Circadian rhythms are believed to be an evolutionary adaptation to daily environmental cycles resulting from Earth’s rotation about its axis. A trait evolved through a process of natural selection is considered as adaptation; therefore, rigorous demonstration of adaptation requires evidence suggesting evolution of a trait by natural selection. Like any other adaptive trait, circadian rhythms are believed to be advantageous to living beings through some perceived function. Circadian rhythms are thought to confer advantage to their owners through scheduling of biological functions at appropriate time of daily environmental cycle (extrinsic advantage), coordination of internal physiology (intrinsic advantage), and through their role in responses to seasonal changes. So far, the adaptive value of circadian rhythms has been tested in several studies and evidence indeed suggests that they confer advantage to their owners. In this review, we have discussed the background for development of the framework currently used to test the hypothesis of adaptive significance of circadian rhythms. Critical examination of evidence reveals that there are several lacunae in our understanding of circadian rhythms as adaptation. Although it is well known that demonstrating a given trait as adaptation (or setting the necessary criteria) is not a trivial task, here we recommend some of the basic criteria and suggest the nature of evidence required to comprehensively understand circadian rhythms as adaptation. Thus, we hope to create some awareness that may benefit future studies in this direction. (Author correspondence: vsharma@jncasr.ac.in or vksharmas@gmail.com)

Keywords: Adaptation, Circadian resonance, Circadian rhythms, Fitness, Natural selection

INTRODUCTION

Organisms ranging from bacteria to mammals exhibit daily rhythms in various behaviors and physiological processes (DeCoursey, 2004). Many of these rhythms persist under constant environmental conditions with near-24-h period, which implies that they are not merely passive responses to daily cyclic changes in light, temperature, and other environmental variables, but are regulated by endogenous rhythm generating systems. Due to their near-24-h period under constant conditions, these endogenous rhythms are called circadian (for about-a-day in Latin) rhythms and the underlying mechanisms, circadian clocks (Johnson et al., 2003). Circadian clocks synchronize and attain stable phase relationships with daily environmental cycles through a process called “entrainment,” using precise and predictable cyclic environmental variables such as light and temperature, known as “zeitgebers” (for time givers in German) (Johnson et al., 2003).

For several promising reasons that will be discussed in subsequent sections of this review, circadian rhythms have been perceived to be of great functional significance to living beings under daily environmental cycles, and consequently it has been a common belief that these rhythms are evolutionary adaptation to earthly rhythmic environment. So far, a large numbers of studies have been conducted in organisms spanning bacteria to mammals to test if circadian rhythms are evolutionary adaptation (reviewed in Johnson, 2005; Paranjpe & Sharma, 2005; Sharma, 2003; Yerushalmi & Green, 2009). It is the primary aim of this review to critically examine available evidence from such studies and comment upon the most parsimonious inference regarding the adaptive value of circadian rhythms. As a part of this exercise, we will first discuss what “adaptation” is, and what can be used as evidence for adaptation. We will then discuss the development of adaptive hypotheses about circadian rhythms. Subsequently, we will examine the available evidence in the light of criteria for adaptation and comment upon our current state of understanding about circadian rhythms as adaptation. At the end, we recommend experiments that we feel are essential to rigorously test the adaptive value of circadian rhythms, hoping that they may serve as directions for future studies.
ADAPTATION

Literally, the word “adaptation” refers to suitability of one thing over another. In the biological context, adaptation refers to suitability of peculiarities of living organisms (such as behavior, morphology, and physiology) to its biotic/abiotic environment. Adaptation could be categorized into two types: (1) physiological adaptation—for example, adaptation of human eye to light intensity is a change that occurs within a lifetime of organism; and (2) evolutionary adaptation—which occurs at the level of population over several generations through change in genetic architecture under the influence of natural selection. In this review, we will primarily focus on evolutionary adaptation and not physiological adaptation. Although definition of adaptation discussed above implies “a state of being adapted,” it is a common practice in evolutionary biology to use the word adaptation to refer to evolutionary processes that lead to the state of being adapted.

It is an ancient idea that living organisms are adaptation to their environments and its origin can be traced back to teleological explanations dominant since the days of Aristotle, which advocated that purpose or function underlies peculiarities of living beings and adaptations were assumed to be a work of “divine powers” or “creator” (Amundson, 1996). Darwin’s theory of evolution by natural selection (Darwin, 1859) provided the first fully naturalistic explanation for how biological adaptations could come into existence and is now universally regarded as the primary process underlying apparent adaptation of living beings to their environment (Amundson, 1996). Darwin’s theory about the process of adaptation rests on two premises: that organismal design and hence its peculiarities (traits) exhibit variation, and that this variation is heritable. In the struggle for survival in a world with limited resources, individuals with favorable (suited to environmental conditions) heritable variations manage to survive, reproduce, and hence leave more offspring to the next generation than those with less favorable heritable variations. Darwin referred to this filtering of trait variation through the process of performance-based differential reproductive success (fitness) as “natural selection.” Therefore, in principle, a trait evolved by a process of natural selection under a given set of environmental conditions suggests its suitability under that environment and therefore can be referred to as an adaptation. Therefore, to call any trait as adaptation or to test its adaptive value, evidence suggesting evolution of a trait by a process of natural selection is sought. Since adaptive traits are those that are retained by natural selection by virtue of their higher fitness compared with other variants, they are, by default, expected to enhance fitness of their owners through some perceived function. Therefore, the demonstration of adaptive value of a trait involves collecting evidence which suggest its proposed functional significance. There are several empirical methods that provide such evidence for adaptation and they will be elaborated in this review while discussing appropriate evidence for the adaptive value of circadian rhythms.

FUNCTIONAL SIGNIFICANCE OF CIRCADIAN RHYTHMS:
DEVELOPMENT OF THE IDEA

Although theory of evolution by natural selection provides a general framework for testing adaptive significance of any given trait, the nature of adaptive hypothesis is largely dependent on perceived functional significance of the trait. Circadian rhythms, too, are believed to be useful to living beings in multiple ways. The idea of usefulness of circadian rhythms to their owners has been evolving continuously with growth in our understanding of circadian rhythms as an organization and their functions. Therefore, we feel it is a prerequisite to overview the development of this idea to fully appreciate the propositions of the adaptive value of circadian rhythms.

Multioscillatory Organization of Circadian Clocks and Extrinsic Advantage

Continuously changing light intensity, temperature, and humidity in a rhythmic manner with a period of 24-h is a fundamental feature of abiotic environment on the earth’s surface. Such cyclic variation in the levels of abiotic variables makes each cycle a complex mosaic of favorable and unfavorable phases. Therefore, it is quite intuitive to perceive potential advantages of diurnal rhythms for the survival of organisms under natural conditions. The earliest reported notion of adaptive advantage of diurnal rhythms could be traced back to Charles Darwin’s views about his observations on 24-h rhythms of plant leaf movements (Darwin & Darwin, 1980). In his book The Power of Movement in Plants, Darwin proposed an adaptive role for the 24-h rhythm in leaf movements (Darwin & Darwin, 1980). Darwin observed patterns in upward or downward movement of leaves at night in many species of plants. With the knowledge of the detrimental effects of radiation frost occurring during the night on the dorsal surface of leaves, he reasoned that rhythmic leaf movements are adaptation to challenges posed by rhythmic occurrence of radiation frost (Darwin & Darwin, 1880). Since Darwin, a large number of studies on several organisms have claimed an adaptive role for diurnal rhythms in many biological processes by conjecturing their direct or indirect relation with survival under rhythmic abiotic environment (Aschoff, 1964; Cloudsley-Thompson, 1960). Diurnal rhythms are also thought to be advantageous under 24-h cycles of biotic environment, as it helps in achieving appropriate phase relationship of behavioral/physiological rhythms with members of the same or other species for a variety of reasons, such as (1) greater chances of finding mates by synchronizing activity schedules with conspecific individuals, (2) avoiding predators, and (3) scheduling activity based on food availability (Cloudsley-Thompson, 1960).
Recognition of potential advantages of daily rhythms appears to be a source of the notion that endogenous rhythms in behavioral and physiological processes are evolutionary adaptation to rhythmic environment on earth. Unfolding of several features of endogenous near-24-h rhythms suggesting their usefulness as reliable timekeepers made this perception grow stronger. Some elegant experiments on bee and bird navigation had implicated the involvement of endogenous timekeeping systems, which were subsequently shown to be based on endogenous circadian oscillators. Experiments by von Frisch on honeybees suggested the involvement of endogenous system capable of measuring passage of time in suncompass-mediated navigation (von Frisch, 1950). Around the same time, Gustav Kramer and others reached a similar conclusion based on their studies on the use of endogenous clocks by starlings to navigate during seasonal migration. In addition, these studies succeeded in providing convincing evidence to suggest that navigational clock and circadian oscillations were indeed the same (Hoffmann, 1953, 1954; Kramer, 1950). These experiments demonstrated the use of endogenous rhythms by bees and birds to estimate passage of time, just as we use our timers. Subsequent studies on endogenous circadian oscillators or “clocks” aimed at understanding the nature of these rhythms revealed their close resemblance to the functioning of physical oscillators (Pittendrigh, 1958). Apparent temperature refractoriness of the period of these biological oscillations (temperature compensation; Pittendrigh, 1954) and their ability to synchronize and attain stable phase relationships (ability to entrain) with 24-h cycles of earthly environmental zeitgebers (time cues) such as light and temperature (Pittendrigh, 1960) were recognized to be of clear functional significance for them to act as reliable timekeepers in scheduling biological processes at fixed times of the day (Pittendrigh, 1961). Occurrence of innate, endogenous, temperature-compensated, entrainable circadian oscillators in a wide range of organisms (from protists to mammals through plants, insects, and birds) was taken as evidence for the central importance of circadian rhythms in coping with challenges posed by daily environmental cycles, leading to the perception that circadian clocks are the fundamental adaptive property of life on earth (Pittendrigh, 1960). Thus, the belief that circadian clocks confer adaptive advantage to living beings by timing their biological functions at favorable phases, and/or by avoiding certain behavioral functions at unfavorable phases, appears to be a natural consequence of their recognition as clocks or as a program capable of scheduling biological functions.

