to the upper left, and the growth ridges curve forward rather than backward as in Fig. 1B (*9*). The arrow indicates a section through one of the numerous pores found in the growth ridges; Fig. 2, A and B, are enlargements of similar sections through such pores.

The pores seen in Fig. 2, A and B, are not open to the surface, but are sealed on each end by a thin layer (arrows) of some material. Shells treated with Clorox (*10*) have open pores, indicating that this material is organic. This layer is the periostracum; it is present on the outer (upper) surface as the initial shell layer, and on the inner surface as an artifact of the calcification process, left behind by the retreating mantle (see Fig. 1B).

The upper surface of the growth ridge seen in Fig. 2, A and B, is the first part to be calcified; the periostracum here appears draped over crystals arranged in seemingly random fashion. Beneath this surface the crystallization becomes more uniform, with a suggestion of layering; this is particularly prominent in Fig. 2B. In general the layering marks the development of the calcite laths typical of the outer shell layer, but at least one layer (best seen about 2 μm from the base of the pore at the right side of Fig. 2A) may represent a brief interruption in calcification due to a retraction of the mantle. Much the same sequence can be seen in the region of the interspace; in Fig. 2C (an enlargement of the upper left corner of Fig. 2D) the crystals at the outer surface of the shell (arrow) are irregular; only after 5 to 10 μm of shell thickness have accumulated do the lath-like crystals typical of the outer shell layer begin to form.

These SEM observations are in good agreement with time lapse observations. The irregular, apparently random, crystal arrangement near the surface probably reflects the disruption of the earliest crystallites during mantle movements, before their incorporation into the stable shell margin. Similarly, the uniform, lath-like crystallization encountered a few microns below the shell surface probably reflects the influence of a stable shell on subsequent calcification.

This interpretation of marginal growth in *Pecten diegensis* has implications for the role of the substrate in general. First, it appears that substrate stability is a requirement for orderly shell growth; but if stability is lacking shell growth can still proceed in a disorderly manner. Second, it does not seem likely that even orderly shell growth requires anything beyond stability (and, of course, isolation from seawater) from its substrate; this conclusion is based on the observation that if the crystals in the outer, disorganized zone were directing the orientation of new crystal growth, the new growth would itself be disorganized; instead, it becomes quite orderly as soon as the substrate becomes stable.

These implications lead in turn to the conclusion that the mantle itself, acting through the organic matrix, provides the continuity and orientation to the new growth. If so, we come full circle from the early views of shell calcification as an essentially inorganic precipitation to the point of recognizing it to be a highly sophisticated biochemical process.

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7. They were dredged from a depth of approximately 50 m from the R.V. *Velero IV* on 1 and 2 July 1970, through the courtesy of D. S. Gorsline, University of Southern California.
8. The periostracum was not visible at the resolution of this apparatus.
9. In most specimens of *Pecten diegensis*, first formed growth ridges curve forward while later ones curve backward.
10. Clorox is a commercial solution of 5.25 percent sodium hypochlorite; this has the useful property of dissolving organic matter without harming calcium carbonate.
11. Supported by NSF grant GB-20692. The time lapse work was done at Kerckhoff Marine Laboratory, Corona del Mar, California, through the courtesy of the Division of Biological Sciences, California Institute of Technology. Instrument time on the SEM and valuable assistance were graciously provided by J. W. Schopf, University of California, Los Angeles (NSF grant GA-23741).

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Suppressing Drosophila Circadian Rhythm with Dim Light

Abstract. Drosophila *larvae were reared and allowed to pupate in continuous bright white light. The pupae were then transferred to a much dimmer blue light. In continuous blue light of intensity below 0.001 erg per square centimeter per second, adult flies emerged in pulses 24.7 hours apart, each pulse occupying about 6 hours. But in continuous light of intensity exceeding 0.1 erg per square centimeter per second, they emerged at a steady rate. This intensity range from effective darkness to effective light is roughly from starlight to moonlight. Inside this range, the emergence peaks broaden for about a week with little change of period.*

In a wide diversity of organisms, physiological and behavioral activities exhibit an approximate 24-hour periodism even in the absence of external cues. Although these rhythms are only circadian when isolated from light or temperature cycles, they synchronize to the earth's rotation when exposed to the day-night cycle. In many organisms, circadian rhythms decay in continuous light. For example, as little as 0.2 erg cm^{-2} sec^{-1} of blue light (450 nm) suffices to eliminate circadian variations of growth rate in the fungus *Neurospora* within 3 days (*1*).

