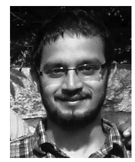
Circadian Rhythms

4. Why do Living Organisms Have Them?

Koustubh M Vaze, K L Nikhil and Vijay Kumar Sharma

Over the series of articles on circadian rhythms, we saw that living organisms exhibit daily rhythms in multiple behaviours and physiological processes. Although some rhythms may be passive responses to 24 h environmental cycles, many are regulated by endogenous-rhythm generating machinery called circadian clocks. We have introduced the concept of circadian clocks, their organization and the underlying molecular mechanisms. In this article, we will dwell upon one of the most fundamental questions pertaining to circadian clocks, 'Why do we have them?'. The aim of this article is not to provide answers, but to discuss the efforts and strategies used to address this question and critically evaluate explanations proposed till date.



(left) Koustubh Vaze is a post-doctoral researcher in the Department of Neurobiology and Genetics, University of Würzburg, Germany. Currently, he is working on the role of circadian clocks in respect of photoperiodism.

(right) Nikhil K L is pursuing his PhD at JNCASR, Bangalore. His research is on understanding the role of circadian clocks in timing adult emergence in *D. melanogaster*.

(right) Vijay Kumar Sharma is a Professor at JNCASR, Bangalore. His research interests are in understanding circadian organization of fruit flies and ants, adaptive significance of circadian clocks, neurogenetics of circadian egg-laying rhythm in fruit flies and timekeeping under natural conditions.

Introduction

The kinds of questions asked by a biologist interested in diving into and exploring the vast ocean of biology can be broadly classified into two categories: 'How?' and 'Why?'. For instance, a biologist interested in studying the peacock's tail would look into two seemingly different, yet related aspects. 'How do peacocks achieve such brilliantly coloured spots on their tails and what are the physiological mechanisms underlying it?' and 'Why did peacocks evolve such brilliantly coloured tails in the first place? Does such ornamentation[1] serve any purpose? What are the fitness[2] consequences of evolving such ornamentation?' and so on. Such 'how' questions which are directed towards dissect-

[1] Ornamentation or biological ornaments are exaggerated traits observed in animals which mostly serve decorative purpose and is used to attract member of the opposite sex. Ornaments are believed to have evolved as a consequence of sexual selection and are used by mates as a proxy to assess the quality of individual harbouring it before deciding to mate with it.

ing the mechanistic basis of phenomena are termed 'proximate'. The 'why?' questions that arise out of curiosity to explore and explain the reason behind the presence or evolution of a behavioural/physiological trait, are termed 'ultimate'. In one of our earlier articles, we discussed several aspects of the molecular basis of circadian rhythms[3] and thus were in the domain of 'proximate' questions. However, in this article we will address the 'ultimate' question pertaining to chronobiology and more specifically circadian rhythms: 'Why do living organisms exhibit circadian rhythms?'

[2] Fitness is a measure of the ability of an individual to survive and reproduce so as to contribute to the gene pool of the next generation. In other words, the more progeny an individual has, the more its contribution to the next generation's gene pool and thus, the more fitter it is believed to be. Some of the commonly used measures of fitness (also called fitness parameters) include lifespan/survivorship, fecundity, stress resistance, growth rate, and pre-adult development time.

[3] See *Resonance*, Vol. 18, No. 9, 2013.

Keywords
Adaptation, fitness, circadian resonance, latitudinal clines, experimental evolution.

Are Circadian Rhythms an Adaptation?

Darwin's theory of natural selection (*Box* 1) provided a powerful explanation for the evolution of biological traits and is now universally regarded as the primary process of biological evolution. According to Darwin's theory of evolution, the process of natural selection leads to adaptation (*Box* 1). To demonstrate that a given trait is adaptive, one must gather evidence (a) in favour of

Box 1. Adaptation from Darwinian Perspective

We started this article with a question – "Why do living organisms have circadian clocks?". Although we are talking about circadian clocks, the same question can be asked about any trait. Questions like this have been addressed since ancient times and naturally, some perceived purpose or a function has been invoked to explain the existence of a trait. Consequently, one might propose that a trait X exists in an organism because it is functionally important to its bearer. Such propositions are known as 'adaptations'. In the pre-Darwin era, such useful traits had been taken as evidence to suggest the presence of an intelligent designer/supernatural power that creates organisms and their peculiarities. In stark contrast to the previous explanations, Darwin proposed that traits come into being through a long and gradual, but a natural/realistic process. In his book *On the Origin of Species* (1859), Darwin went on to propose his theory of evolution which hypothesised the basic nature of this process.

