Action Spectra for Phase Shifts of a Circadian Rhythm in Drosophila

Abstract. *Action spectra were determined for light-induced phase shifts of the circadian rhythm of adult emergence in* Drosophila pseudoobscura. *The action spectra for advance and delay phase shifts are similar; the most effective wavelengths are in the blue region, with a sharp cutoff above 500 nanometers. This similarity suggests that the same photoreceptive pigment mediates both advance and delay phase shifts.*

Circadian rhythms are entrained by light cycles, phase shifted by single light pulses, and inhibited by continuous light. Action spectra for these light effects on circadian rhythms have been determined in plants (*1*, *2*) and animals (*3*–*5*) to characterize the photoreceptive pigments; however, these studies have not been conclusive. Furthermore, they have not compared the action spectrum for advance phase shifts with that for delay phase shifts. Action spectra for light-induced phase shifts (both advance and delay) of the circadian rhythm of adult emergence in *Drosophila pseudoobscura* were determined; the experimental technique was similar to that reported earlier (*6*).

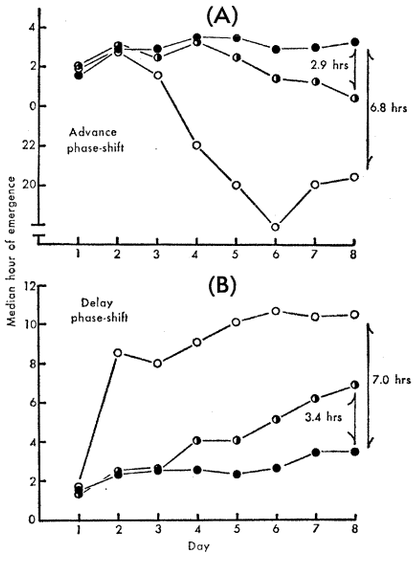

Fig. 1. Median time of adult emergence in free-run and phase-shifted populations of *Drosophila* pupae. Each graph represents three populations of pupae released into constant darkness for 8 days. Each point denotes the median hour of adult emergence for the corresponding day, where day 1 is the first day of constant darkness, and hour 0 is the time dawn would have occurred if the entraining light-dark cycle had been continued. Phase shifts were induced by single 15-minute light signals which interrupted the period of darkness. (A) Advance phase shifts induced by signals given at circadian time 20 on day 1. (B) Delay phase shifts induced by signals given at circadian time 17 on day 1. Each of the three populations is identified according to the light signal it received. Open circles indicate the white light control population (intense, white light signal); solid circles, the free-run control population (no light signal); and half-solid circles, the experimental population which received a dim, blue light signal (wavelength 483 nm; intensity, 1.3×10^{11} quanta sec^{-1} cm^{-2}). On day 8, experimental phase shifts were 50 percent of the size of white light control phase shifts.

Large populations of insects were reared to the pupal stage in a daily cycle of 12-hours light and 12-hours dark. The pupae were then harvested and glued to six brass plates (144 cm^2) coated with flat black paint. The plates were mounted in individual automated collection devices which provided an hourly record of adult emergence. The pupae were left in light until the end of the final hour of the entraining light-dark cycle; thereafter, they were kept in constant dark, except for a 15-minute light exposure to be described. The temperature was constant throughout within 0.5° of 20°C. Since the pupae were of mixed developmental ages and the duration of pupal life was 8 to 9 days, we could assay the emergence rhythm for 8 days.

In order to obtain colored light pulses of different intensities, four monochromatic projectors were constructed. Each projector contained a 500-watt projection lamp (G.E. CZX); an interference filter compounded with colored glass cutoff filters (Photovolt Corp.) (*7*); neutral-density filters (Kodak Wratten, Bausch and Lomb), when needed; and two lenses. Mounted on a collection device, such a projector uniformly illuminated approximately 1500 pupae glued to the brass plate. For each projection source, light intensity was measured by a radiometer (Yellow Springs Instrument Co.) built into a replica of the collection devices (*8*). When light intensity was decreased by neutral-density filters, the final intensity was calculated from the filter transmittance measured by a Cary-15 spectrophotometer and from the initial intensity measured by the radiometer.

