EVOLUTION

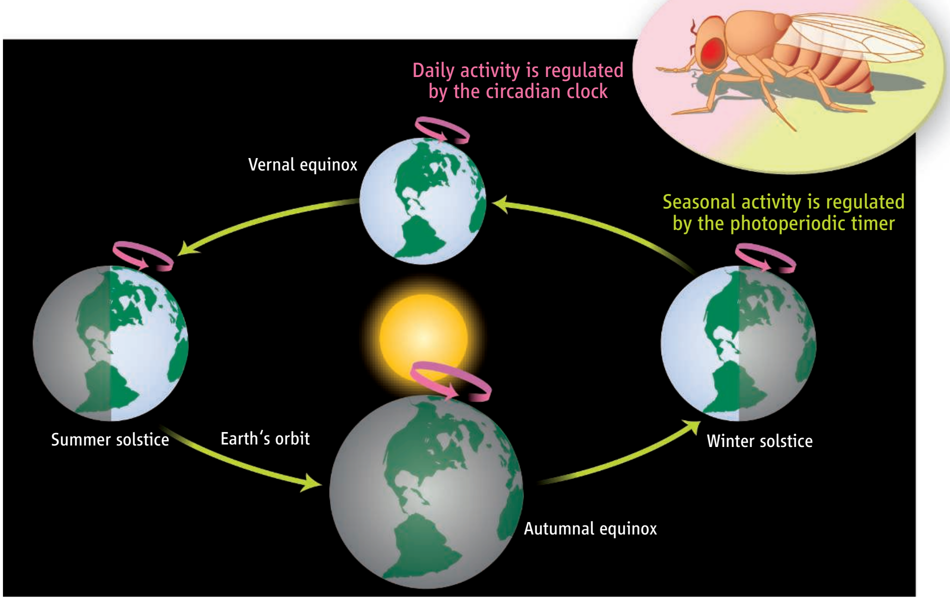
Tantalizing *timeless*

William Bradshaw and Christina Holzapfel

The circadian clock regulating daily activities is distinct from the photoperiodic timer that regulates seasonal activities in *Drosophila melanogaster*.

There are two great rhythms of the biosphere: the daily cycle caused by Earth's rotation about its own axis and the annual cycle of the seasons caused by Earth's rotation about the Sun. Plants and animals use an internal circadian clock that is set by light to time many daily biochemical, physiological, and behavioral activities, and they use the length of day (photoperiodism) to time development, reproduction, migration, and diapause (dormancy) in anticipation of the changing seasons (see the figure) (*1*). A great deal is known about the molecular basis of circadian rhythmicity, especially in the fruit fly *Drosophila melanogaster* (*2*), but the molecular basis of photoperiodic timing of seasonal activities remains largely unknown in animals (*3*–*6*). On pages 1895 (*7*) and 1898 (*8*) of this issue, Kyriacou and colleagues have shed light on a decades-old controversy rooted in the argument that the ubiquitous circadian clock must provide the underlying mechanism for the seasonal photoperiodic timer (*9*). This argument is both intuitive and parsimonious. However, in a series of elegant experiments, Kyriacou and colleagues show that, in fact, the daily clock and the seasonal timer are genetically distinct processes in *D. melanogaster*. A further interesting twist is that a crucial circadian clock gene, *timeless*, affects the incidence of a seasonal event—diapause—but does so without involving the photoperiodic timer.

Tauber *et al.* have found that European *D. melanogaster* are photoperiodic for the initiation of diapause (*7*). In accord with other arthropods (*10*), the incidence of diapause in these flies—that is, the frequency with which dormancy occurs in a population of flies—is high when the length of day is short; incidence of diapause decreases with increasing day length. The authors also found that the incidence of diapause is positively correlated with latitude. Thus, in northern Europe, the incidence of diapause is higher than in southern Europe. Kyriacou and colleagues identified two naturally segregating alleles of *timeless*, a central circadian clock gene in *Drosophila*. One is a new allele that originated 8000 to 10,000 years ago during the postglacial invasion of Europe by *D. melanogaster*. Ancestrally, *timeless* was represented by the single allele, *s-tim*, which codes for S-TIM, a short form of the TIMELESS protein. The other naturally segregating allele, *ls-tim*, codes both for ancestral S-TIM and for L-TIM, a long form of TIMELESS. Unlike mutations that are induced in laboratory stock colonies, this polymorphism at the *timeless* locus represents a spontaneous, single-nucleotide mutation in natural populations that has been maintained by selection. Sandrelli *et al.* show that relative to ancestral S-TIM, the derived L-TIM binds more tightly with the circadian photoreceptor CRYPTOCHROME and thereby attenuates the photosensitivity of the circadian clock (*8*). The question then remains as to whether allelic variation in *timeless* affects the photoperiodic timer.



Daily and seasonal cycles on Earth. The timing of daily physiological processes in plants and animals is regulated by the internal circadian clock that is set by dawn and dusk transitions as Earth rotates about its axis. The timing of seasonal development, reproduction, migration, and dormancy are regulated by long and short days (photoperiodism) that signal seasonal changes as Earth rotates about the Sun. Of interest is whether or not there is a genetic connection between the circadian clock and the photoperiodic timer and if they evolve independently over the vast climatic gradients of Earth.