The theory of natural selection rests on two premises: (1) biological traits exhibit variation, and (2) this variation is heritable. Darwin proposed that individuals with variations which are *better suited to the environment they inhabit* manage to survive, reproduce and in turn leave more offspring (and thus more contribution to the gene pool) to the next generation than those with less suitable or harmful variations. He referred to this process of selective enrichment of advantageous variation as natural selection. Therefore, a trait which comes into being through a process of natural selection under a given environment suggests its suitability to that environment and is referred to as an adaptation.

influence of the trait on reproductive fitness and (b) for the action of natural selection on the trait. The fact that the circadian clock period also called as the free running period (τ), matches closely with that of the daily cycles of environmental variables such as light, temperature and humidity hints towards the possibility of evolution of circadian rhythms in relation to the environment[4] [1]. Thus, if circadian rhythms are an adaptation to the cyclic environment that organisms have evolved in, then they are expected to influence the fitness of organisms and also evolve in response to selection pressures.

[4] See *Resonance,* Vol. 18, No. 7, 2013.

Early studies on circadian rhythms focussed on unravelling the fundamental properties and functional principles of circadian clocks which was followed by a period of exploration of its multi-oscillatory organization[5]. As part of the effort to explore the adaptive value of circadian clocks and drawing knowledge from our understanding of its properties and underlying organization, circadian clocks are thought to confer adaptive advantage to living organisms in two ways [1]:

[5] Multi-oscillatory organization of circadian clocks indicates that the circadian rhythms are not governed by a single clock/oscillator but comprises a network of interacting oscillators as revealed by many studies. Initially, the network was considered to be hierarchical in nature (comprising a master oscillator that drives other slave oscillators) but recent evidence suggests that there exist two way interactions between oscillators.

Extrinsic Advantage

Circadian rhythms entrain to cycles of environmental variables and assume a stable phase-relationship with environmental cycles[4]. As a result, behaviours and physiological processes under the control of circadian clocks occur at specific times of the day. From the perspective of the ecology of organisms, appropriate timing of biological functions would enhance the organism's chances of survival (by avoiding harsh conditions), procuring more mates, finding more food and so on. For example, probability of successful mating can be increased by synchronizing activity schedules of conspecifics and the chances of being preyed upon can be reduced by scheduling activity at a time when predators are asleep. Thus, circadian clocks are believed to confer fitness advantage to organisms by scheduling various essential biological functions at favourable times of the day (dependent on extrinsic environmental factors).

Synchronization of internal rhythms is an essential aspect of physiology, and circadian rhythms benefit living beings by bringing about such temporal order.

Intrinsic Advantage

Individuals exhibit circadian rhythms in multiple biological variables such as sleep/wake, hormone concentration, immune response, metabolic enzymes [1]. Such rhythms are thought to be governed by a network of oscillators interacting with each other and the observed stable temporal relationship (fixed time difference) amongst themselves is believed to be functionally important. For instance, let us consider two biological phenomena such as sleep and metabolic processes involved in oxidation of food, both of which exhibit circadian rhythmicity. We all sleep at night and feed during day. What if both these cycles (sleeping and feeding) occurred in phase or at the same time? The body reaches its lowest temperature at night when we are asleep, the blood pressure drops and the rate of metabolic reactions is low. Therefore, it would be physiologically incompatible if we experienced hunger and sleep at the same time as the body would not be in the right state to metabolise food efficiently. Thus, synchronization of internal rhythms is an essential aspect of physiology, and circadian rhythms benefit living beings by bringing about such temporal order. In other words, circadian rhythms are thought to benefit organisms by coordinating their internal metabolic processes.

Having discussed how clocks can be advantageous to organisms, we will explore various studies performed to test these hypotheses and to discuss the merits and drawbacks associated with such studies.