In every set of experiments, two of the six populations of pupae served as controls. One population, designated as "free-run control," received no light signal after release into constant dark. The median hour of emergence of free-run control flies for each day occurred shortly after hour 0, that is, shortly after the time when dawn would have occurred if the light-dark cycle had been continued (Fig. 1). The second control population, designated as the "white light control," received a 15-minute intense white light signal (1100 lu/m^2, white fluorescent) at circadian time (CT) 17 or 20 during the first day of constant darkness; that is, the signal was given 17 or 20 hours after the time when dawn would have occurred if the light-dark cycle had been continued. As noted earlier, such light signals induce a phase shift in the emergence rhythm; the signal applied at CT 17 induces delay phase shifts, whereas the signal applied at CT 20 induces advance phase shifts (Fig. 1). Delay phase shifts begin the day after the signal, but advance phase shifts do not begin for several days (*9*).

The remaining four populations of

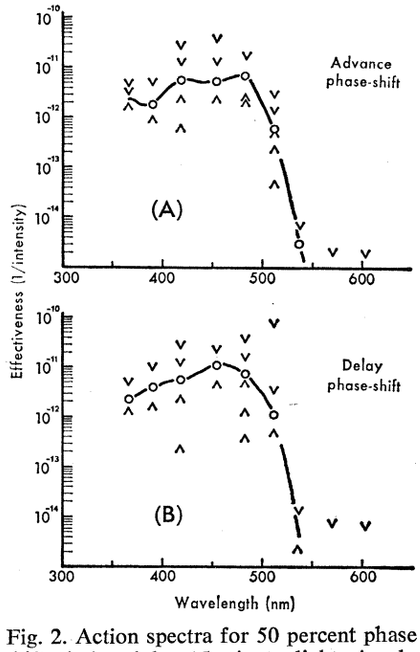

Fig. 2. Action spectra for 50 percent phase shifts induced by 15-minute light signals. (A) Advance phase shifts; (B) delay phase shifts. The ordinate is a measure of effectiveness, and is expressed as the reciprocal of the light signal intensity. Light intensity is expressed as quanta sec^{-1} cm^{-2}. Intensities indicated by open circles induced phase shifts between 40 and 60 percent on day 8 of the experiment. Arrows pointing upward indicate intensities which induced more than 60 percent phase shifts on day 8. Arrows pointing downward indicate intensities which induced less than 40 percent phase shifts on day 8.

pupae received 15-minute monochromatic light signals simultaneously with the signal given to the white light control population. The phase shift caused by the monochromatic signal was expressed as a percentage of the phase shift caused by the white light signal. In successive experiments, the intensity of the monochromatic light signals was altered until the phase shift 7 days after the signal equaled approximately 50 percent (Fig. 2). The spectral regions tested extended from 367 nm into the infrared (*10*).

Differences in wavelength did not affect the direction of phase shift—that is, whether the phase was advanced or delayed. The magnitude of the phase shift increased with increased light intensity, but reached a maximum that was equivalent to the phase shift caused by the intense white light control signal. Finally, during the 7-day period after the light signals, phase shifts caused by dim light signals changed relative to phase shifts caused by intense signals, such as the white light control signal (Fig. 1).

The action spectrum for delay phase shifts is similar to the action spectrum for advance phase shifts (Fig. 2). In both cases, the most effective wavelengths were between 420 and 480 nm; there was a slightly reduced response to the shorter wavelengths, and a sharp cutoff above 500 nm. Wavelengths of 570 nm and longer induced neither advance nor delay phase shifts, even at very high intensities. Phase shifts could not be induced by intense, nonmonochromatic light spanning a broad spectral region from 600 nm into the infrared (*10*). Finally, the two curves are superimposable within the limits of experimental resolution (Fig. 2).

