Adaptive Significance of Circadian Rhythms

Biological Clocks and Darwinian Fitness in Cyanobacteria

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Many physiological and behavioural processes within living organisms are rhythmic, and occur with periodicities of about a day; such biological rhythms are referred to as *circadian* (from the Latin words *circa* = about, *dies* = day). Other such rhythmic physiological and behavioural processes show periodicities of about a month (*circalunar*), or about a year (*circannual*). The periodicities of these biological rhythms closely match those of daily light/dark cycles, or the seasonal cycles of climate, caused by the rotation of the earth around its axis, and around the sun. Marine and intertidal organisms, moreover, are also under the influence of tidal cycles with periodicities of about 12.4 hrs. The most pervasive and widely studied among these biological rhythms are the ones with periods on the order of one day, namely circadian rhythms. These rhythms have been found in organisms ranging from simple unicellular prokaryotic cyanobacteria (blue-green algae) to flowering plants and mammals. In humans, too, many bodily functions, from sleep-wake patterns to core body temperature, exhibit circadian cycles. Flowering in plants shows a circadian pattern, as does foraging activity in many animals. The ubiquitous nature of circadian rhythms strongly suggests that they confer an adaptive advantage to the organism, in terms of adjusting its physiology and behaviour in anticipation of changes in the environment. Yet, there has been little direct experimental evidence in support of such a view, although the adaptive significance of circadian rhythms in nature has been inferred from observations of geographical variation in circadian rhythm parameters, in various species, that is correlated with differences in day length at different latitudes.

In some organisms, like fruitflies and some plants, longevity, growth and developmental rate (which are indirect measures of fitness) have been seen to be higher when individuals were maintained under environments whose periodicities were comparable to the endogenous circadian period of the organism. It was hypothesized that, in such a situation, the biological clock underlying these rhythms is in 'resonance' (no pun intended) with the environmental periodicity and this is beneficial for the physiological well being of the organism. These studies on the possible adaptive significance of circadian rhythms were all carried out on organisms with endogenous periodicities close to 24 hrs. The endogenous periodicity, also called free-running period (FRP), is the periodicity exhibited in a biological rhythm when the organism is kept in an aperiodic environment (e.g. constant light or constant darkness). However, a more rigorous way of determining the adaptive significance of circadian rhythmicity could be to estimate fitness parameters of individuals with altered circadian periodicities that differ considerably from 24 hrs.

In a recent study published in the *Proceedings of the National Academy of Sciences,* USA [1], a group of scientists from USA and Japan has investigated for the first time the relative fitness of individuals with widely differing endogenous periodicities, when reared under environmental light/dark cycles of varying periodicity. In this study, these scientists used various asexual strains of the cyanobacterium *Synechococcus* that exhibit different endogenous periodicities in their luminescence rhythm. In asexual microorganisms, the relative growth of one strain when placed in competition with another is a good measure of reproductive fitness. In this study, two of the four strains of cyanobacteria used expressed wild type endogenous periodicity (FRP=25 hrs), while the other two were mutant strains expressing free-running periodicities of 20 hrs and 30 hrs, respectively. These mutants were derived from the wild type strains by chemical mutagenesis, and carried point mutations within the *kaiC* gene which is one of a cluster of genes, *kaiABC*, that has been shown to play a crucial role in the circadian organisation of *Synechococcus*.

When the strains were grown in pure cultures, no differences in growth rate were seen among the four strains, regardless of whether they were grown in continuous light (LL), light-dark cycles (LD) 12:12 hr, LD 11:11hr, or LD 15:15hr, although the growth rate of all strains was accelerated in LL. This implies that the environmental light/dark cycle *per se,* did not differentially affect the growth rates of these strains. On the other hand, when the different strains were competed against each other in various pair-wise trials, the outcome of competition was seen to be strongly dependent on the endogenous periodicities of the competing strains, and the periodicity of the imposed LD cycle.

These competition experiments done by Ouyang and others were initiated by mixing two strains in equal proportions in a culture, and then tracking their relative proportions over time, in order to assess which strain was the superior competitor (the proportion of the superior competitor in such mixed cultures would be expected to increase over time: this is a standard technique of assessing Darwinian fitness in microorganisms). After being initiated with equal proportions of the two strains, the mixed cultures were exposed to two different LD cycles, LD 11:11hr and LD 15:15hr. When the two wild type (FRP = 25 hrs) strains were competed with each other, they maintained roughly equal proportions for many generations in both types of LD cycles. However, when wild type strains (FRP = 25 hrs) were competed with the short period mutant strain (FRP = 23 hrs), the mutant out-competed the wild type strains in the 22 hr LD (11:11hr) cycle, while the wild type strains out-competed the mutant in the 30 hr LD (15:15hr) cycle. Similarly, when the wild type strains (FRP = 25 hrs) were competed with the long period mutant (FRP = 30 hrs), the wild type strains were the superior competitors in the 22 hr LD cycle, while in the 30hr LD cycle the long period mutant out-competed the wild type strains. When the two mutants (FRP=23 hrs and FRP=30 hrs) were made to compete with each other, the short period mutant was the superior competitor in the 22 hr LD cycle, while the long period mutant out-competed the short period mutant in the 30 hr LD cycle.

These results clearly suggest that the strain whose endogenous periodicity most closely matched the environmental periodicity had a fitness advantage over others in that environment, and was thus favoured by natural selection. Moreover, when the same combinations of strains were grown in mixed cultures in LL, in all cases both strains maintained themselves in equal proportions, indicating that in the absence of a periodic cycle, neither of the two strains had any intrinsic advantage over the other. Although the physiological mechanism by which one strain out-competes the other is not as yet known, the authors speculate that it may be due to competition for limiting resources like light, nutrients and carbon dioxide. Another possible explanation for the results is that these cyanobacterial strains rhythmically secrete diffusible factors that could inhibit the growth of other strains and thus the strain which times its secretion earlier, as a result of differences in its endogenous periodicity, may be at an advantage.

The work by Ouyang and co-workers [1] can be considered as the first step towards rigorously addressing the issue of adaptive significance of circadian rhythms. There are two basic aspects of circadian rhythms, the study of which could give a clear picture of the adaptive significance of these rhythms, namely the free-running nature of these rhythms, and the phase locking to the environmental LD cycles. The outcome of the experiments described here addresses the second point quite clearly. The importance of such phase locking seems to be apparent even in nature, where we often find that different organisms have distinct temporal niches during which they carry out specific activities. The possible adaptive significance of possessing circadian periodicity *per se* is not, however, addressed by the study described here. It has been hypothesized in the past that there may be fitness benefits of possessing a biological clock that are quite independent of the phase locking aspect of circadian rhythms. Such benefits, it is thought, may accrue as a result of being able to synchronise various internal metabolic processes among themselves. Clearly many different lines of work will have to be pursued in order to obtain a full picture of how various aspects of circadian rhythms confer an adaptive advantage to the organisms possessing them; the elegant study of Ouyang and co-workers is a very welcome first step in this direction.

Suggested Reading

[1] **Y Ouyang, C R Andersson, T Kondo, S S Golden and C H Johnson. Resonating circadian clocks enhance fitness in cyanobacteria. *Proc. Natl. Acad. Sci. (USA)* 95, 8660–8664, 1998.**

[2] **M K Chandrashekaran. *Biological Clocks.* Bharatiya Vidya Bhavan, Bombay, 1985.**

[3] **M C Moore-Ede, F M Sulzman and C A Fuller. *The Clocks That Time Us.* Harvard University Press, Cambridge, MA, USA, 1982.**

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