Empirical Approaches Used to Test the Adaptive Nature of Circadian Rhythms

Manipulation of Circadian Phenotype[6] or Cyclic Environment

[6] Circadian phenotype refers to the phenotype of an organism in terms of behaviour and physiological variables regulated by circadian clocks.

The manipulation of circadian phenotype or cyclic environment has been the strategy in several studies performed to test the adaptive value of circadian rhythms. Typically, such studies have involved testing the effect of the manipulation of the circadian phenotype or the rhythmic environment on the measurable components of fitness such as lifespan, reproductive output, etc.

Circadian Resonance: If circadian rhythms are an adaptation to 24 h cycles of environmental variables on Earth, when subjected to environmental cycles with periodicities deviating from 24 h, the circadian systems may not confer the expected benefits. In other words, circadian clocks confer maximum advantage to bearers when entrained to environmental cycles whose periodicities match (resonate) with the clock period. This hypothesis is more commonly known as 'circadian resonance' [1].

Consequences of circadian resonance have been tested in several model organisms by studying the effects of rearing wild-type strains (τ close to 24 h) under LD cycles of a wide range of periodicities (16 h to 32 h) on various fitness parameters such as growth rate, body mass and longevity. Evidence from such studies suggests that organisms with τ close to 24 h exhibit higher reproductive fitness under LD cycles of 24 h period as compared to non-24 h LD cycles. In addition to wild-type strains, mutant short ($\tau < 24$ h) and long ($\tau > 24$ h) period strains have been used to comprehensively test circadian resonance. One such study on the photosynthetic cyanobacteria (*Synechococcus sp.*) (*Box* 2) has provided one of the most convincing evidence so far in support of the circadian resonance hypothesis.

The study involved the use of four cyanobacteria strains – short-period (SP22; $\tau = 23$ h) and long-period (P28; $\tau = 30$ h) strains that were isolated through chemical mutagenesis screens[3] and a wild-type (WT; $\tau = 25$ h) strain. These strains were subjected to pair-wise competition assays where batches of any two strains were mixed in certain proportions and each batch was maintained in short-day (LD11:11 h) or long-day (LD15:15 h) light regimes following which the growth rate of each strain in the mixed cultures was assessed. Based on the circadian resonance hypothesis, the short-period (SP22) strain should have higher fitness under LD11:11 h and the long-period (P28) strain under LD15:15 h. It was observed that the P22 strain indeed had higher fitness and outperformed the other two strains under LD11:11 h

Box 2. Circadian Rhythms in Cyanobacteria

Several strains of nitrogen-fixing cyanobacteria have been reported to exhibit circadian rhythms. Photosynthesis during the light phase releases oxygen from water, but the nitrogenase enzyme (required for nitrogen fixation) harboured by the bacteria is sensitive to oxygen. Thus, cyanobacteria have evolved mechanisms to isolate the nitrogenase enzyme from oxygen. The photosynthetic cycle and the nitrogen fixation cycle are temporally segregated such that the vegetative cells produce oxygen through photosynthesis when nitrogen fixation is shut down while the photosynthesis system is turned off when heterocysts (nitrogen fixing cells) express the nitrogenase enzyme to carry out nitrogen fixation. This temporal segregation is essential for the cell and is believed to be governed by circadian clocks. Rhythms of nitrogenase activity and amino acid uptake have also been reported in several strains of cyanobacteria and also, the period of these rhythms are temperature compensated, and the rhythms are light sensitive. Due to the relative simplicity of prokaryotic organization over eukaryotes, circadian rhythms in cyanobacteria has drawn attention of many chronobiologists interested in the mechanisms of circadian clocks. A recent study on molecular clocks in cyanobacteria has revealed an interesting property of its clocks. The study reported that circadian oscillations in the three core clock proteins (KaiA, KaiB and KaiC) in cyanobacteria can be set up in a test tube mixture comprising the three proteins and ATP (Adenosine-5'-triphosphate) suggesting that post-translational events alone can sustain circadian rhythms and do not require earlier events.

but not in LD15:15 h (*Figure* 1) and *vice versa* for the P28 strain (*Figure* 1) thus indicating that an organism would be maximally fit under cyclic environment whose period matches with that of its own. This study provided strong evidence in support of the circadian resonance hypothesis which was later shown to be valid in other organisms as well.