I measured the threshold for photosuppression in the fruit fly *Drosophila pseudoobscura*, using the circadian rhythm of eclosion of pupal populations (*2, 3*). Chandrashekaran and Loher (*4*) had already found that 0.3-lux white light [containing about 0.2 erg cm^{-2} sec^{-1} in the effective band, 400 to 500 nm (*5*)] suffices to eliminate circadian variations of pupal eclosion rate in *Drosophila* populations within 3 days. The rate of damping is about the same even under bright light. How much light is needed for this photosuppression? Extrapolation from measurements of rephasing by a single light pulse of 100 erg cm^{-2} sec^{-1}, by means of a theoretical model of clock kinetics, suggested that as little as 0.01 erg cm^{-2} sec^{-1} might suffice, and that under suppressing intensities there should be at most a slight increase of period (*2, 6*).

I reared and harvested *Drosophila pseudoobscura* pupae under continuous bright white light, then transferred them to dim light (400 to 500 nm). After 3 to 8 days, depending on the age of the individual pupa at harvest, metamorphosis terminated in eclosion. These emerging flies were automatically recorded hourly for 7 days in the 12 independent experiments

shown in Fig. 1, each at a different light intensity.

The transition from bright white light to dim blue light immediately initiates rhythmicity in every experiment of Fig. 1, at a mean period of 24.7 hours. Behavior in the first 3 days after a perturbation affecting the circadian "clock" is commonly attributed to complex physiological "transients" (*7*), including a 10- to 20-fold dark adaptation (*2*); these have not been studied in great detail. I focus attention on behavior after day 2. There is a hint of some broadening of peaks even at intensities less than 0.002 erg cm^{-2} sec^{-1} (Fig. 2). Rhythmicity is suppressed by continuous light as dim as 0.01 erg cm^{-2} sec^{-1}; it is suppressed faster and more completely by higher intensities. There may be a slight increase of period with increasing light intensity (estimated by regression of peak centroids), but, if so, the increase is less than 2 percent (*6*). It should not be inferred that rhythmicity would remain suppressed in a diurnally varying light regime always greater than 0.01 erg cm^{-2} sec^{-1} (*2*, *8*).

Some comparisons may be helpful. Full moonlight typically illuminates the earth's surface with about 0.1 erg cm^{-2} sec^{-1} in the relevant band (400 to 500 nm); on a moonless night, the stars provide about 0.001 erg cm^{-2} sec^{-1} in this band (*9*). Light of 0.01 erg cm^{-2} sec^{-1} is too dim to induce a measurable electrical response in the eye of adult *Drosophila* (*10*). Even at 0.1 erg cm^{-2} sec^{-1}, flies immediately fall from the ceiling where they eclose in my emergence monitor, whereas this almost never happens in normal room lighting. Zimmerman and Goldsmith (*10*) argued that the clock photoreceptor in *Drosophila* is not a carotenoid derivative. There seems little reason to suspect that the effects of light on the circadian rhythm in *Drosophila* are mediated by the familiar visual system.

At 0.01 erg cm^{-2} sec^{-1}, for even the most absorbent biological pigment, a week will elapse before half the molecules will have absorbed a photon (*2*); because of tissue absorption, the waiting time is undoubtedly longer. Thus, it seems implausible that the photoreceptor substance plays a direct stoichiometric role in whatever biochemical oscillation underlies the circadian time sense. For example, it is unlikely that the "clock" operates by accumulation of a photolabile product to some critical concentration that triggers complete destruction of the product once a day.

Even with this sensitivity, *Drosophila*'s circadian time sense may be only slightly affected by light in the pupal stage outside the laboratory: When given an opportunity, the larvae I studied pupate 1 to 3 cm under the soil. In a light-dark cycle, they do this chiefly by night, so all are synchronously phased by the preceding dusk rather than by the time of burrowing of each individual. In such a dark place, accurate phasing of emergence 9 days later may depend upon a spontaneously recycling clock accurately tuned to 24 hours—features for which

