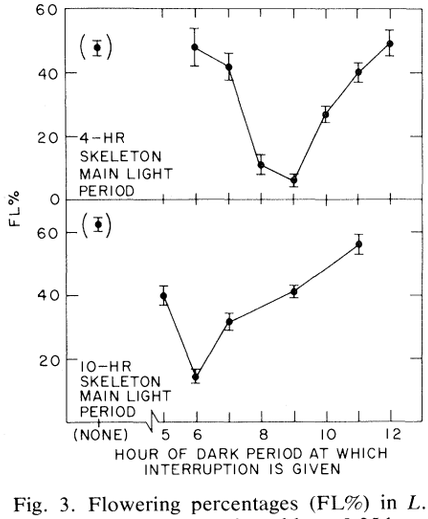

Fig. 3. Flowering percentages (FL%) in *L. perpusilla* cultures as affected by a 0.25-hour red light interruption of the night (long dark period) at various times in 24-hour schedules with red-filled skeleton main light periods 4 or 10 hours long (*34*).

Analyzing Photoperiodism Through Metabolism

A relation between the effects of skeleton photoperiods on flowering in *Lemna* and a rhythm in *Drosophila* leaves something to be desired in the way of formal analysis; to relate photoperiodic response and rhythmic process in a single organism would be better. But the rhythm with which most work on higher plants has dealt is that of leaf movements, a process that, admittedly, may be beyond the capacity of duckweeds. Given the heterotrophic nature of the system, CO_2 output rate seemed a reasonable alternative. This view was encouraged both by work on circadian rhythmicity of CO_2 exchange in succulents (*30*) and by the realization that hardly anything else could be measured nondestructively with equal ease.

With appropriate techniques, evidence was forthcoming that a portion of the CO_2 output of *L. perpusilla* cultures is controlled by a circadian rhythm. The responses to skeleton photoperiods of the time course of CO_2 output on the one hand, and of flowering on the other, show sufficient parallels to confirm the hypothesis that the same circadian timer affects both (*31*). More than such parallels is required, however, if one wishes to go beyond formal analysis to a study of mechanism.

Under a standard *light* : dark regime, *0.25* : 23.75, the pattern of CO_2 output established after several days varies, depending on the nitrogen source in the medium. With no nitrogen, naturally little growth occurs, but CO_2 output remains high for many days, adopting a simple sine-wave course with a daily maximum roughly 11 hours after the light pulse. The same pattern occurs whether the daily light is red or far-red. With nitrate in the medium, however, the situation changes: the pattern under red light shows two peaks, roughly 5 and 16 hours after the light pulse, and the effects of red and far-red differ markedly (Fig. 2). These patterns are specific to nitrate and not, as one might at first suppose, characteristic merely of nitrogen-sufficient and growing cultures in comparison to nitrogen-deficient ones. If ammonium is supplied instead, patterns similar to, but not the same as, those on nitrate are obtained (*32*). If nitrogen is supplied as aspartate, glutamate, or glutamine, all of which support healthy growth, patterns characteristic of these substances occur (*12, 19*).

Nitrogen sources thus modify the detectability of a phytochrome response in CO_2 output. They affect the response to

timing as well. For example, on transfer from continuous light to darkness, output on nitrogen-free or ammonium media exhibits substantially more circadian oscillations than output on nitrate, which damps rapidly. On the other hand, output on nitrogen-free medium takes on a weak 12-hour periodicity under a *light* : dark schedule of *0.25* : 11.75, while the same schedule elicits a clear 24-hour periodicity on nitrate or ammonium (*32*).

In general terms, the basis of these phenomena seems clear enough, bearing in mind that at most 50 percent of the CO_2 output is affected by the light regimes used. Given the relation of nitrogen metabolism to organic acid pools, the various nitrogen sources probably affect the relative proportions of intermediates involved in CO_2 flux, with the result that different reactions may be limiting or "displayed" in different circumstances. In the analysis of photoperiodism, it would then be useful if one or more of these reactions could be linked specifically to the photoperiodic timing mechanism.