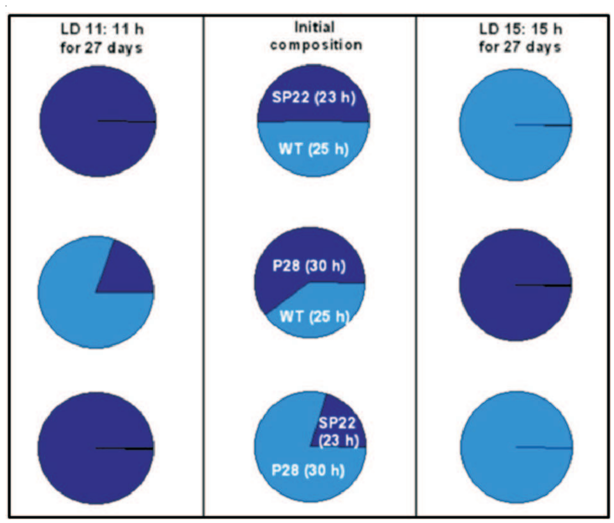


Figure 1. The picture depicts the strain composition following mixing of strains and rearing them under different LD regimes. Short-period (SPP22) strains outperformed both wild-type (WT) and long-period (P28) strains under LD 11:11 h, while the P28 strains dominated over other strains under LD 15:15 regime and this was independent of initial composition ratios of the strains. (Figure modified after [2].)

The other strategy employed to test the adaptive significance of circadian rhythms stems from the idea that if circadian rhythms are beneficial to organisms, then lack of such rhythms should be disadvantageous (would result in reduced fitness). But how does one render an organism arrhythmic? Circadian rhythms can be abolished by ablation of the cells/tissues comprising the clocks [1]. Also, several mutants that exhibit arrhythmic phenotypes[3] have been isolated and have proved to be extremely useful in circadian rhythm research.

In one such study, loss of function mutant strains of the core clock genes *period* (*per*^{0}), *timeless* (*tim*^{0}) and *cycle* (*cyc*^{0}) in fruit flies *Drosophila melanogaster* lacking activity/rest rhythm[3] were used and fitness of these arrhythmic mutants was assessed under laboratory LD cycles. It was observed that the reproductive output of mutant males and females (estimated by measuring sperm counts and egg-output respectively) was lower than that of wild-type strain. However, even though the results would tempt the reader to believe that circadian clocks may influence the fitness of organisms, it is equally likely that the reduction in reproductive output observed in the clock mutants is a pleiotropic effect[7] of the mutation in the genes, independent of the clock or the oscillatory phenomena governed by it. Also, since most mutants are inbred[8], the observed reduction in reproductive output may be a consequence of inbreeding depression thus posing counter-arguments against the interpretation of the role of circadian clocks in the regulation of reproductive output.

Another study tested the consequences of lack of circadian clocks under natural conditions in chipmunks *Tamias striatus*. In this study, chipmunks were rendered arrhythmic by surgical removal (ablation) of the master clock Suprachiasmatic Nuclei (SCN)[9], and were released into a semi-natural desert enclosure (the chipmunks were released into an enclosed arena of their natural habitat, but were confined to an area within which they could move around).

Interestingly, arrhythmic animals lacking SCN showed reduced

[7] A gene is believed to be pleiotropic when it influences multiple traits. Consequently, a mutation in the gene would influence all the traits governed by it. One of the common examples of pleiotropy in humans is Phenylketonuria (PKU) caused by deficiency of the enzyme phenylalanine hydroxylase. A mutation in the gene for this enzyme manifests in multiple phenotypes in addition to PKU and includes eczema, mental retardation, and pigment loss.

[8] Inbreeding occurs when two closely (genetically) related individuals mate and produce offsprings. The progeny of such a cross exhibit increased homozygosity for various recessive (and deleterious) loci thus leading to reduced fitness resulting in 'inbreeding depression'.

[9] The suprachiasmatic nucleus is a densely packed region of around 20,000 neurons located bilaterally beneath the hypothalamus and above the optic chiasm. SCN receives direct light inputs from the retinal photoreceptors through the Retinohypothalamic tract (RHT) and is rich in various neurotransmitters. Ablation of the SCN renders the organism arrhythmic and thus is considered the central/master circadian clock that governs circadian rhythms in mammals.