Timing systems consisting of hourglass-like mechanisms, also, in principle, should be able to do the job of scheduling biological functions at favorable times of the day, thereby conferring advantages just as oscillator-based circadian systems would do (Aschoff, 1964). Such systems could schedule biological functions by estimating time using nonoscillatory phenomena such as accumulation or degradation of a substance whose threshold concentrations may trigger biological processes (Rensing, 2002). Being inherently nonperiodic, they are thought to be dependent on some environmental triggers such as “dawn” or “dusk” to initiate time measurement. However, timing systems based on endogenous, self-sustained oscillations with period approximately equal to that of environmental cycles are thought to be superior to hourglasses in at least two respects: (1) they allow organisms to track time of the day without being totally reliant on environmental time cues in every cycle, which at times organisms may fail to detect due to environmental noise, for example, cloud cover or animal’s own behavior such as sleep and postures; and (2) organisms may respond more efficiently to environmental challenges by being prepared in anticipation of environmental changes. Therefore, oscillator-based systems are likely to be more foolproof than hourglass-like mechanisms (Aschoff, 1964).

In essence, perceived significance of circadian rhythms for organisms in tracking time of the day appears to be the central theme behind the extrinsic advantage hypothesis. According to the extrinsic advantage hypothesis, circadian rhythms confer adaptive advantage to living beings by programming their biological functions at favorable times of the day, thereby helping them respond efficiently to daily challenges.

Multioscillatory Organization of Circadian Clocks and Intrinsic Advantage

The potential benefits of circadian rhythms through appropriate scheduling of behavioral or physiological processes that are of direct relevance to the survival of organisms under rhythmic environmental challenge are quite obvious. However, circadian clocks are also thought to confer advantage to living beings by maintaining a temporal order in their internal milieu. Development of this view has been concurrent with the recognition of circadian rhythms as an integral and pervasive element of living organization as a whole. Observations suggesting coexistence of 24-h rhythms at multiple levels of biological organization appears to be the starting point of development of this thought process (Pittendrigh, 1960). Extensive studies on mammalian systems, especially mice and humans, revealed the presence of 24-h rhythms at multiple levels of biological organization such as locomotor activity, body temperature, susceptibility to toxins at the level of whole organism, blood sugar, eosinophil counts, adrenal activity, liver glycogen content at the level of tissues and organs, and at the level of basic cellular physiology such as RNA and DNA metabolism (Halberg, 1960). Observations of robust mutual phase relationship among various rhythms implied their significance for smooth functioning of internal physiology and consequently their probable advantages to the organism as a whole. Indeed, this idea was convincingly supported by the findings of an early study on cockroaches by Harker (1956,
K. M. Vaze and V. K. Sharma

1958). This study made use of the known causal role of autonomous circadian oscillations of neurosecretions of subesophageal ganglion (SG) in activity/rest rhythm, and a standardized procedure of SG transplantation in reinstating behavioral rhythmicity in arrhythmic individuals (obtained by ablation of the same ganglion). In this study, SG transplantation was carried out between animals entrained to anti-phasic light-dark (LD) cycles. The study found development of tumors in these subjects compared with controls in which SG transplantation was done between individuals entrained to in-phase LD cycles (Harker, 1956). Although this study did not point out the identity of probable biochemical rhythms responsible for the adverse effects, the results implied lack of coordination among biochemical rhythms as the cause (Pittendrigh, 1961). Considering the coexistence of multiple rhythms in a single organism (Halberg, 1959) and some direct but scattered evidence available from studies on tissues and organ cultures, suggesting the possible existence of multiple autonomous oscillators such as those in isolated segments of hamster intestine (Bünning, 1958; Enderle, 1951), it was hypothesized that multiple rhythms could be a reflection of coupled multiple oscillators, and therefore an organism could be viewed as a population of autonomous circadian oscillators (Pavlidis, 1973; Pittendrigh, 1960). Evidence suggesting multioscillatory circadian organization and functional significance of mutual phase relationships among multiple biochemical oscillations can be considered as foundation for the notion that circadian rhythms are elementary features of a grand physiological organization. This notion implies that circadian rhythms confer adaptive benefit to organisms through temporal coordination of their internal physiology, which is referred as intrinsic advantage of circadian rhythms. As a corollary, circadian clocks being a fundamental component of physiological organization, any threat to the temporal coordination would ultimately cause an adverse effect on the organism’s physiological well-being.

Role of Circadian Clocks in Photoperiodism

The potential utility of 24-h endogenous rhythms of behavior and physiology in coping with 24-h environmental cycles is a reasonable expectation, but circadian clocks are also thought to be causally involved in adaptive responses of organisms to environmental cycles of much longer periodicity (i.e., annual cycles) and are thus believed to be important in coping with seasonal cycles (Hut & Beersma, 2011). Cyclic seasonal changes are important features of the abiotic environment on earth and are primarily results of the inclined position of the earth’s north-south axis with respect to the plane of elliptical orbit of Earth’s rotation around the Sun (Daan, 2009). Seasonal changes in abiotic environment every year make each annual cycle a mosaic of favorable and harsh episodes for life on earth. Severity of seasonal changes increase with latitudes away from the equator; thus, they are more prominent in temperate latitudes and polar region than in the tropics. Impact of seasonal variation on various life forms is quite apparent from annual cycles of behavior and physiology in organisms from diverse taxa. Behavioral and physiological responses of organisms to seasonal changes in their environment are thus a prominent feature of life on earth. Organisms have evolved multiple adaptive strategies to survive under harsh conditions and of scheduling reproduction during favorable seasons so as to maximize survival of their offspring. These include strategies such as aestivation/diapause in insects, hibernation in mammals, migration in insects, birds, and mammals, and a temporal programming of reproductive physiology aimed at facilitating reproduction in favorable seasons such as timing of flowering in plants, growth of gonads, and development of secondary sexual characters in animals. Despite enormous diversity of strategies used by organisms, all of them invariably depend upon day length for survival strategies or initiation of reproductive phase. Strong correlation of day length with constellation of other changes in the abiotic environment (which are associated with season change) makes day length the most reliable predictor of seasonal changes. Because of strict dependence of these annual cycles of behavioral or physiological responses of living organisms on day length (daily photoperiod), they are known as photoperiodic responses. Photoperiodic responses are thus considered as adaptation to seasonal changes due to their direct implications in survival and reproductive success.