Tauber *et al.* noted that at any given photoperiod, the incidence of diapause in *s-tim* flies is higher in a northern population (Netherlands) than in two southern populations (Italy). Also, at all latitudes, the incidence of diapause is higher in *ls-tim* flies compared to *s-tim* flies. As the authors point out, this pattern is consistent with an adaptive advantage that the *ls-tim* allele imparts in the highly seasonal European climate. However, there is no significant effect of an interaction between photoperiod and the different *timeless* alleles on the incidence of diapause, either in natural populations or in genetically transformed flies. Photoperiod and *timeless* exert their influence on diapause independently, both within and between populations. Therefore, *timeless* in European *D. melanogaster* serves two functions: It plays a central role in the circadian clock and, ancillary to its clock function, it affects the incidence of diapause directly, without going through the photoperiodic timer.

A similar conclusion follows from studies on *period*, another central circadian clock gene. Mutant flies that lack *period* have a dysfunctional circadian clock but remain photoperiodic (*11*) and maintain cycling levels of TIMELESS protein (*12*, *13*). In wild-type flies, *timeless* is transcribed and translated rhythmically. TIMELESS protein binds to the circadian photoreceptor protein CRYPTOCHROME, and in the presence of light, TIMELESS is then degraded (*2*). In mutant flies that lack *period*, *timeless* continues to be transcribed and translated into TIMELESS protein, but the TIMELESS protein still binds to CRYPTOCHROME and is degraded in the

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light (*12*, *13*). Consequently, in normal cycles of day and night, TIMELESS could theoretically convey day-length information, even in flies with a dysfunctional circadian clock. Kyriacou and colleagues' results show that this tantalizing possibility is highly unlikely in natural populations of European *Drosophila* because they find no significant *timeless* genotype × photoperiod interaction in the induction of reproductive diapause. Both naturally segregating alleles at the *timeless* locus, as well as induced mutations at the *period* locus, indicate that the circadian clock that regulates daily activities and the photoperiodic timer that controls seasonal activities in *D. melanogaster* are distinct molecular and physiological processes.

The distinction between the daily circadian clock and the seasonal photoperiodic timer is important because a wide variety of animals, from rotifers to rodents, use day length to time their seasonal life-history events. As seasonality changes with geography, so also does response to day length (*10*). Evolution of photoperiodism therefore constitutes the major adaptation of animal populations when dispersing in temperate and polar regions or when confronting the growing challenge of rapid climate change (*14*). Had there been a causal connection between the circadian clock and the photoperiodic timer, then understanding the biochemical and molecular mechanisms of circadian rhythmicity would have provided insight into the biochemical and molecular mechanisms underlying seasonal adaptations along geographical climatic gradients, as well as insights into regional solutions to rapid climate change.

The search for understanding the photoperiodic timer by exhaustive studies of specific circadian clock genes has shown little promise. As in the case of Kyriacou and his colleagues, we have learned a great deal more about circadian genes themselves, but the genetic mechanisms underlying photoperiodic response remain elusive. Future searches for the mechanistic basis of photoperiodism and its evolution should therefore focus on approaches such as fine-scale mapping of genes on chromosomes or microarrays showing differential gene expression that are unbiased by the assumption of a causal connection with the circadian clock.

References and Notes

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ASTRONOMY

Inside a Cosmic Train Wreck

Complementary experiments and calculations reveal how black holes behave in the opaque central region of a galactic collision.

Paolo Coppi

When young galaxies crash into each other, the result is often not a pretty sight. The violent gravitational forces of the encounter rip apart the beautiful galactic spiral arms, and gas and stars shoot out into intergalactic space at high velocity (see the figure). Yet we are only realizing now that the most important result of such an encounter is often not visible to us at all.

Much of the gas in the collision is not flung out but instead cools quickly, collapsing to the center of the system. Eventually tens of billions of solar masses of gas can pile up into a region only a few hundred light-years across. The gas becomes so dense that it blocks most light and so compact that standard ground-based telescopes cannot resolve the details of the collapse due to blurring by Earth's atmosphere. The same density and compactness that make the gas collapse so hard to study observationally also make it hard to study theoretically. Two papers in this issue begin to lift the veil on this unexplored central region. On page 1877, Max *et al*. (*1*) report an advance in ground-based imaging that permits us to directly observe black holes in the densest areas of the collapse, and on page 1874, Mayer *et al*. (*2*) present high-resolution simulations showing how black holes in the colliding galaxies follow and respond to the collapsing gas.

To penetrate the dense gas, Max *et al*. used a detector operating at infrared wavelengths. To achieve high spatial resolution, they used an adaptive optics technique in which the shape of the telescope mirror is modified in real time to compensate for jittering of the image due to atmospheric turbulence. This combination enables Max *et al.* to present one of the highest-resolution observations yet of the central, "nuclear" region of the NGC 6240 galaxy merger, mapping out its distribution of stellar light and unambiguously reconciling the different estimates for the positions of the two supermassive black holes that lurk there.

Mayer *et al*. present complementary theoretical calculations that are some of the most realistic to date of the gas distribution at the center of a merger. Although several important physical effects, in particular the "feedback" of energy from the luminous central stars and black holes back into the collapsing gas, ultimately require better modeling, the calculation already seems accurate enough to resolve a long-standing puzzle: Rather than wander forever around the center of the merger, two black holes in a system like NGC 6240 should quickly merge to emit a potentially detectable blast of gravitational wave radiation.

Why is so much effort going into understanding what happens when gas-rich galaxies, and in particular massive ones, collide? Comparison of data from experiments such as the Wilkinson Microwave Anisotropy Probe, which tells us what primordial density fluctuations looked like, to data from galaxy surveys like the Sloan Digital Sky Survey, which tells us what those density fluctuations have evolved into today, strongly suggests that we live in a universe where the matter density is

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