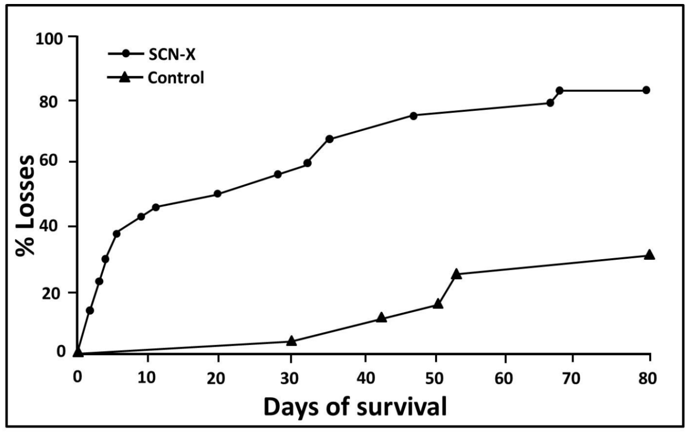


Figure 2. Mortality of chipmunks with ablated supra-chiasmatic nucleus (SCN-X) and the respective controls. The survivability of the SCN-X chipmunks was considerably lower than that of the controls with close to 50 % mortality within the first 10 days following release into the semi-natural enclosure indicating that the intact SCN and therefore a function circadian clock is ecologically relevant for the survivability of the organisms. (Figure modified after [3].)

survival than their rhythmic counterparts (*Figure* 2). Once again, it can be argued that the reduced survival in the arrhythmic animals is a consequence of SCN ablation on the general health of the animals (any sort of physical ablation of a body organ does have consequence on the physiology of the organism). However, careful analysis revealed that deaths of most arrhythmic individuals were due to predator attacks and not due to compromised physiology. Thus it was hypothesised that clocks conferred extrinsic advantage by helping the chipmunks schedule their activity at an appropriate time of the day so as to avoid being attacked by predators.

Correlation Between Clock Properties and Environmental Variables

As you might have observed, dogs (and other animals) adapted to high altitude or cold regions generally have more fur cover than the same breeds in lower altitudes or warmer regions. This is because they have evolved and adapted to the local environmental conditions. Similarly, it is reasonable to assume that if circadian rhythms are adaptations to rhythmic environmental pressures, then properties (discussed later) of circadian timing systems should also vary with the local environmental conditions. Several studies have investigated signatures of such local adaptations by looking at how clock properties vary as a function of geographical location (and thus local environment).

Arrhythmic animals lacking SCN showed reduced survival than their rhythmic counterparts.

***Latitudinal Clines in Clock Properties*:** The shape of the earth and the axial tilt has a consequence for the day length at any given place on the earth and at any given day of the year. Therefore, even though almost all places on the earth (except polar regions) experience light/dark cycles throughout the year, day length at any given time of the year varies with latitude. This latitude-dependent variation in day length in turn has consequences for the properties of daily cycles of temperature and humidity, which together influence the quality and strength of rhythmic selection pressures.

A small stretch of the core clock gene *period* in fruit flies *D. melanogaster* codes for the Threonine-Glycine (Thr-Gly) di-peptide repeat. Natural populations are found to be polymorphic for these repeat number alleles and the two Thr-Gly_{17} and Thr-Gly_{20} alleles which code for 17 and 20 di-peptide repeats respectively constitute about 90% of the Thr-Gly repeat number variation in the European populations. A survey of the European fruit fly populations collected from locations spanning latitudes 30°N to 55°N showed a systematic change in allelic composition with latitude. The frequency of the Thr-Gly_{17} allele showed a gradual decrease from south to north whereas Thr-Gly_{20} allele showed an increasing south to north trend (*Figure* 3). The correlation between Thr-Gly allele frequency and latitude indicates that the phenotypic effect of Thr-Gly repeat number is under natural

The correlation between Thr-Gly allele frequency and latitude indicates that the phenotypic effect of Thr-Gly repeat number is under natural selection. Further investigations to understand the functional significance of Thr-Gly repeat alleles showed that the repeat number influences the temperature-compensation ability of circadian clocks.