Circadian clocks have been implicated in photoperiodic responses. Earlier, photoperiodic responses were believed to be phenomena purely based on hourglass-like mechanism, later Bünning proposed a causal role for endogenous circadian rhythms (then, endodiurnal rhythms) in photoperiodic responses (Bünning, 1936). Following Bünning, several studies investigated the role of circadian clocks in photoperiodic responses, and now there is ample evidence suggesting the involvement of circadian clocks in mediating photoperiodic responses in organisms ranging from plants to mammals (Pittendrigh, 1966, 1972; Pittendrigh et al., 1984; Vaz Nunes & Saunders, 1999; Vaz Nunes & Veerman, 1982). Many models have been proposed to explain the role of circadian clocks in photoperiodic responses. Some models consider the role of circadian systems in photoperiodic responses as non-clock-like (e.g., resonance model; Pittendrigh, 1972), whereas other models consider that circadian rhythms play an active role in photoperiodic time measurement (e.g., external, internal coincidence model; Pittendrigh, 1966; Pittendrigh et al., 1984). Involvement of circadian rhythms in photoperiodic time measurement through “resonance” and/or “coincidence” has been tested in a few early studies (Bollig et al., 1976; Veerman & Vaz Nunes, 1980). Circadian rhythms are thus believed to confer adaptive advantage to living beings by helping them cope with seasonal changes in addition to their significance in dealing with daily rhythms.
In summary, circadian clocks have been hypothesized to confer advantage to living beings (1) by appropriately scheduling their behavioral and physiological processes in relation to cycles of environmental challenges and opportunities (extrinsic advantage); (2) by achieving temporal order in internal physiology (intrinsic advantage); and (3) through the role of circadian clocks in mediating responses to seasonal changes. These hypotheses have been tested using a variety of alternative empirical methods, which will be discussed in the following section.

**EMPIRICAL METHODS AND EVIDENCE FOR THE ADAPTIVE VALUE OF CIRCADIAN RHYTHMS**

Several empirical methods are in use to collect evidence concerning adaptive hypotheses (Endler, 1986; Rose & Lauder, 1996). In this section, we will discuss, one by one, the empirical approaches that have been employed for testing the adaptive value of circadian rhythms (Box 1) and evidence available from each of those approaches.

**BOX 1 Empirical approaches that have been employed for testing adaptive a value of circadian rhythms**

(A) **Comparative Analysis**

Convergent evolution – the evidence for evolution of similar traits in unrelated species living under similar habitats are considered as strong evidence suggesting evolution of a trait by the action of natural selection (Endler, 1986; Larson & Losos, 1996).

(B) **Experimental Manipulation of Trait/Ecology**

A hypothesis regarding benefits conferred by a trait is framed by taking into consideration the ecology of the organism. Adaptive hypothesis is tested by studying the effects of manipulation of trait or the ecology on the reproductive fitness or its components (Sinervo & Basolo, 1996).

(C) **Correlation Between Trait and Ecological/Environmental Variables**

It is a commonly used approach to test the adaptive hypothesis in natural populations. Correlation between variation in a trait and the variation in ecological variables is taken as evidence suggesting the adaptive value of a trait (Endler, 1995).

(D) **Studying Evolution in Real Time**

Laboratory or wild populations are exposed to selection pressures, tailored according to the adaptive hypothesis concerning a trait. Selected and unselected control populations are then monitored for change in trait(s) over several generations. Comparison of trait(s) between selected and control populations can unequivocally establish imposed selection as a cause of trait evolution. Evolution of a trait of interest under relevant selection pressure may be taken as strong evidence suggesting the adaptive value of a trait (Rose et al., 1996).

(A) **Comparative Analysis**

Evidence suggesting evolution of similar trait(s) in unrelated species inhabiting similar biotic/abiotic habitats is one of the oldest methods of testing adaptive hypothesis. Evolution of similar trait(s) in unrelated species excludes the possibility of trait similarity due to shared genes/developmental processes (Endler, 1986; Larson & Losos, 1996) and thereby indicates independent evolution of similar traits. Independent evolution of similar trait(s) could thus be attributed to action of equivalent natural selections.

Circadian rhythms are ubiquitous in nature. Organisms ranging from bacteria to mammals exhibit circadian rhythms in various behaviors and physiological processes (DeCoursey, 2004). Across the phylogenetic spectrum, basic features of circadian rhythms such as temperature compensation, phase-dependent effects of zeitgebers are remarkably conserved. Genetic analysis of circadian rhythms has led to the identification of several genes controlling circadian rhythms from bacteria to mammals (Bell-Pedersen et al., 2005). Clock gene homology studies suggest that many genes are shared among phylogenetic groups for their clock functions. There are many orthologous clock genes between mammals and insects (Young & Kay, 2001; Yu & Hardin, 2006). However, as yet there is no evidence of homology between bacterial and eukaryotic clock genes (Bell-Pedersen et al., 2005; Young & Kay, 2001), suggesting that circadian rhythms evolved at least two times during the history of life on earth (Rosbash, 2009). Moreover, although products of unrelated genes constitute molecular clocks, underlying molecular mechanisms appear to be highly conserved (Young & Kay, 2001). All this evidence suggests that circadian clocks are evolutionary adaptation to the rhythmic environment on earth.

(B) **Experimental Manipulation of Trait/Ecology**

We saw in the section on adaptation that adaptive traits are thought to be advantageous to living beings (by enhancing fitness of their owners) through some perceived function. Therefore, the adaptive value of a trait is assessed in this approach through testing proposed functional significance of a trait. Functional significance of a trait is assessed by studying the effects of experimental manipulation of trait(s) or ecological factor(s) relevant to the proposed function of a trait on the fitness (Sinervo & Basolo, 1996). Fitness is often assessed by measuring fecundity, longevity, or morphological and behavioral traits such as body size (mass) and growth rate/development time (Pekkalala et al., 2011). In principle, the effect of trait manipulation on fitness allows detection of natural selection acting on the trait, whereas the effect of manipulation of organism’s ecology on the fitness helps identify ecological factors acting as natural selection. The strength of this approach thus lies in its ability to detect action of natural selection and to identify ecological factors acting as forces of natural selection.
(Sinervo & Basolo, 1996). This approach has been employed to test the adaptive value of circadian clocks in several studies by manipulating either circadian phenotypes and/or rhythmic ecology.

**Experimental Manipulation of Light Regimes**

Cyclic variations in the level and quality of light are an important aspect of rhythmic ecology; therefore, experimental manipulation of light-dark (LD) cycles can potentially mimic alteration of rhythmic ecology. Exposing organisms to constant light (LL) or non-24-h LD cycles (T-cycles) has been some of the most commonly used light regime manipulations to test adaptive significance of circadian rhythms. However, experimental manipulations of light regimes under laboratory conditions could end up in manipulation of (a) circadian phenotype alone, or (b) circadian phenotype and organism’s ecology, and thus have implications for inference that can be drawn about the possible means by which circadian rhythms confer advantage (intrinsic, extrinsic).

Circadian systems of many organisms are capable of entraining to LD cycles of a range of periodicities, and thus could be driven at frequencies of the entraining LD cycles (Johnson et al., 2003). Light regime manipulation, therefore, has been used as a means of manipulating circadian phenotype to test the adaptive value of circadian rhythms by assessing its effects on fitness. Exposing organisms to T-cycles has been used to test a hypothesis that is commonly known as “circadian resonance hypothesis.” Basic framework of the hypothesis is as follows: Near-24-h periods of endogenous rhythms strongly suggest that circadian rhythms are adaptation to 24-h environmental cycles. Therefore, it is believed that, although circadian rhythms are capable of entraining to non-24-h cycles, the extent of adaptive benefits under such non-natural cycles may not match those under 24-h cycles. This proposed suboptimal performance of circadian systems under non-24-h cycles could result from system’s inability to gain intrinsic and extrinsic advantages.

**Effects on Intrinsic Advantage.** Negative effects of non-natural LD cycles on intrinsic advantage could be visualized in the light of multioscillatory model of circadian clocks proposed by Pittendrigh (1960). Even though the term circadian resonance was first used by Pittendrigh and Minis (1972), its underlying proposition is deeply rooted in the multioscillatory framework of circadian organization developed much earlier by Pittendrigh (1960). Circadian resonance is the clearest form of this proposition and states that organisms function effectively or perform better when their endogenous circadian system is driven by zeitgeber cycles of frequencies close to their innate frequency (i.e., 24-h) and hence the term resonance. The starting point of the proposition underlying circadian resonance could be seen in the form of Pittendrigh’s interpretation of the observations on plant growth rate under rhythmic and arrhythmic light and temperature regimes (from Earhart laboratory; Went, 1960) using his multioscillatory model of circadian clocks. General results from the early plant studies were (1) growth rate was lower when plants were reared under LL but such light-induced malfunction was avoidable by simultaneous use of 24-h temperature cycles (Hillman, 1956); and (2) under periodic light regimes, growth rates were optimal when the period of LD cycles was close to 24-h and showed lower rates when plants were reared under LD cycles far removed from 24-h (Highkin & Hanson, 1954; Went, 1960). Pittendrigh invoked multioscillatory model of circadian organization to explain these observations (Pittendrigh, 1960). He stated that running of all the constituent oscillators (believed to be controlling rhythms of different biological functions) with identical period and appropriate mutual phasing of the constituent oscillators was key to optimal functioning of the system (Pittendrigh, 1960). Consequently under LL, constituent oscillators no longer remain synchronized in the absence of zeitgebers and fail to maintain appropriate mutual phasing, leading to the breakdown of the entire circadian organization, which can be circumvented by providing temperature cycles under LL. To explain the adverse effects of non-24-h LD cycles, Pittendrigh invoked a model comprising a possibility of the presence of light-sensitive and light-insensitive oscillators, and the role of light-sensitive oscillator as synchronizer to maintain appropriate mutual phasing among all the constituent oscillators of the system. According to this model, light-sensitive oscillator would entrain constituent oscillators only if its own frequency is close to the endogenous frequency of the constituent oscillators owing to the limits of entrainment (Bruce, 1960). Entrainment of light-sensitive oscillator to LD cycle far removed from 24-h would thus fail to maintain synchrony and appropriate mutual phasing among constituent oscillators, causing a collapse of circadian organization. Pittendrigh (1960) thus attributed the adverse effects of LL or non-24-h LD cycles to the possible lack of coordination in internal physiology resulting from the breakdown of multioscillatory circadian organization, and thus in turn inability to gain intrinsic advantage.