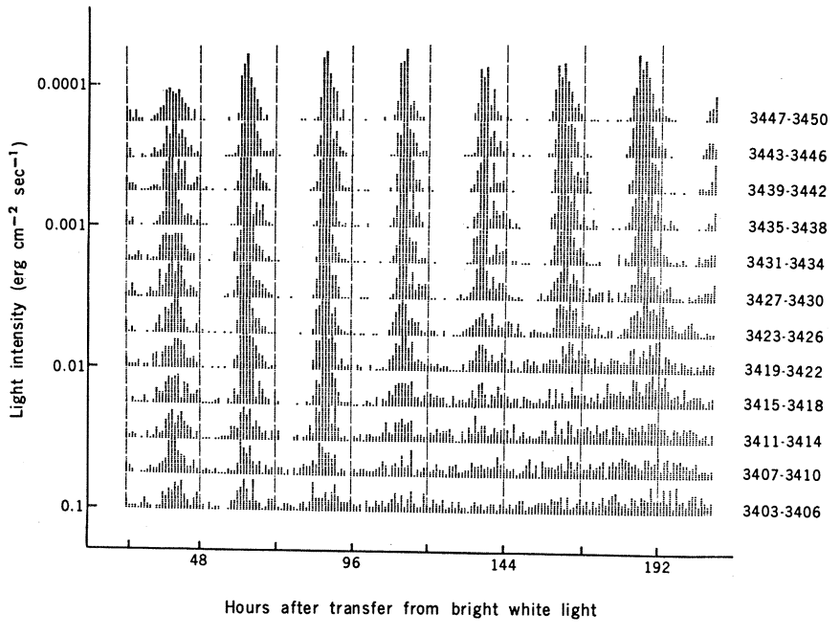
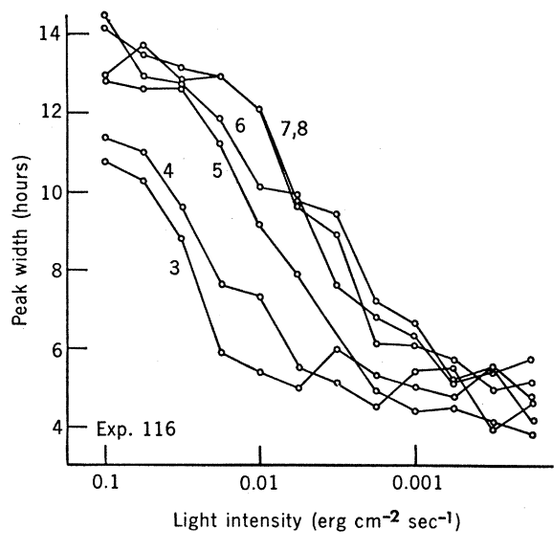


Fig. 1. Emergence histogram of a *Drosophila* population at each of 12 blue light intensities from 0.1 to 0.0001 erg cm^{-2} sec^{-1}. Data are plotted from hour 24 to hour 210 after transfer from bright white light. Each dot represents the emergence time of one adult fly. Except for the top histogram, each histogram has been cropped at 17 flies per hour by the one above it. The numbers to the right of each histogram are computer file numbers; each histogram results from pooling four (indistinguishable) experiments at two adjacent light intensities, two with the yellow-bodied sex-ratio mutant females used in (*2*) and two with wild-type flies of both sexes.

Fig. 2. Peak width in emergence histograms in Fig. 1. Peak width is given as twice the standard deviation of fly counts about a mean for each of six successive 24-hour intervals in Fig. 1, beginning at hour 54. For example, curve 3 (day 3 after transfer from bright white light) represents the second column of complete peaks in Fig. 1; curve 4 (day 4) represents the third column of peaks; and so forth. By day 7 or 8, peak widths seem to be stabilized at each intensity. The theoretical maximum peak width, for constant rate of emergence, is



$$2\sigma = 2\left(\int_{12}^{0} x^2 dx/12\right)^{1/2} =$$

$$2[12^3/(3 \times 12)]^{1/2} =$$

$$8(3)^{1/2} \text{ hours} = 13.9 \text{ hours}$$

where σ is the standard deviation and x is hours. The random jumble of peak widths below 0.001 erg cm^{-2} sec^{-1} is typical of populations emerging in continuous dark.

Drosophila is cherished as a laboratory subject.

Light of 0.01 erg cm^{-2} sec^{-1} approximates the minimum intensity reported as sufficient for photoperiodic determination of season from day length by insects (*11*, *12*), a process in which circadian rhythmicity has been implicated (*11*, *13*). Seasonal control of development in *Drosophila* has been reported, although little studied (*14*).