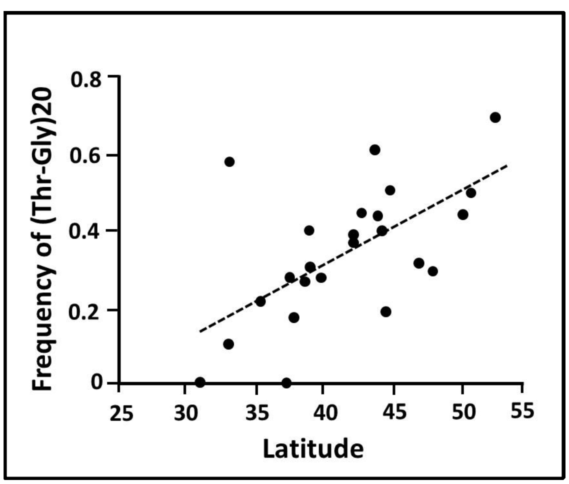


Figure 3. Frequency of (Thr-$Gly)_{20}$ repeat length alleles in *Drosophila melanogaster period* gene in natural populations across latitudes in Europe. (Figure modified after [3].)

selection. Further investigations to understand the functional significance of Thr-Gly repeat alleles showed that the repeat number influences the temperature-compensation ability of circadian clocks [1]. Latitudinal variation in Thr-Gly repeat number alleles is thus thought to be the result of selection acting on the thermal stability of circadian clocks strongly suggesting its adaptive value. There have been multiple such latitudinal cline studies along similar lines and will not be discussed in detail here. However, such latitudinal correlations have been observed with respect to clock properties such as period and phase-relationship of adult emergence rhythms in *Drosophila* sp. in Europe, amplitude and phase response curves in Japan. Furthermore, correlations between clock period and day length has been observed in *Arabidopsis thaliana*. These studies have been discussed extensively elsewhere [1, 3].

Circadian Clocks Under Constant and Aperiodic Environments: If circadian rhythms are adaptation to rhythmic environment, then these rhythms will not have any significance to organisms inhabiting constant environments. Multiple studies were directed towards studying clock properties in organisms inhabiting areas such as deep sea vents and underground water caves which are devoid of any cyclic light or temperature cycles.

Studies on the circadian rhythms of many behaviours and physiological variables in underground cave-dwelling fish populations have reported (1) loss of circadian rhythmicity in one or more behaviours and physiological variables, (2) persistence of circadian rhythms with greater period variation and (3) circadian rhythms refractory to LD cycles due to degenerated photoreceptors. Increased variation or complete loss in functionally important properties of circadian rhythms in cave-dwelling populations of fish can be attributed to weakening or absence of rhythmic selection pressures thus leading to its regression. Such observations also indicate that these cave-dwelling fish populations evolved from ancestors who probably inhabited the upper regions of the water bodies where they were exposed to cyclic environment and had evolved functional circadian clocks. Regressive evolution of circadian rhythms in constant environments thus supports the notion of adaptive value of circadian rhythms.

Studies on similar lines were also performed on populations of *Drosophila* sp. maintained (and thus evolved) in the laboratory under constant light, temperature and humidity conditions for over 600 generations and yet were found to exhibit robust circadian rhythms in adult emergence (eclosion), locomotor activity and oviposition (egg-laying). Persistence of circadian rhythms (in both the cases discussed above) in spite of evolution under constant conditions for several generations can be interpreted in multiple ways. Considering only the extrinsic advantage hypothesis, it can be argued that given the enormously long evolutionary time scales, such rhythms are in the process of regression and would require many more decades to be completely eliminated from the populations. Or, such rhythms possibly continue to persist in the organisms due to the intrinsic advantage they confer. Either ways, the evidence is suggestive of the adaptive value of circadian clocks.

Observing Evolution of Clocks in Real Time: In the previous section, we speculated that the observed correlations/trend may be an adaptation to local environmental conditions. But do clocks really evolve in response to selection pressures? Is it possible to simulate evolution in a controlled laboratory set-up? If so, then one can easily subject organisms to such simulated conditions and assess the evolution of clock properties over generations which would provide more convincing evidence to support the notion that circadian clocks evolve in response to selection pressures. Fortunately, studying evolution in real time is no more a sci-fi movie script and is achievable through a technique called 'laboratory selection' (refer *Box* 3).