**Effects on Extrinsic Advantage.** Although Pittendrigh (1960) attributed the adverse effects of non-24-h LD cycles to the possible lack of coordination in internal physiology, it is plausible that such effects are the result of being deprived of benefits resulting from the occurrence of rhythmic processes at an appropriate time of the environmental cycle (i.e., being deprived of extrinsic advantage). Although circadian systems are entrainable to non-24-h environmental cycles, their phase relationship with environmental cycles are likely to be abnormal and, hence, adverse effects of light regime manipulation are plausible as a result of occurrence of rhythmic processes at wrong (nonadaptive) time of the environmental cycle, or, in
isms can only be ascribed to lack of intrinsic advantage, whereas in case of phototrophic organisms could be attributed to the inability to gain extrinsic advantages in addition to their effects on intrinsic advantages. Although light is also an important aspect of rhythmic ecology of all nonphototrophic organisms living in the wild, laboratory manipulations of light regimes are unlikely to mimic natural conditions can be attributed to lack of intrinsic advantages alone, whereas in case of phototrophic organisms they could be attributed to lack of intrinsic as well as extrinsic advantages.

**Experimental Manipulation of Circadian Phenotype Through Methods Other Than Light Regime Manipulation**

Understanding of anatomical and genetic bases of circadian clocks has made it possible to manipulate circadian phenotypes, which has been employed to test adaptive significance of circadian clocks in several studies (reviewed in Johnson, 2005; Sharma & Joshi, 2002; Sharma, 2003). In mammals, the region of the brain known as suprachiasmatic nucleus (SCN) has been identified as a seat of the central circadian pacemaker regulating various behavioral and physiological processes. Surgical ablation of SCN is known to cause arrhythmicity in various behavioral and physiological processes (Ralph & Menaker, 1988; Stephan & Zucker, 1972). Gene mutations causing abnormal circadian phenotypes such as arrhythmicity, longer or shorter than normal endogenous periodicities, have been identified over last four decades in model organisms ranging from bacteria to mammals (Bell-Pedersen et al., 2005). Consequently, SCN ablation and gene mutations have been used as means of manipulating circadian phenotype to test the adaptive value of circadian rhythms in several studies. As discussed earlier, the fitness consequences of such circadian manipulations assessed under laboratory conditions in case of better non-phototrophic organisms can only be ascribed to lack of intrinsic advantage, as laboratory conditions represent very limited aspects of natural ecology and fitness effects measured under natural conditions can be attributed to lack of intrinsic and extrinsic advantages.

**Evidence From Experimental Manipulation of Circadian Rhythms and/or Rhythmic Ecology**

**Testing for circadian resonance.** Two studies, one by Pittendrigh and Minis (1972) in fruit flies D. melanogaster and the other in blow flies Phormia terraenovae by von Saint Paul and Aschoff (1978), could be considered as among the earliest formal tests of circadian resonance, which examined the fitness consequences of LD cycles of different periodicities or of aperiodic light regime using adult longevity as the measure of fitness. In the study on fruit flies, the life span of adult flies maintained under LD12:12 was compared with those maintained under non-24-h LD cycles (LD10.5:10.5 and LD13.5:13.5) and LL, whereas in the study on blow flies Phormia terraenovae, the longevity of adult flies was examined under LL and LD cycles of periodicities ranging from 20 to 28-h. Both studies showed a clear trend of reduced longevity under LD cycles with non-24-h period and LL, compared with that under LD12:12. This deleterious effect was attributed to the loss of appropriate mutual phase relationship among constituent circadian oscillators (Pittendrigh & Minis, 1972; von Saint Paul & Aschoff, 1978). Thus, reduced longevity under non-24-h LD cycles and LL compared with that under LD12:12 provided evidence that is in line with the expectations of circadian resonance hypothesis and suggests the intrinsic adaptive value of circadian clocks.

Saunders (1972) examined the effect of LD cycles of periodicities ranging from 21 to 72-h on the preadult development time in flesh fly Sarcophaga aryszostoma. Preadult development was found to be longer under LD cycles with periods close to 24-h (natural frequency) or its multiples than under non-24-h LD cycles with periods that were not multiples of 24-h. The effect of LD periodicities (which drive circadian rhythms at their frequencies) on development time suggests implications of circadian organization for developmental processes.

Although circadian resonance has been studied by comparing the fitness of wild-type strains (with a period of 24-h) under 24-h and non-24-h LD cycles, a more complete and rigorous test of circadian resonance would be to test whether match between endogenous period and the period of LD cycles improves fitness. Availability of circadian-period mutants (shorter or longer than 24-h) in various model organisms has made it possible to examine fitness consequences of interactions between endogenous period and the period of LD cycles. In a first reported study of its kind (Ouyang et al., 1998; Figure 1), a wild-type strain (AMC149 and AMC343; $\tau = 25$-h) of cyanobacteria and two mutant strains, SP22 and P28, with shorter ($\tau = 23$-h) and longer ($\tau = 30$-h) periods, respectively,
is remarkable to see that short- and long-period strains exhibit lesser fitness than wild-type strains; however, it formed like wild-type strain. Usually mutant strains when competed with a wild-type strain under allele with wild-type allele, and the rescued strain P28R, mutation was rescued (P28) by replacing the mutant ous under growth competition, the long-period clocks and not due to any secondary mutation deleterious under light-dark (LD) cycles whose periods matched their environmental cycles out-performed other strains. To further validate whether the advantage of circadian rhythms in cyanobacteria, it need not be taken as a general rule across the spectrum of living beings.

Another study tested circadian resonance in the plant Arabidopsis thaliana using a similar approach (Dodd et al., 2005). In this study, wild-type strain col-o (with τ of 24-h), long-period mutant strain ztl-l (with τ ranging from 27.1 to 32.5-h), and short-period mutant locl-l (with τ of 20.7-h) grown under LD cycles with periods of 20 (T20), 24 (T24), and 28 (T28) h were assessed for their performance in terms of photosynthetic activity and growth rate. Chlorophyll content was found to be higher when plants were grown under LD cycles whose periods matched their τ. Wild-type strain (col-o) showed significantly higher chlorophyll content under T24 than in T20 or T28. Similarly, mutant strains (ztl-l and locl-l) had higher chlorophyll content when grown under LD cycles that matched their endogenous periods than in T24. Consequently, plant strains grown under LD cycles whose periods matched their τ showed higher CO2 fixation and greater biomass. In a reciprocal competition between short- and long-τ mutants by growing their mixed populations under T20 and T28, plants grown under LD cycles with periodicities matching their τ showed higher chlorophyll content, leaf number, and aerial biomass. Together, these results suggest that having circadian clocks with periods matching the environmental cycles enhances growth in plants, thus providing evidence in support of the circadian resonance hypothesis.
Recent study in Arabidopsis tested circadian resonance using a novel method of estimating fitness (Yerushalmi et al., 2011). In this study, first the population of Arabidopsis plants with a variation for $\tau$ was created from F2 progeny of a cross between long-$\tau$ (prr7prr9, $\tau = 36.55$-h) and short-$\tau$ (prr5prr7, $\tau = 22$-h) mutant strains. The $\tau$ in F2 population was found to vary between 20.79 and 45-h. F2 generation plants were then reared under short- and long-period LD cycles of 20-h (LD13:7; T20) and 28-h (LD19:9; T28) periods, respectively. After one generation of selection, F3 plants were examined for $\tau$ and frequencies of prr5prr7 and prr7prr9 alleles as a measure of reproductive success of short- and long-$\tau$ variants. Average $\tau$ of F3 plants was positively correlated with the period of the $T$-cycles (T20 or T28) in which their F2 parents were reared. Moreover, frequency of long-$\tau$ allele prr7prr9 was found to be significantly greater in F3 population whose parents were reared under T28, but frequency of short-$\tau$ allele prr5prr7 did not differ significantly between F3 populations coming from F2 parents reared under T20 or T28. These results were taken to suggest that long-$\tau$ phenotypes are favored under T28 and short-$\tau$ phenotypes are favored under T20, and thus provide evidence in support of circadian resonance.