Perhaps the most precise way of studying photoperiodic timing is through the time of maximal sensitivity to a light break during an inductive dark period. Thus, in a short-day plant such as *L. perpusilla*, the possibility that some treatment affects photoperiodic timing can be tested by seeing whether it shifts the time at which a standard light break inhibits flowering. If it does, the further hypothesis that a second process depends on the same timer can be approached by asking whether that process undergoes a quantitatively similar shift. It was on this basis that Halaban, working with *Coleus*, concluded that photoperiodic timing in that plant depends on the same circadian rhythm that controls leaf movement. In response to a given change in light period length, the time of maximal sensitivity to a light break shifts by the same amount of time as does the time of the daily minimum in leaf position (*33*). As to *Lemna*, the question is whether some aspect of CO_2 output might similarly serve as an indicator, in this case a metabolic indicator, of photoperiodic timing.

Here again, as in the previously planned study on phytochrome content, photoperiodic control with minimal light exposure is desirable. Because of the difficulties (described above) associated with simple skeleton schedules having light only at the beginning and end of the intended "light period," schedules were established with a 15-minute red pulse at least every 3 hours. For example, designating the beginning of each light period as hour 0, a 4-hour span is represented by 15 minutes of red light starting at hours 0, 1.75, 2.75, and 3.75, and a 10-hour span by red at hours 0, 3.25, 6.5, and 9.75; additional pulses at hours 12.75 and 15.75 convert the 10-hour schedule to a 16-hour span. Data on both flowering and CO_2 output show that such "filled skeletons" have none of the complex properties of their simpler counterparts (*19, 25, 31, 34*); in particular, those representing 10-, 12-, 14-, and 16-hour light spans give essentially the same critical daylength curve as do light regimes with solid light periods (*19*). In addition, the critical day length in such experiments is not affected by the nitrogen source in the medium, indicating that only CO_2 output pattern, not photoperiodism itself, is modifiable through nitrogen metabolism.

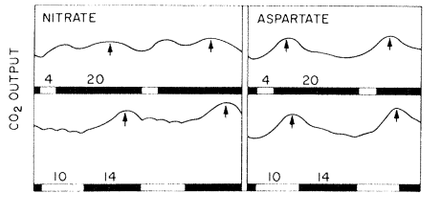

Fig. 4. Carbon dioxide output by *L. perpusilla* cultures on nitrate or aspartate media under the indicated red-filled skeleton light regimes. The 4 hours indicated actually represent 0.25 hour of red light at hours 0, 1.75, 2.75, and 3.75; the 10 hours indicated actually represent 0.25 hour of red light at hours 0, 3.25, 6.5, and 9.75. The results for nitrate are for days 5 and 6, and those for aspartate are for days 6 and 7.

Experiments were performed in this manner to determine the time of maximal sensitivity to a light break in conjunction with 4- and 10-hour "main light periods." Typical results are shown in Fig. 3. Overall means of many such experiments give times of 12.8 hours after the start of the 4-hour light period (hour 8.8 of the corresponding dark period) and 16.4 hours after the start of the 10-hour light period (hour 6.4 of the corresponding dark period). The 6-hour increase in the length of the light period can thus be viewed as delaying the time of maximal sensitivity by 3.6 hours, measured from the start of each light period, or advancing it 2.4 hours as measured from the start of each dark period (*34*).

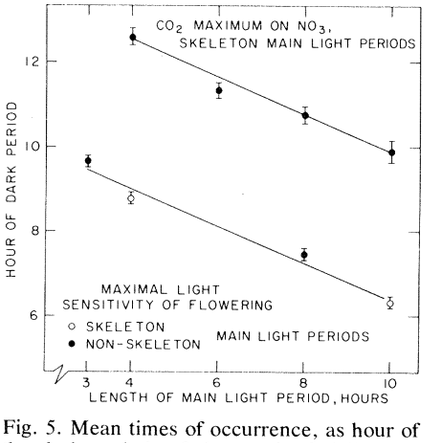

Fig. 5. Mean times of occurrence, as hour of the dark period, of the maximal daily CO_2 output on nitrate medium and of the maximal sensitivity of flowering to a night interruption as affected by the length of the main light period. Brackets are equal to twice the standard error of each mean. The slope (± standard deviation) of the CO_2 curve is -0.425 ± 0.049, that of the light sensitivity curve is -0.439 ± 0.024 (*34*).