Let us assume that you want to look at response to selection for larger body size in a population of *D. melanogaster*. On calculating the body size of all individuals, you will observe a distribution of body sizes with a large proportion of individuals having

Box 3. Laboratory Selection

Laboratory selection studies are basically simulation of evolutionary change in a trait through the process of natural selection, on laboratory populations under controlled laboratory conditions. In laboratory selection studies, populations harbouring substantial variation for a trait of interest are subjected to selection, which is imposed on every generation by choosing individuals exhibiting a particular range of trait values. In a typical laboratory selection study, two sets of replicate populations are monitored for trait(s) of interest over several generations. One set of populations is subjected to selection whereas the other set is maintained in an identical manner but without selection to serve as control populations. Comparison of a generation-wise change in the trait value between the selected and control populations allows testing for the effects of imposed selection on the trait evolution. Although one cannot recreate all the causal factors in nature which lead to the evolution of a trait, in its current state; evolution of a trait in experimental populations in the laboratory or field in response to relevant selection pressures imposed by the experimenter provides strong evidence for its adaptive value.

some body size around the mean of the distribution while very few individuals make up the extremes (*Figure* 4a). Based on this, you set a cut-off value of body size (indicated by *dashed line* in *Figure* 4b) and individuals above the cut-off value will be selected and bred, thus simulating an environment where large individuals are favoured. By accepting the larger individuals and rejecting the smaller ones, you are filtering out smaller individuals from the population similar to the traits/individuals being filtered through natural selection (*Box* 1). While these populations serve as 'experimental' populations, you will have to maintain control populations with no such selection imposed. If this process is continued

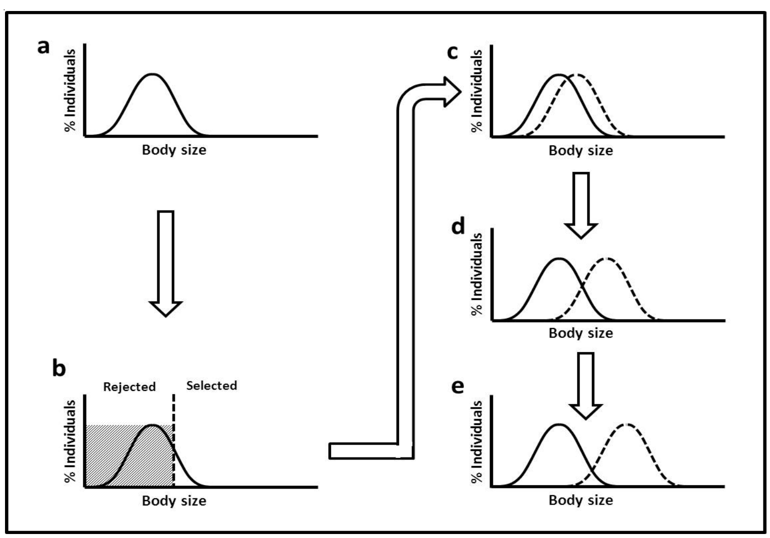


Figure 4. Pictorial representation of how trait values change in response to laboratory selection. Upon obtaining a distribution of trait value (say body size) in a population (a), a cut-off trait value should be decided so as to know which individuals to be selected and which ones to be rejected as represented by the highlighted region in (b). (In this case, large body size individuals are favored and small individuals rejected.) In every generation, the individuals falling in the selection region of (b) will be selected and the others rejected. If this process is continued over several generations, the distribution of trait values moves away from the rejection region towards the selection region.

over several generations, you will observe that while the distribution of body size in the 'control' populations remains unchanged, gradually the distribution is displaced towards the right indicating that the proportion of large body size individuals is increasing in the population as a response to selection (*Figure* 4c–e). In other words, genes/alleles governing body size are being selected and the frequency of those genes/alleles increases over time.