In a study in fruit flies D. melanogaster, wild-type ($per^{+}$; $\tau \sim 24$-h) and short-period ($per^{1}$; $\tau \sim 16$-h) and long-period ($per^{2}$; $\tau \sim 28$-h) mutant strains were assayed for lifespan under LD8:8 and LD12:12 (Karsfeld & Rouyer, 1998). Male flies of short- and long-period mutants lived shorter than wild-type flies. Moreover, life span reduction was found to be larger in $per^{1}$ flies whose $\tau$ deviates from wild-type more than $per^{2}$. Analysis of the effect of genotype and rearing LD cycles on life span showed a significant main effect of genotype and genotype $\times$ light regime interaction but not of light regime. Significant genotype $\times$ light regime interaction indicates that the effect of clock mutations on life span was dependent on the period of LD cycles. Comparison of difference between longevity of $per^{+}$ ($\tau \sim 24$-h) and $per^{2}$ ($\tau \sim 16$-h) under LD8:8 (T16) and LD12:12 (T24) showed that difference was smaller under T24 than in T28, suggesting that T16 is less deleterious for $per^{+}$ flies compared with T24. Although these results were taken as evidence supporting circadian resonance (not explicitly), the longevity of $per^{1}$ flies did not show the trend expected under circadian resonance.

A recent study tested the effects of concordance of endogenous period with environmental period (circadian resonance) on the reproductive fitness in natural populations of pitcher plant mosquito Wyeomyia smithii (Emerson et al., 2008). Pitcher plant mosquitoes are a temperate species that lay eggs and complete their preadult development inside water-filled leaves of purple pitcher plant. Pitcher plant mosquito undergoes larval dormancy (diapause) as adaptive response to harsh winter conditions. Diapause is initiated, maintained by short-photorperiod conditions, and terminated under long photoperiods. Populations of pitcher plant mosquito subjected to diapause-maintaining short-photorperiod light regimes of periodicities ranging from 24 to 72-h (LD10:14 to LD10:62) exhibit adaptive response (maintenance of diapauses) under LD periodicities of 24-h or its multiples, suggesting involvement of circadian clocks in regulation of seasonal responses (Bradshaw et al., 2003). Having found implications of circadian clocks in regulation of diapauses, Emerson et al. (2008) exposed natural populations of pitcher plant mosquito to three short-photorperiod diapause-maintaining LD cycles, LD10:14, LD10:36, and LD10:25, and studied their fitness using a composite estimate based on four different components—pupal survivorship, fecundity, embryonic viability, and adult longevity. Mosquito populations exposed to LD regimes with a period of 24-h (LD10:14) or multiples of 24-h (LD10:36) had higher fitness than those exposed to LD cycles with a periodicity that was nonmultiples of 24-h (LD10:25).

A recent study on two species of Camponotus ants (Lone et al., 2010) reported that preadult development was faster in LD cycles of period of 24-h than in cycles with periodicities of 20 and 28-h. Faster preadult development is regarded as advantageous in insects (Prasad & Joshi, 2003); therefore, faster development under 24-h LD cycles than non-24-h LD cycles was interpreted as an adaptive strategy, possibly through circadian resonance.

Clock-less in the laboratory. Several studies have tested adaptive significance of circadian rhythms by examining the effect of loss of circadian integrity on reproductive fitness. A study on the short-period mutant golden hamsters, tau ($\tau = 20$-h), tested the effect of environmentally induced rhythm disruption on longevity (Hurd & Ralph, 1998). Previous study had shown that the effect of environmental light regime on activity/rest rhythm was dependent on hamster genotype with respect to tau locus, i.e., wild-type, homozygous (tau/tau: $\tau = 20$-h), or heterozygous (tau/+: $\tau = 22$-h; Osiel et al., 1998). Although heterozygous hamsters ($\tau = 22$-h) were able to entrain to 24-h LD cycles, their activity and rest phases were highly fragmented compared with wild-type and homozygous hamsters. To test the effect of this light-regime-induced rhythm disruption on fitness, longevity was measured under LD14:10. Heterozygous (tau/+: $\tau = 22$-h) hamsters showed reduced life span compared with the homozygous (tau/tau: $\tau = 20$-h) and wild-type (WT: $\tau = 24$-h) animals. Consolidated activity rhythm and normal longevity was rescued in heterozygous animals by transplanting SCN from a wild-type animal. Although these results suggest fragmentation of activity as a primary cause of reduced longevity in the heterozygous hamsters, inconsistency in the effect of period on longevity makes it difficult to conclude whether the observed effects on longevity were due to disruption of circadian organization or due to some non-clock effects of tau mutation.
In a study on fruit fly *D. melanogaster*, it was demonstrated that fruit flies lacking consolidated activity/rest behavior under DD lived shorter than their rhythmic counterparts (Kumar et al., 2005), supporting the view that circadian rhythms are advantageous. Many studies have tested the consequence of light-regime-induced arrhythmicity on the fitness of *Drosophila*. The longevity of *D. melanogaster* maintained under LL or LD12:12 was lower than under DD (Allemand et al., 1973). In a separate study on four large, outbred, replicate populations of *D. melanogaster* maintained under LL for several hundred generations, the longevity of flies was found to be significantly reduced in LL compared with LD12:12 or DD (Sheeba et al., 2000). Reduction in longevity under LL, however, was found to be accompanied by enhanced reproductive output, suggesting that multiple fitness components should be taken into account to draw any conclusion on adaptive advantage of circadian rhythms.

Loss-of-function mutations in the core clock genes *period* (*per*), *timeless* (*tim*), *clock* (*clk*), and *cycle* (*cyc*) in *D. melanogaster* are known to cause disruption of activity/rest rhythms. Beaver et al. (2002) tested the effect of loss-of-function mutations in the core clock genes *period* (*per*), *timeless* (*tim*), *clock* (*clk*), and *cycle* (*cyc*) in *D. melanogaster* on fecundity. Egg output in loss-of-function mutants showed ~40% reduction compared with wild-type flies. In the same study, wild-type females mated with mutant males showed reduced fecundity, suggesting effect of clock mutations on male fecundity and, indeed, clock mutant males (*per*01 and *tim*01) showed lesser sperm counts than wild-type males. Moreover, although oscillatory expression of clock genes in the male reproductive tract suggested the presence of functional circadian clocks, circadian rhythm was not detectable in sperm release (Beaver et al., 2002). Thus, these studies, at most, establish the role of core clock genes in the regulation of reproductive output; however, it appears that lower reproductive output in the null mutants is likely to be a result of non-clock-like function of clock genes, which is also consistent with another report suggesting a role for clock genes in non-clock functions such as regulation of oogenesis (Beaver et al., 2003).

A recent study has demonstrated the fitness costs of circadian rhythm disruption to the malaria parasite *Plasmodium chabaudi*, implying adaptive advantages of having circadian rhythms for the malaria parasite (O’Donnell et al., 2011). Completion of the life cycle of the malaria parasite involves its passage through vertebrate hosts in which it replicates asexually and a mosquito vector wherein it replicates sexually. Asexual cycle of replication in vertebrate hosts involves occurrence of various developmental stages (ring, trophozoite, and schizont) in a sequential manner that exhibits 24-h period (or its multiples) and the appearance of these developmental stages occurs at specific times of the day. The mature parasite stage, schizonts, appears in the host blood circulation primarily during the night. To test whether synchronization of the parasite cell cycle with that of the host carries any fitness advantage, parasites from one host were introduced into the recipient hosts entrained to LD cycles that were 180° out of phase with that of the donor host. It was found that desynchrony between parasite and host rhythms due to the parasite moving from one host to another reduced in-host survival and between-host transmission potential of parasite. Although the underlying mechanisms are yet to be revealed, these results suggest the importance of circadian rhythms for the parasite to maximize its reproductive fitness (O’Donnell et al., 2011).