It is this shift for which a parallel in CO_2 output was sought through concurrent experiments with "main light periods" of various lengths and on various nitrogen sources. From some typical data (Fig. 4), it is evident that, with nitrate in the medium, increasing the length of the light period from 4 to 6 hours delays the major peak substantially in terms of time from the start of the light period, while with aspartate the peak is not so affected. Initial results of this kind suggested that the peak with nitrate might shift in the same way as the time of maximal sensitivity. That this is indeed the case is shown by Fig. 5, in which the mean time of the CO_2 maximum on nitrate in many experiments is plotted—as hour of the dark period—as a function of light period length. Also plotted is the mean time of maximal light sensitivity in flowering experiments for both nonskeleton and filled skeleton main light periods; within the limits of error, the values from both types of experiment describe the same line. The major observation here is that the two lines—that for light sensitivity and that for the CO_2 maximum on nitrate—have essentially identical slopes, signifying that the timing represented by each is the same. The simplest conclusion, although not the only one possible, is that both processes are timed by the same timer (*19, 34*).

Further experiments show that the daily maximum on ammonium medium, which falls roughly 2 hours earlier than that on nitrate medium, is timed in the same way. That on aspartate, however, as already suggested by Fig. 4, is not. The lines in Fig. 5 have slopes of about -0.4, and these slopes differ significantly ($P < .01$) from both 0 and -1. On the same axes, the aspartate data would determine a slope of about -1, indicating that the peak comes at an approximately constant time (about 7.5 hours) after the start of each light period (*17, 34*). Described another way, the aspartate peak seems dependent only on a "dawn" or

"light-on" signal (*35*). In the same terminology, an event coming at a constant hour of the dark period no matter what the associated light period (a slope of 0 in Fig. 5) would be said to depend solely on a dusk or light-off signal, but no such timing has been observed in this series of experiments. Clearly, the timing in Fig. 5 represents an interaction of both dawn and dusk signals, whatever these may be.

It is of course possible that the parallel between the timing of the daily CO_2 maximum on nitrate or ammonium on the one hand, and that of the maximum photoperiodic sensitivity to a light break on the other, is mere coincidence, and that the processes are entirely unrelated. However, it is at least a reasonable working hypothesis that the parallel exists because both processes are in some way connected to the same reaction or series of reactions. On this hypothesis, the CO_2 maximum is a metabolic indicator of photoperiodic timing and one that can, in effect, be coupled or uncoupled by a procedure readily definable in biochemical terms—the use of different nitrogen sources. Hence, by appropriate work on organic acids, related nitrogen compounds (*36*), and the enzymes and cofactors concerned, it may be possible to distinguish reactions closely coupled to photoperiodic timing from those not so coupled, and thus to specify its components. On the evidence already discussed, one of those components must oscillate in a circadian fashion. In addition, however, certain aspects of the CO_2 experiments (*32, 34*) are consistent with the suggestion of several investigators (*35, 37*) that photoperiodic timing also involves a linear, nonoscillating ("hourglass") component. The opportunity now offered by the *Lemna* system, and the challenge, is that of giving concrete biochemical identity to "processes" and "components" that are at present undefined.

Other Prospects

It would obviously be useful to combine many of the lines of work already described. To what insights, for example, might studies of photoperiodic timing through CO_2 output patterns converge with those on inhibitors such as copper ion? Other investigations are also relevant, such as those on oscillations in the respiratory metabolism of *L. gibba*, possibly related to photoperiodic timing (*38*), and others on the complex effects of quality and intensity of both main and subsidiary light periods in *L. perpusilla* (*39*), effects analogous to those known on *Xanthium* and *Pharbitis*. Studies on photoperiodism and metabolism in *Chenopodium* may also be closely related (*40*). The possibility of augmenting the work already done with accurate phytochrome determinations may yet exist (*41*), and common chemical controls should be elucidated by work with flowering in cultures of *Wolffia microscopica* (*42*), *Spirodela polyrhiza* (*43*), and the many strains that seem totally unable to flower.

Experimental organisms are, in a sense, scientific instruments. Systems involving duckweeds are highly sensitive and correspondingly powerful. Although they can yield results that are hard to interpret, to avoid them because of that is like avoiding electron microscopes for the same reason.

Summary

The roles of photoperiodism and related light-dependent and hormonal processes in plants are not well understood. Rapid growth, aquatic habit, and adaptability to axenic culture make the Lemnaceae, or duckweeds, excellent material for investigating these topics and others in which highly defined conditions or the presence of organic substances are crucial. As a major example among several that are described, recent work with one species suggests a relation between some features of carbon dioxide flux and the photoperiodic timing mechanism, thus providing a system in which the biochemical basis of the latter may be explored.

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