Several studies have used this approach to study how clock properties evolve in response to environmental selection pressures. Several insects exhibit diurnal rhythm in adult emergence, and in *D. melanogaster* the peak of emergence lies around dawn [1]. A peak at dawn is believed to have evolved in response to selection acting on the timing of emergence (timed by circadian clock) as emerging early in the morning could increase the chances of survival (reduced predation and desiccation risk due to low temperature and high humidity). A laboratory selection study for timing of adult emergence was carried out in which the authors selected flies emerging early in the morning ('early') and late in the evening ('late'). As a response to selection, 'early' populations evolved increased morning emergence and 'late' populations, an increased evening emergence. Also, the 'early' and 'late' populations evolved a shorter and longer τ respectively, thus indicating that selection in Nature may act on the timing of behaviours (governed by clocks) and results in evolution of underlying circadian clocks as well as renders support for the extrinsic advantage hypothesis.

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In another laboratory selection experiment on melon fly *Bactrocera cucurbitae,* populations were selected for faster or slower pre-adult development (the time duration from egg laying to adult emergence from pupae). Consequently, the faster and slower developing populations evolved shorter and longer clock periods (τ) respectively. A similar study on populations of *D. melanogaster* selected faster developing flies and reported correlated change in τ (shorter by 0.5 h) of the faster developing flies. Both these studies indicate that apart from daily rhythms in adults, circadian clocks also mediate developmental rates (probably by governing rhythms in various growth hormones). In yet another selection study on *Bactrocera cucurbitae,* populations were selected for earlier age reproduction ('young' lines) and old age reproduction ('old' lines). It was observed that the 'old age lines' lived longer, exhibited longer τ, and mated later in the day as compared to the young lines indicating that the influence of circadian clocks spans a plethora of behaviours and processes across the lifetime of an individual thus highlighting its importance. Observing evolution of clocks in response to various selection pressures under controlled laboratory set-up indicates that a similar process might be at work in Nature.

Concluding Remarks

In the preceding sections, we tried to introduce the concept of adaptation and various strategies used and studies performed on different organisms, all trying to decipher one common riddle, "Why do we have circadian clocks?" and convince you that establishing that any given trait is actually an adaptation is far more tedious than merely drawing speculations about its adaptive value (as is the case with circadian clocks). To summarise our understanding from the studies discussed above:

1. Loss of circadian clocks is deleterious to organisms and results in reduced reproductive output and lifespan under controlled laboratory conditions and reduced survivability in Nature.
2. Clocks help organisms perform better under conditions when endogenous clocks resonate with environmental cycles thus suggesting a possible direct effect of daily cycles on the physiology of organisms.
3. Latitudinal clines in clock properties suggest a possible local adaptation to best suit the environment that the organisms inhabit.
4. In addition, persistence of rhythms in organisms inhabiting aperiodic environments is suggestive of the physiological importance of circadian clocks to help time various internal/ metabolic cycles.

However, in all these studies the adaptive value of clocks has been inferred on the basis of suggestive evidence and not through direct evidence for the evolution that could be tracked in real time.

5. Laboratory selection studies provided concrete evidence to indicate that circadian clocks indeed evolve in response to selection pressures acting on various aspects of clock-governed behaviours.

Taken together, these studies leave us in a position to claim that circadian clocks do have adaptive value and thus almost all organisms on earth exhibit circadian rhythms in one way or the other in accordance with their environment.

Previous articles:
1. Koustubh M Vaze and Vijay Kumar Sharma, 'From Daily Rhythms to Biological Clocks', *Resonance,* Vol.18, No.7, pp.662–672, 2013.
2. Nikhil K L and Vijay Kumar Sharma, 'The Underlying Molecular Mechanisms', *Resonance,* Vol.18, No.9, pp.832–844, 2013.
3. Koustubh M Vaze, Nikhil K L and Vijay Kumar Sharma, 'Why do Living Organisms have Them', *Resonance,* Vol.18, No.11, pp.1032–1050, 2013.

Suggested Reading

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Address for Correspondence
Vijay Kumar Sharma
Chronobiology Laboratory
Evolutionary and Organismal Biology Unit
Jawaharlal Nehru Centre for Advanced Scientific Research
Jakkur, PO Box 6436,
Bangalore 560 064, India.
Email:vsharma@jncasr.ac.in