Clock-less in the wild. All the studies on adaptive value of circadian rhythms discussed till now have been performed under controlled laboratory conditions. As laboratory light-dark or temperature cycles represent very limited aspects of 24-h cycles under natural conditions, the effects of circadian disruption on fitness under laboratory conditions cannot test extrinsic advantages conferred by circadian rhythms. Therefore, many studies have tested the effects of circadian rhythm disruption on fitness under seminatural conditions. Adaptive advantage of circadian rhythms is also evident from survival of animals with or without intact activity/rest rhythms under wild conditions. In a study on the ground squirrels, survivorship of SCN-lesioned and intact control animals was studied in desert enclosures. SCN-lesioned animals were highly vulnerable to predation by feral cats and as a consequence were found to incur greater mortality compared with intact controls (DeCoursey et al., 1997). The authors compared these results with the absence of evidence for adverse effects on survival of SCN-lesioned animals under laboratory conditions from other studies (Menaker & Vogelbaum, 1993; Ruby et al., 1996), and concluded that although lack of circadian clocks may not incur any physiological disadvantage under laboratory conditions, it may have adverse effects on the survival under natural conditions. In a similar study on chipmunks *Tamias striatus*, SCN-lesioned animals suffered significantly greater mortality compared with the intact controls under seminatural conditions (DeCoursey & Kralus, 1998; DeCoursey et al., 2000). A careful analysis of the activity/rest behavior revealed that SCN-lesioned animals were more vulnerable to attacks by predators, which was speculated to be due to increased nighttime restlessness. Although it is clear that SCN-lesioned animals incurred greater mortality, we do not yet know if this is because these animals were poor in terms of their physical ability to escape predators.

Another recent study (Daan et al., 2011) tested the effects of circadian dysfunction on survival under seminatural conditions in mice. Mouse clock mutation *Per2Brdm1* is known to cause compromised circadian organization and entrainment. In this study, replicate populations of mice containing individuals that were...
homzygous, heterozygous, and wild-type (in approximate Mendelian ratios) were monitored for two successive years under seminatural enclosures. Over 2 yrs, frequencies of two mutant and wild-type genotypes varied were found to be sex dependent. In the first year, the frequency of mutant genotypes was reduced but recovered to their frequencies at the time of release in seminatural enclosure by the end of second year. This experiment could be considered as a step forward towards understanding the fitness advantage of circadian clocks under natural conditions. Although this study shows differential survival in clock mutant and wild-type genotypes, its causes are still unknown; thus, in general, the results are not conclusive regarding the utility of circadian clocks in the wild.

(C) Correlation Between Trait and Ecological/Environmental Factors

By definition, adaptation means suitability of a trait to its biotic/abiotic environment. Populations evolved under different environmental conditions are thus expected to exhibit variation in their phenotypes. Therefore, association between phenotypic/genetic polymorphism and potentially selective environments in nature is often interpreted as evidence suggesting the action of natural selection on a trait and consequently as adaptation of a trait to that environment. Such “correlational approach” has been used extensively to test adaptive hypotheses regarding a variety of traits in natural populations (Endler, 1995). This approach has been used in several studies to test the adaptive value of natural variation in clock properties.

Evidence From Correlational Approach

Latitudinal clines. Although every point on the earth’s surface experiences robust 24-h cycles of abiotic factors such as light, temperature, and humidity, several features of such diurnal cycles vary from one location to another. Day length (photoperiod), time course, and amplitude of daily fluctuations of light, temperature, and humidity are some of the features that exhibit remarkable gradient across latitudes and thus result in latitude-dependent variation in the quality of rhythmic selection pressure. As scheduling of various biological functions at appropriate phases of environmental cycles through the process of entrainment is thought to be one of the ways by which circadian rhythms confer advantage to living beings, correlation between polymorphism in clock properties or its underlying genetic polymorphism with latitude has been used as evidence for adaptive significance of circadian clocks.

Lankinen (1986, 1993) reported latitudinal variation in phase relationship and circadian period of adult emergence rhythm in strains of two species of *Drosophila*—*Drosophila littoralis* (30–70°N) and *D. subobscura* (56–63°N). Another study that surveyed populations of *D. auraria* native to latitudes ranging from 34.2°N to 42.9°N in Japan reported a latitudinal cline in phase, amplitude, period, and phase response curve (PRC) of circadian adult emergence rhythm (Pittendrigh & Takamura, 1989).

Clock gene *per* is a core component of *Drosophila* circadian molecular clockwork (Allada & Chung, 2010). A stretch of *per* locus codes for repeats of the amino acid pair Thr-Gly (Costa et al., 1992). Natural populations of *D. melanogaster* and *D. simulans* are polymorphic for these Thr-Gly repeat-number alleles. Thr-Gly dipeptide repeat-number alleles (Thr-Gly)$_{17}$ and (Thr-Gly)$_{20}$ (which code for 17 and 20 dipeptides, respectively) are predominant in the European populations (over 90%; Costa et al., 1992; Rosato et al., 1994). A study conducted on natural populations of *D. melanogaster* collected from different latitudes of Europe showed correlation between latitude and the frequency of (Thr-Gly)$_{17}$ and (Thr-Gly)$_{20}$ alleles. These studies showed that (Thr-Gly)$_{17}$ is predominant in the southern parts, whereas (Thr-Gly)$_{20}$ is more common in northern Europe, suggesting latitudinal cline in *per* Thr-Gly repeat-number polymorphism (Figure 2). Subsequent studies undertaken to examine functional significance of Thr-Gly repeat-number variation found its role in thermal stability of circadian behavior. Thermal stability of circadian rhythms is believed to be an important property for it to act as time-keeper in the face of fluctuating temperature conditions and which is measured as index called temperature compensation (Pittendrigh, 1954). Temperature compensation of *Drosophila* lines homozygous for (Thr-Gly)$_{20}$ allele exhibited better temperature stability than lines with (Thr-Gly)$_{17}$, suggesting that the latitudinal distribution of Thr-Gly repeat alleles of *per* gene was shaped by gradient of mean temperature across latitudes (Sawyer et al., 1997). Similar studies on flies sampled from different latitudes in Australia revealed a correlation between the frequency of (Thr-Gly)$_{20}$ and (Thr-Gly)$_{23}$ alleles and latitude. Frequencies of (Thr-Gly)$_{20}$ were positively correlated with latitude as seen in Europe. Such parallel cline in Australia strengthens the notion that Thr-Gly polymorphism at *per* locus is under
selection for thermal stability of circadian rhythms (Sawyer et al., 2006). However, independent study by Weeks et al. (2006) could not establish latitudinal cline of Thr-Gly allele frequencies in Australian populations.

In a study on populations of Arabidopsis thaliana derived from natural populations inhabiting locations ranging from 20°N to 60°N, plants were studied for various clock properties (Michael et al., 2003). This study found a positive correlation between τ and day length, suggesting association of τ with latitude.

In a recent study, frequencies of alleles of the core clock gene timeless (tim) in samples of fruit fly D. melanogaster collected from populations native to different latitudes were found to vary with latitude. In D. melanogaster, the gene tim codes for a light-responsive element of the circadian molecular oscillator, and some laboratory strains were found to exhibit polymorphism in two alleles, ls-tim and s-tim, which code for both long and short peptides and only short peptide, respectively (Rosato et al., 1997). ls-tim allele frequencies in populations collected from different latitudes across Europe (from southern Italy to Sweden) showed a strong latitudinal cline. ls-tim frequency was highest in southern Italy and gradually decreased northwards. When ls-tim allele frequencies of samples from Italy and southwards were also included in the analysis, correlation between latitude and allele frequency lost significance. When the same allele frequencies of samples inclusive of Italy and southwards were plotted against direct distance and overland distance from the putative site of origin of the allele (Novoli, southern Italy), the correlation was stronger than that seen with latitude (Tauber et al., 2007), suggesting that the observed clinal variation might be due to spread of the allele in all directions from its site of origin and not because of natural selection. Application of Tajima D test to tease apart these possibilities suggested strong contribution of natural selection in the observed clinal variation. Functional analysis of tim polymorphism revealed a causal role of tim alleles in the regulation of incidence of ovarian diapause (Tauber et al., 2007). Is-tim, which is predominant in southern Europe, showed higher incidence of diapause than s-tim genotypes. Ovarian diapause is a strategy in insects to survive extreme low temperatures during winters. Northern Europe experiences much lower temperatures than the southern locations. Therefore, higher incidence of diapause in Is-tim genotype, which is predominant in southern locations, seems contradictory to the notion that Is-tim allele is under selection for ovarian diapause. Although based on these data, it was proposed that the observed clinal variation in Is-tim frequency is an outcome of spreading of recently emerged ls-tim allele in southern Europe by directional selection, it is not yet clear whether latitudinal distribution of tim alleles is a result of natural selection acting on consequences of tim alleles for incidence of diapause or clock function.

