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NEWS AND COMMENTARY

Open season on the Bünning hypothesis and seasonal timing

What kind of insights can quantitative genetics provide us about this controversial hypothesis?

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The huge amount of data generated by microarrays and high-throughput sequencing technologies led to the reincarnation of system biology. Yet good old quantitative genetics and biometric analysis of phenotypic variation remains a powerful approach to unravel the genetic architecture of a trait, and the interactions among different genetic circuits. In a recent paper in *Heredity*, Bradshaw *et al.* (2012) have taken this elegant approach to test for genetic links between two major genetic networks, the circadian and the photoperiodic clocks in the pitcher plant mosquito *Wyeomyia smithii*.

The circadian clock is an evolutionary conserved adaptation that allows organisms to respond to the daily fluctuations of their environment. The clock circuit comprises a network of interacting genes, organised in positive and negative feedback loops, of which many components have been identified. The photoperiodic clock is another, equally-important timing mechanism, which allows the monitoring of the annual change in day length, and is used for timing of specific seasonal responses. Many insect species, for example, particularly in temperate regions, use the photoperiodic clock to detect the autumnal shortening of the day and to trigger diapause (a developmental or reproductive arrest) for surviving the winter.

There are some significant differences between the circadian and the photoperiodic clocks. The circadian pacemaker is capable of generating $\sim$24 h rhythm even in the absence of environmental cues, while, by definition, light–dark cycles are essential for photoperiodic timing. Also, the circadian system is designed to keep a fixed cycle rhythm in a wide temperature range, whereas photoperiodic timing is temperature sensitive. Although both clocks have been intensively studied for more than six decades, the circadian clock is far better characterised at the molecular level compared with the photoperiodic timer, which is largely uncharted territory.

Way before the molecular genetics era, Erwin Bünning formulated the idea that photoperiodic timing is mediated by the interaction of light, either directly or indirectly, with the circadian pacemaker. This was an alternative to the 'hour-glass' model that postulates that the timer is entirely driven by the external light–dark cycle, and is reset every day. Over the years, the Bünning hypothesis received considerable experimental support in various organisms. The Nanda–Hamner (NH) protocol, named after its developers, is one of the most popular experiments for testing the Bünning hypothesis. Subjects are exposed to light–dark cycles of abnormal periods (for example, T=72 h), made by combining a fixed duration of light with an extended period of darkness. Under these conditions, many animal and plant species exhibit regularly timed multiple peaks of their seasonal response, rather than a single maximum, as would have been expected under the 'hour-glass' model. Importantly, these peaks are spaced at 24 h intervals (for example, T=24, 48, 72), echoing the underlying circadian oscillation. However, there are many examples where this protocol fails to show a circadian rhythm. Overall, the Bünning hypothesis is still a major debated question in chronobiology.

Wild populations of overwintering insects such as *W. smithii* show substantial variation in the critical photoperiod (CPP), which is the day length required to set off their median seasonal response. Usually a population's CPP correlates with latitude and altitude (CPP gets longer at higher latitudes so diapause is triggered earlier in the year). *W. smithii* also show a robust rhythmic response in NH experiments, which would suggest a causative role for the circadian clock in photoperiodic timing, but a series of studies by the Oregon group of Bill Bradshaw and Christina Holzapfel begs to differ.

Using the rhythm of the NH response as a readout for the circadian clock, Bradshaw and his colleagues found that variation in CPP among populations was not associated with any variation in the period of circadian rhythm, and that artificial selection for lines with long or short CPP was not accompanied by changes in the period length of the circadian clock (Bradshaw *et al.*, 2003). In some populations, however, the amplitude of the NH rhythm, a parameter that may reflect the light sensitivity of the circadian pacemaker, did show a negative genetic correlation with CPP. Does this correlation reflect a role of clock genes in photoperiodism (pleiotropy), or merely a by-product of linkage disequilibrium between components of the two clocks? To address this question, Bradshaw *et al.* (2012) used an antagonistic selection on both traits (CPP and rhythm amplitude). In just five cycles of selection, the direction of the previously reported genetic correlation was reversed, indicating that the two traits can evolve independently, and that it is unlikely that the circadian clock is causally involved in photoperiodic timing.

The question, however, remains, whether the NH rhythm (particularly, the rhythm amplitude) is a reliable proxy of the circadian pacemaker. Further study of the circadian system of *W. smithiii* is required to verify the significance of the NH rhythm and its different parameters. It is also possible that local adjustments of both the circadian

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rhythm and CPP are carried by selection targeting mainly downstream components, whereas the core circuits are tightly linked and are not amenable for selection. A more direct approach to test the role of the circadian system in photoperiodism would be to genetically silence the clock. This was indeed attempted in *Drosophila* using clock mutants, and in other insects using dsRNAi knockdown. Excluding the *period* of null mutants, whose photoperiodic response appears intact, the photoperiodic behaviour of other mutant/transgenic animals is disrupted (for example, Ikeno *et al.*, 2011), supporting Bünning hypothesis. On the other hand, these results may be interpreted merely as pleiotropic effects of single clock genes on diapause rather than a direct connection between circadian and photoperiodic mechanisms.

Although the controversy surrounding the Bünning hypothesis still remains, the general message from Bradshaw *et al.* (2012) about the importance of circadian-unbiased, forward genetic approaches in studying the molecular basis of photoperiodism is well taken. In principle, molecular approaches, including transgenic, that were once limited to *Drosophila*, are now applicable to non-model insects such as *Wyeomyia*, and would permit a more direct dissection of any association between photoperiodic and circadian timers.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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