Interpretation of the action spectra is difficult because of lack of knowledge concerning the precise photoreceptive site within the insect and the light-transmitting effects of interfering pigments. However, the *Drosophila* action spectra are similar to action spectra for regulation of circadian rhythms in the fungus *Neurospora* and the moth *Pectinophora* (*2, 5*). The insensitivity of the *Drosophila, Neurospora,* and *Pectinophora* rhythms to wavelengths above 570 nm is noteworthy, because action spectra for light effects on circadian rhythms in other organisms show a small but distinct peak between 600 and 700 nm after a sharp decrease near 450 nm (*3, 11*). Insensitivity above 600 nm is evidence against the possible involvement of phytochrome, a photoreceptive pigment associated with photoperiodism in plants (*12*).

The similarity of the action spectra for advance and delay phase shifts strongly supports the view that the same kind of photoreceptive pigment mediates the two shifts. The action spectra are consistent with the possibility that a carotenoid is the photoreceptor, but they do not rule out pterins, flavins, or other compounds.

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7. The light transmission curves of the interference filters were determined on a Cary-14 spectrophotometer. The regions of transmitted light were 8 to 15 nm wide at one-half peak transmission.
8. The procedure for measuring light intensity in the visible region of the spectrum was as follows. With the light source on, the radiometer was zeroed using an infrared pass, visible absorbing filter (Kodak Wratten 89B) in series with the interference filter. The infrared pass filter was then replaced by a transparent blank filter (Kodak Wratten 0), and the intensity was read on the radiometer. The blank was removed before the projector was used to irradiate populations of pupae, and a factor of 10 percent was added to the measured intensity values to compensate for incomplete transmission of the blank. This procedure corrected for the small quantity of infrared light which was not entirely blocked by the interference filter.
9. C. S. Pittendrigh and D. H. Minis, *Amer. Natur.* **98**, 261 (1964).
10. A single red and infrared pass filter (Corning CS2-61) was used to isolate a broad spectral region from 600 nm into the infrared. It replaced the interference filter in the light projector.
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13. Research supported by NSF grant GB-5819 (to Amherst College and W.F.Z.) and based on a thesis presented by K.D.F. to the biology department. We thank B. Spear (Amherst College) for assisting in the experiments and Dr. C. S. Pittendrigh (Princeton University) for helpful suggestions.
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Cycasin: Detection of Associated Mutagenic Activity in vivo

Abstract. *Cycasin and its lagycone, methylazoxymethanol, increase the mutant frequency of* Salmonella typhimurium *histidine auxotrophs when tested in the host-mediated assay. As expected, the degree of cycasin-related mutagenic activity depends on the facility with which the compound can be enzymatically deglucosylated by the normal intestinal flora.*

Plants in the family Cycadaceae, genus *Cycas*, contain a β-glucoside, cycasin, which is toxic (*1*) and carcinogenic (*2*) when administered orally to mammals. Comparative studies with gnotobiotic and conventional rats have shown that cycasin must be enzymatically converted to its aglycone, methylazoxymethanol, by the normal intestinal flora before death or tumors can be induced (*3, 4*).

Methylazoxymethanol, in contrast to cycasin, is mutagenic for *Salmonella typhimurium* (*5*) and causes chromosomal aberrations in onion root-tip cells (*6*), but little is known about the formation or retention of mutagenic activity in vivo.

The recently introduced "host-mediated" assay (*7*), which incorporates a microbial indicator in a murine host, presents an ideal system for studying this problem. Histidine auxotrophs of *Salmonella typhimurium* are injected intraperitoneally, and the test compound is administered directly (orally, intramuscularly, and so forth) to the host. Thus the compound potentially

Table 1. Effect of a reduced intestinal bacteria population on the mutagenic capacity of cycasin in the host-mediated assay. Intestinal flora and *Salmonella* were counted as viable organisms per milliliter. *Salmonella* were recovered from the peritoneal cavity.

Group	Treatment		Viable organisms (No./ml)		
	Ampicillin	Cycasin	Intestinal flora	*Salmonella*	Mutant frequency
Positive control	−	+	1.86×10^7	6.91×10^7	4.9×10^{-7}
Ampicillin control	+	−	5.79×10^4	1.51×10^8	3.3×10^{-8}
Negative control	−	−	5.38×10^6	6.89×10^7	2.9×10^{-8}
Experimental	+	+	3.46×10^4	1.29×10^8	5.4×10^{-8}