Circadian clocks under constant environments. Corollary to the proposition that circadian rhythms are advantageous to living organisms in cyclic environments, it is reasonable to expect that these rhythms would be of no obvious significance to animals inhabiting aperiodic environments such as deep sea vents and caves where environmental factors such as light and temperature are virtually stable. Therefore, evolutionary regression of circadian rhythms has been predicted in organisms inhabiting such environments that are virtually devoid of rhythmic selection pressure (Bünning, 1973; Sharma, 2003). In fact, there is some evidence in favor of evolutionary regression of circadian rhythms from the observations on cave-dwelling animals (troglobites). Blume and coworkers (1962) reported lack of activity/rest rhythm in cave-dwelling eyeless crayfish Niphargus puteanus, with low frequency of rhythmic individuals exhibiting periodicities ranging from 10 to 57-h. In another study, although cave-dwelling amblyopsid fish were also reported to have completely lost activity rhythm, fish population exhibited circadian rhythm in oxygen consumption without the ability to entrain to LD cycles (Poulsen & White, 1969). Many studies have reported persistence of one or other circadian rhythm in troglobites, for example, circadian rhythm of air-gulping behavior in the cave-dwelling fish Oreonectus evezardi (Biswas et al., 1990), and activity in the catfish Tricomycerus sp. (Trajano & Menna-Barreto, 1995, 1996) and the cave-dwelling millipedes Glyphiulthus cavernicola sulu (Koilraj et al., 2000). Although these studies reported persistence of circadian rhythms in troglobite populations, the frequency of individuals showing circadian rhythms was relatively low and was accompanied by individuals showing ultradian and infradian components and arrhythmicity. Higher incidence of periodicities deviating from 24-h and arrhythmicity in troglobite populations is thus clearly in line with the predictions of evolutionary regression of circadian rhythms in the absence of rhythmic selection pressures and thus supports extrinsic advantage of circadian rhythms. Persistence of rhythms in troglobites have often been attributed to causes such as (1) evolutionary time under cave conditions has not been sufficient for complete regression of circadian rhythms, and (2) some sections of troglobite populations may be exposed to zeitgeber cycles such as temperature (Trajano & Menna-Barreto, 1995, 1996). Regression of circadian rhythms in troglobite populations has been regarded as evidence supporting the extrinsic advantage hypothesis on one hand, and persistence of circadian rhythms has been interpreted as evidence for intrinsic advantage on the other hand. Persistence of circadian rhythms in laboratory-bred populations maintained under constant conditions for several hundreds of generations has been taken as evidence supporting intrinsic advantage of circadian rhythms in many studies. Populations of fruit fly D. melanogaster maintained under constant light, temperature, and humidity conditions for more than 600 generations.
were found to exhibit circadian rhythms in adult emergence, activity/rest, and egg-laying behaviors (Sheeba et al., 1999, 2001, 2002). Flies from these populations also showed remarkable ability to entrain to a wide range of LD cycles, much like their counterparts from the wild (Paranjpe et al., 2005). This evidence was taken to suggest intrinsic adaptive advantage of circadian rhythms. Another recent study has reported persistence of endogenous circadian rhythm of activity in populations of fruit fly D. melanogaster Oregon R strains reared under DD for over 50 yrs (∼1300 generations) (Imafuku & Haramura, 2011). Whichever way one interprets these findings, be it evolutionary regression or persistence of circadian rhythms under constant conditions, the findings suggest the adaptive value of circadian rhythms.

(D) Selection Studies—Studying Evolution in Real Time

By definition, a trait is considered as an adaptation if it has evolved as a response to natural selection (Amundson, 1996). Therefore, ideally, only the evidence for evolution of a trait in response to natural selection would serve as a hard evidence for adaptation. Most of the empirical methods that are employed to test adaptive significance of a trait usually infer evolution by the process of natural selection on the basis of evidence such as influence of a trait on reproductive fitness or its components, nonrandom distribution of a polymorphic trait in space and time, or other evidence suggesting the action of natural selection on the trait in the past (Reznik & Travis, 1996). Although it is not unreasonable to infer adaptation from such evidence, it needs to be kept in mind that this type of evidence is ultimately circumstantial and does not really serve as direct evidence for natural selection in action and for particular agent(s) of natural selection as a cause of trait evolution. As it is impossible to recreate the historical events that led to the evolution of a trait in its current form, there is no option but to rely on indirect evidence and power of speculations to reconstruct evolutionary history of a trait (Reznik & Travis, 1996). Evolution is a continuous process; therefore, although we cannot possibly collect direct evidence for natural selection in action in the past, in principle it should be possible to collect such evidence from the ongoing process of trait evolution. Experimental evolution in the field or under laboratory conditions is a common means of obtaining such evidence.

A typical experimental evolution study consists of multigeneration monitoring of populations under selective regime(s). Dynamics of a trait of interest in populations under selective regimes is compared with unselected control populations. Replication at the level of evolving populations allows testing of the repeatability of evolution and statistical significance of difference between mean trait values of selected and control populations. Possibility of testing statistical significance of difference between mean trait values of selected and control populations allows researchers to judge the causal role of selective regime(s) in the evolution of a trait. Such experiments are commonly known as selection studies (Bennett, 2003; Futuyama & Bennet, 2009). It is plausible to run selection experiments in the field or under laboratory conditions and there are advantages and disadvantages associated with each approach (Irschick & Reznick, 2009). Although there are many limitations of the interpretability of results from laboratory selection experiments to real-world conditions, they are superior to field selection experiments in several respects: (1) possibility of keeping adequate number of true independent replicate populations; (2) better control over or possibility of detecting confounding effects arising from sources external to population (environmental correlates) or internal to populations (population ancestry, linkage); and (3) better control over population size, which may influence the outcome of evolution through genetic drift. Possibility of maintaining adequately large population sizes over generations of selection can minimize the role of drift in the evolution of a trait. Taken together, in laboratory selection studies, as a result of greater control over experimental variables there is a possibility of drawing clearer conclusions with a greater degree of confidence than from studies in natural conditions (Futuyama & Bennett, 2009). Moreover, it is possible to follow changes in a variety of relevant organismal traits (including fitness traits) while they occur, and the evolved populations are available for further functional genetic analysis, thereby opening a possibility of elucidating mechanistic link underlying correlation between trait and reproductive fitness (Futuyama & Bennett, 2009).

Thus, selection experiments are not just a powerful empirical tool for fair investigation of the role of natural selection in the evolution of a trait, but also a means of understanding adaptation as a process and as an outcome in a more complete sense (Rose et al., 1996). With so many attractive features, it is not surprising that laboratory selection has been used extensively to test adaptive hypotheses about a large spectrum of biological traits covering behavior, morphology, physiology, and life history in diverse model systems.

Properties of endogenous circadian rhythms such as τ and PRC are known to be key determinants of entrained phases of the rhythms (Johnson et al., 2003). Circadian rhythms are believed to confer advantage to their owners by scheduling biological functions at appropriate phase(s) of the environmental cycle through a process of entrainment, which is commonly known as extrinsic advantage (Sharma, 2003). Therefore, direct evidence for evolution of circadian clocks in response to natural selection acting on timing of biological functions would serve as a strong proof of the extrinsic adaptive value of circadian rhythms. However, there is very little direct evidence for evolution of circadian clocks (clock properties such as τ and PRC) in response to selection for timing of biological functions and most of which came from laboratory selection studies.
Evidence From Laboratory Selection Studies

Many insect species are known to exhibit diurnal rhythm of adult emergence (emergence from pupal case), which is characterized by a large number of flies emerging during morning hours followed by a gradual reduction in the incidence of emergence as the day progresses (Saunders, 1992). Robust rhythm in adult emergence also persists under laboratory LD and DD conditions, and their circadian control has been well established (Saunders, 1992). It has been hypothesized that occurrence of emergence peak around morning—coolest and most humid part of the day—is an adaptation to increase the chances of survival. Wings of newly emerged adult individuals are in folded state and it is believed that maximum emergence during morning hours reduces chance of death due to drying up of folded wings (Pittendrigh, 1958). Although it is still unclear whether timing of adult emergence is under selection in nature and what environmental factors act as agents of selection, several laboratory selection experiments have been conducted by imposing selection on the timing of adult emergence. In all these experiments, adults emerging during the selection windows form breeding populations; hence, only those adults contribute offspring to the next generation. Although all such studies were based on the principle of selection, not all were initiated with the intention of testing the adaptive value of circadian rhythms. In many selection experiments, insect populations were selected for adult emergence during morning or evening hours under laboratory LD cycles and were named as “early” and “late” populations to indicate selection for emergence early in the morning and late in the evening than wild-type (directional section). In some of the earliest laboratory selection experiments, after 50 generations of selection in fruit fly D. pseudoobscura and after 9 generations in the moth Pectinophora gossypiella, early and late populations diverged in median phase of emergence by 4 and 5-h, respectively (Pittendrigh, 1967; Pittendrigh & Minis, 1971). In both fruit fly and moth, although early populations evolved longer \( \tau \) and late populations evolved shorter \( \tau \) of emergence rhythm compared with parent populations, according to the authors this difference was of too small a magnitude as to be causal to the differences in their emergence phenotypes. Moreover, PRC of emergence rhythms was not different among early and late populations. In any of these selection studies, there was no replication at the level of population and no information was provided about population size; therefore, it is not possible to conclude that the evolution of early and late emergence phenotypes was adaptation to selection, considering the possibility of evolution by genetic drift.