Unfortunately, there is nothing magical about moonlight (0.1 lux), for the light intensity required to photosuppress circadian rhythmicity in some other organisms is much greater. For example, the Queensland fruit fly, *Dacus tryoni*, is virtually insensitive to light while pupating (*15*), and 50 lux is required to suppress rhythmicity in sparrows (*16*). Action spectra also vary markedly (*17*).

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3. The blue light (400 to 500 nm) came from a distant incandescent filament, filtered by a Kodak Wratten 47 celluloid to an intensity adjusted to 1/1000 of my standard for 100 erg cm^{-2} sec^{-1} with the same spectral distribution [A. T. Winfree, *J. Theor. Biol.* **28**, 327 (1970)]. This was further attenuated by 0 to 23 thicknesses of Morilla No. 82 tracing paper. Calibration against the standard was carried out with an RCA S-11 blue-green photomultiplier tube in the linear range of voltage and intensity. The final intensity gradient, under a 23-step staircase of tracing paper, was checked by timing exposures to Kodabromide F-3 emulsion; the exposure was attenuated by a factor of 10 per eight steps. Since the pupal photoreceptor is probably in the brain, and pupae are glued in all orientations under unilateral illumination, they probably are subjected to a wide range of intensities under each step.
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Disruption of Gypsy Moth Mating with Microencapsulated Disparlure

Abstract. *Broadcast applications of microencapsulated disparlure at rates of 2.5 to 15.0 grams per hectare are capable of reducing successful mating of wild gypsy moths under field conditions. In test plots, population densities were as high as 32 pairs of pupae in an area of about 700 square meters.*

Disparlure (*1*), the synthetic sex attractant for the gypsy moth, *Porthetria dispar* (L.), is a potentially valuable agent for containing or manipulating field populations of this insect (*2*, *3*). The gypsy moth has caused an average of 198,801 ha of forest defoliation annually over the past 10 years in the northeastern United States, with a high of 582,433 ha in 1971 (*4*). Field tests of an aerially applied microencapsulated formulation of disparlure (*5*) against laboratory-reared insects in simulated infestions in August 1972 provided encouraging evidence that mating could be suppressed (*3*). Since significant differences were found in lure release rates and behavioral characteristics of laboratory-reared and wild gypsy moths (*6*), tests with wild insects were required. We report here the results of follow-up field tests in 1973, in which for the first time mating success of wild gypsy moth adults was adequately suppressed or prevented in simulated new infestations.

Test plots, 400 m square (16 ha), located in central Pennsylvania, were sprayed on 30 June to 2 July and 13 to 14 July 1973 (*7*). The formulation contained 5 g of disparlure in a total volume of 1.42 liters; rates of lure other than 5 g/ha were obtained by either halving or tripling the volume of formulation applied per hectare. Gypsy moth pupae collected in the field were placed at designated points in the test plots (*8*) on 2 July (test 1) and 18 July (test 2), and each was examined daily to record adult emergence. The condition and location of each female adult were recorded daily until an egg mass was deposited. Females that had not laid eggs were left in the field, frequently 5 to 6 days after emergence, until they disappeared or the test ended. When eggs were laid, both the egg mass and, if possible, its associated female were recovered; all females were collected at the end of an 11- to 12-day test. Females were dissected as soon as possible, and the bursa copula-

Table 1. Mating success of female *Porthetria dispar* in plots treated with microencapsulated disparlure, Huntingdon County, Pennsylvania, in 1973.

Insect dispersion and density	Treatment (g/ha)	Treated		Control		
		Fertility tests (No.)*	Fertile (%)	Fertility tests (No.)	Fertile (%)	χ^2
		Test 1				
Random, 2 pair/ha	5	17	5.9	23	17.4	0.37
Random, 8 pair/ha	5	68	5.9	81	63.0	49.30†
Aggregate, 32 pair/spot	5	94	13.8	82	52.4	28.34†
		Test 2				
Random, 8 pair/ha	2.5	103	16.5	99	71.7	60.36†
Aggregate, 16 pair/spot	2.5	67	13.4	71	47.9	17.50†
Aggregate, 16 pair/spot	5	83	16.9	71	47.9	15.75†
Aggregate, 16 pair/spot	15	51	9.8	71	47.9	18.08†
Aggregate, 32 pair/spot	15	130	5.4	141	53.2	71.01†

* Females or egg masses (or both) recovered were tested for fertility; the numbers represent pooled totals from four replications. † $P < .001$.