

## Clock Mutants of *Drosophila melanogaster*

(eclosion/circadian/rhythms/X chromosome)

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**ABSTRACT** Three mutants have been isolated in which the normal 24-hour rhythm is drastically changed. One mutant is arrhythmic; another has a period of 19 hr; a third has a period of 28 hr. Both the eclosion rhythm of a population and the locomotor activity of individual flies are affected. All these mutations appear to involve the same functional gene on the X chromosome.

Rhythmic variations in behavior are displayed by many organisms, ranging from single cells to man (1). When the rhythm persists under constant conditions, and has a period of around one day, depending little on temperature, the rhythm is called circadian (2). Many experiments have attempted to probe the mechanism (3), but the nature of the underlying oscillation remains unknown (4). Perturbations by inhibitors of RNA or protein synthesis suggest that such molecules are involved (5-8). Biochemical systems that oscillate with much shorter periods have been demonstrated both *in vivo* and *in vitro* (9, 10), but their relation to circadian rhythms is not clear.

An approach that has been successful in unravelling mechanisms in some systems is the use of genetic alterations. Since the expression of a rhythm requires an integrated system, mutation of the genes responsible for development and function of the system could lead to abnormal rhythms. Various aspects of circadian rhythms have indeed been shown to be sensitive to genetic makeup (11-18). For genetic dissection of circadian rhythms in an organism having a nervous system, *Drosophila* offers certain advantages. Much is already known about the rhythm of eclosion (emergence of the adult fly from the pupa), and genetic methodology is readily available. This paper describes the first result of such an analysis.

### MATERIALS AND METHODS

#### Isolation of mutants

*D. melanogaster* of the C-S (Canton-Special) wild strain was maintained on cornmeal medium. Mutagenesis by ethyl methane sulfonate was according to Lewis and Bacher (19), the treated males being mated to virgin attached-X females, so that each F<sub>1</sub> progeny male carried a treated X chromosome received from his father. Each male was mated individually to attached-X females, producing a stock of males bearing identical X chromosomes, plus normal-rhythm, attached-X females. The stocks were reared at constant temperature under LD 12:12 (12 hr of 50 foot-candles or more of white fluorescent light, 12 hr of darkness each day).

To detect X-linked rhythm mutants, the stocks were examined for ones in which males emerged abnormally. The normal females in each bottle served as an internal control, at least twice as many emerging during the light as during the

dark period. In a few bottles, males emerged in approximately equal numbers during day and night. Each mutant candidate was examined in more detail by raising pupae in LD 12:12, then monitoring the adult eclosion rhythm in constant darkness. From a total of about 2000 F<sub>1</sub> males, three rhythm mutants were obtained.

#### Determination of eclosion and locomotor activity rhythms

Eclosion rhythms, free-running in constant darkness, were determined with automatic "bang boxes" (20), generously loaned by Dr. Colin Pittendrigh. Several hundred pupae, raised in LD 12:12, were transferred to the apparatus at the end of a light cycle. The apparatus was thereafter maintained in constant darkness. Fractions were collected every hour, yielding an eclosion profile.

Locomotor activity of individual adult flies was measured by monitoring their movement with infrared light, which does not affect the *Drosophila* clock (21). The devices, designed and built by Dr. Yoshiki Hotta, used a small incandescent lamp, a Wratten No. 87C filter transmitting only wavelengths greater than 800  $\mu$ m, and a chamber (3 mm thick  $\times$  4 mm wide  $\times$  45 mm high) containing the fly, some food, and a cotton plug. Two silicon solar cells were arranged so that one received light transmitted through the upper third of the chamber, the other the lower third; they were wired so that the output voltage was zero when equal light fell on both cells. As the fly moved into or out of the area monitored by either solar cell, the resulting imbalance was converted to an all-or-none response registered on an event recorder. The sharpest rhythms were obtained with young flies (within one week of eclosion) previously exposed to at least three cycles of LD 12:12.

#### Genetic mapping of rhythm mutants

The rhythm mutants had normal morphology, but their abnormal eclosion patterns could be used as markers in recombination experiments. Males bearing an X-linked rhythm mutation were crossed to females homozygous for the visible markers *yellow-2*, *scute*, *vermillion*, *forked*, and a wild-type allele of *yellow* located near the centromere. These markers had no effect upon rhythm. F<sub>1</sub> males, receiving their X chromosomes from their mothers, were all  $y^2 sc v f \cdot y^+$ , and the females were all heterozygous, having the rhythm mutation on one X chromosome and  $y^2 sc v f \cdot y^+$  on the other. These males and females were mated to each other. F<sub>2</sub> males, receiving X chromosomes that had an opportunity to undergo recombination in their mothers, included various recombinants for the rhythm mutation and the morphological markers.