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Clock Mutants of *Drosophila melanogaster*

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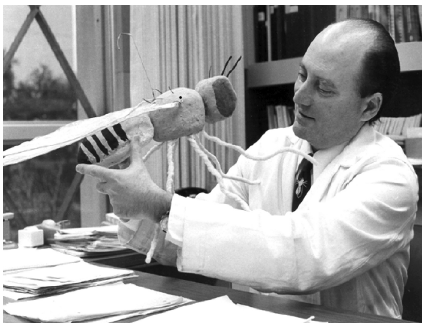
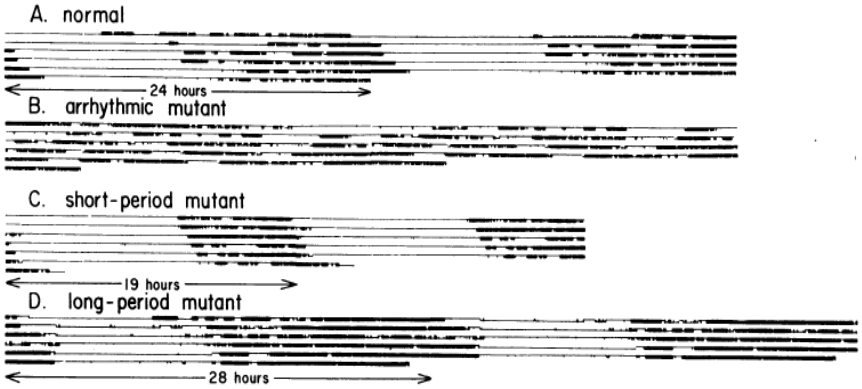
Ron J. Konopka, PhD
Modified from (Takahashi, 2004)



Seymour Benzer, PhD
From (Harris, 2008)

In 1971, Ronald J. Konopka and Seymour Benzer discovered the first mutations affecting circadian phenotypes. By performing a mutagenesis screening, searching for genetic variants affecting flies' behaviour, the two geneticists isolated three novel *Drosophila* mutants characterized by defects in eclosion (moulting) rhythms and locomotor activity. While wild type flies (*CS*) displayed normal eclosion rhythms (24h) and circadian rest-activity cycles (23.8h), these mutants were characterized by faster rhythms (short-period mutant, about 19.2h), slower rhythms (long-period mutant, about 28.5h) or no rhythms at all (arrhythmic mutant).



Circadian rest-activity cycles of wild type and period mutants flies in constant darkness.
(A-D) Actograms showing the activity of flies. Each line shows two intervals of data sequentially represented, and each interval is plotted twice, at the right of the immediately preceding and at the left of the following one. Period *moduli* of 24h, 19h or 28h were used for normal flies and arrhythmic mutant (A-B), short period mutant (C) and long period mutant (D), respectively. From (Konopka & Benzer, 1971).

Genetic mapping and complementation tests, performed with the three mutant lines, identified a single locus on the X chromosome whose mutation resulted in all three changes in rhythmic behaviour. This was the first reported gene affecting the circadian oscillator, a landmark not only for this field but for all of behavioural neuroscience. At the time, many scientists thought that animal behaviour was too complicated to be affected by mutations in a single gene! Konopka and Benzer set out to prove this hypothesis wrong…and it is almost a lucky coincidence that they chose circadian rhythms as their output. (In other papers, Benzer also examined phototaxis and learning.)

Thirteen years later, the gene whose mutation caused these marvellous phenotypes was isolated, using the new technique of P-element mediated rescue (T. A. Bargiello & Young, 1984; T. A. Bargiello et al., 1984; Reddy et al., 1984; Zehring et al., 1984): it was *period*, the core component of the molecular circadian clock. These studies were the first of several major genetic investigations in the fruitfly. Ten years later, a second gene of the fly circadian clock was identified, *timeless* (Sehgal et al., 1994; Vosshall et al., 1994). The forward genetics approach used to search for circadian variants in flies was also been adopted in mammals, leading to the

discovery of the circadian *Clock* gene in mice (Vitaterna et al., 1994). A few years later the homologous *Drosophila* gene was identified, also called *clock.* The work done by Konopka & Benzer thus paved the way for molecular studies on circadian rhythms. *Period, timeless, clock* and *cycle* are all part of a single molecular feedback loop that defines the circadian clock today. The genes and mechanisms controlling it ended up as Nobel-worthy discoveries, and the prize was awarded to Jeffrey C. Hall, Michael Rosbash and Michael Young (Nobel Prize in Physiology or Medicine in 2017). Most investigators in the field are sure that Konopka and/or Benzer would have gotten it too, if they were still alive in 2017!

Clock mechanisms are highly conserved from flies to mammals; the knowledge obtained by studying flies' circadian behaviours supports research in mammalian model organisms and in humans. It is noteworthy that a missense mutation on the *hPER2* gene affects the sleep-wake rhythms in *Homo sapiens* (Toh et al., 2001), mimicking the period-short mutants discovered by Konopka & Benzer. Individuals carrying this point mutation suffer from "Familial Advance Sleep Phase Syndrome": an autosomal dominant variant that causes early sleep onset as well as early awakenings. This illustrates an important concept that has proven true across all organisms studied so far: a mutation that produces a short period length of behaviour or physiology under constant conditions will have an early phase in a normal light-dark cycle of 24 hours, and a mutation that produces a long period length will have a late phase under the same conditions. Thus, having a functional circadian timing system confers adaptive benefits to an organism.
A lot of work has been done since the discovery of these mutants, but the Konopka & Benzer period mutants will always be in the limelight.

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