In a separate study, two strains of D. melanogaster, Oregon R and wild-caught W2, were subjected to selection for morning and evening emergence (Clayton & Paietta, 1972). After 16 generations of selection, the percentage of flies emerging in the morning and evening hours increased significantly. This study, however, did not estimate clock properties (\( \tau \) and PRC) in the selected strains. Like previous studies, there was no mention about replication and population size; therefore, it is not possible to make any assertion about the process of evolution that gave rise to early and late emergence phenotypes.

In a recent study on fruit flies D. melanogaster, replicate early and late populations were derived by imposing selection for morning and evening emergence under laboratory LD cycles from four replicate, large, outbred populations (Kumar et al., 2007b; Figure 3). With increasing generations of selection, early and late populations showed gradual increase in incidence of emergence during morning and evening hours, respectively, as a
direct response to selection and overall divergence of early and late adult emergence waveforms. By the 55th generation, early populations showed increased emergence of ~60% during morning hours compared with ~45% in control populations, whereas late populations showed enhanced emergence of ~25% compared with ~15% in control populations. The early and late populations were created by imposing selection on large, outbred, independent replicate populations; therefore, evolution of divergent adult emergence waveforms in all the replicate populations compared with unselected control populations clearly suggests that early and late emergence phenotypes were adaptation to the imposed selection. Apart from changes in the waveforms of adult emergence rhythm, early and late populations evolved divergent circadian clocks, which were evident from divergence of τ and PRC of their emergence rhythm (Kumar et al., 2007b). The early populations evolved shorter τ (23.4-h), larger phase advances, and smaller phase delays, whereas the late populations evolved longer τ (24.8-h), larger phase delays, and smaller phase advances (Kumar et al., 2007b). Considering the circadian control of adult emergence, divergence of τ and PRC in each of the early and late populations strongly suggests that diverged circadian clocks in early and late populations were causally involved in their diverged adult emergence waveforms. Further studies on these populations also showed evidence suggesting differential evolution of morning and evening oscillators (Kumar et al., 2007a), and utilization of different sections of light phase by early and late populations to entrain their adult emergence rhythm (Vaze et al., 2012). These results show that early and late populations adapted to selection for morning and evening emergence by evolv- ing their circadian clocks and thus provide strong evidence supporting the extrinsic advantage hypothesis.

In another recent study on fruit flies D. melanogaster, populations maintained under LD cycles were selected for emergence within a very short window of 1-h every day that coincided with peak of adult emergence in ancestral control populations (stabilizing selection). Selected populations showed gradual increase in the incidence of emergence during the selection window as a direct response to selection and emergence waveform of selected populations altered compared with control populations. Direct response to selection for emergence in a narrow window of time in replicate, large, outbred populations clearly suggests that it was an adaptation to imposed selection on timing of adult emergence (Kannan et al., 2012a). Direct response to selection was accompanied by changes in circadian clocks underlying rhythmic adult emergence and activity/rest behavior. Selected populations evolved shorter τ of emergence rhythms compared with control populations. More interestingly, selected populations evolved circadian clocks with greater precision, which was evident from lesser day-to-day variation in the timing of the emergence peak in selected populations under DD and LD12:12 compared with controls (Kannan et al., 2012a), and robustness as correlated response to selection (Kannan et al., 2012b). Considering the circadian control of adult emergence rhythm, it is unlikely that increased clock precision in selected populations could be simply a correlated response to selection and thus suggests a causal role in increased precision of populations adapted to selection for emergence in a narrow window of time. This study provides another strong evidence supporting the plausibility of extrinsic advantage of circadian rhythms.

Evidence From Other Approaches
Evidence supporting advantages of circadian rhythms is also available from empirical approaches other than those discussed till now. In a study on three species of Drosophila parasitoids, activity/rest and egg-laying rhythms were monitored and these rhythms were found to be out of phase among three species (Fleury et al., 2000). Competitively inferior species, Asobara tabida showed phase of activity earlier than that of the superior species Leptopilina boulardi. Moreover, inferior species showed shorter period (21.3-h) of activity/rest compared with the superior species (24.3-h). Association of shorter τ with earlier phase of activity in inferior species and near-24-h with later phase of activity in superior species suggests a circadian regulation of activity. Possible clock-mediated temporal segregation of activity phases in sympatric species of Drosophila parasitoids suggests the adaptive value of circadian clocks. A recent study (Wyse et al., 2010) found an inverse relationship between deviation of τ from 24-h (period of natural LD cycles) and life span under laboratory conditions in many strains of laboratory mouse, some rodent, and primate species. This relationship was taken as evidence supporting circadian resonance hypothesis.

SUMMARY—GENERAL IMPRESSION FROM AVAILABLE EVIDENCE
Towards the beginning of this review we discussed what adaptation is and what kind of evidence is essential to call.
any trait as adaptation. We concluded this section by saying that a trait evolved through a process of natural selection is regarded as adaptation; thus, naturally, evidence suggesting evolution of the trait by natural selection is required to declare it as adaptation. Discussion on proposed functional significance of circadian rhythms indicates that circadian rhythms are believed to confer advantage to living beings by scheduling their biological functions at appropriate time of the environmental cycle (extrinsic advantage) and/or by coordinating their internal metabolic cycles (intrinsic advantage). In the subsequent sections, we briefly discussed several commonly used empirical methods to collect evidence suggesting evolution of a trait by process of natural selection (adaptation) (Endler, 1986; Larson and Losos, 1996; Rose et al., 1996; Sinervo and Basolo, 1996) and their application to test the proposed adaptive value of circadian rhythms.

Here we summarize the general impression one gets regarding the adaptive value of circadian rhythms, based on the review of evidence available from different empirical methods.

(A) Comparative analysis: Evidence for convergent evolution of circadian rhythms suggests that circadian rhythms are adaptation to pervasive rhythmic selection.

(B) Fitness consequence of experimental manipulation of circadian phenotypes or of rhythmic ecology of organisms: These studies assessed fitness consequences of experimental manipulation of circadian phenotype or rhythmic components of organism’s ecology by estimating variety of fitness components such as growth rate, fecundity, longevity/survivorship, biomass, and in some cases, using more comprehensive measures of fitness such as change in genotype frequency or per capita expectation of future offspring. Experimental manipulations of circadian phenotype were found to influence fitness components demonstrating relationship between circadian phenotype and fitness. This relationship suggests plausible causal link between variation in properties of circadian rhythms and differential reproductive success and thus indirectly demonstrates the action of natural selection on variation in clock properties. Influence of manipulations of rhythmic ecological factors on fitness components provides evidence in support of the notion that rhythmic environmental factors such as LD cycles act as forces of natural selection. Together this evidence can be taken to suggest functional significance of circadian rhythms in their current form.

(C) Correlation between variation in clock properties and environmental variables: Rhythmic natural environmental factors such as light and temperature are believed to be primary components of rhythmic natural selection. Although of 24-h period, environmental cycles vary systematically with latitude in terms of day length, and mean annual temperature; thus, rhythmic selection too varies systematically with geographic location. Therefore, many studies surveyed variation in properties of circadian rhythms or frequencies of allelic variation at clock loci in natural populations to test if such variations are independent of latitude. The presence of correlation between phenotypic or genotypic variations representing circadian rhythms and latitude strongly suggests that such variation has been shaped by geographically varying rhythmic selection. Such correlations suggest the adaptive value of circadian rhythms.

(D) Laboratory selection studies: Systematic laboratory selection experiments on large, random-mating, replicate populations with unselected control populations is a powerful approach to test the causal relationship between imposed selection and trait evolution. Moreover, the possibility of following the evolution of a trait with generations of selection provides direct evidence for trait evolution. Evolution of clock properties in laboratory populations in response to selection for timing of biological functions underscores the importance of circadian organization for scheduling biological processes at appropriate time of environmental cycles and thus suggest the adaptive value of circadian clocks.

Taken together, evidence from multiple independent empirical approaches indeed suggests the adaptive value of circadian rhythms.

CRITICAL LOOK AT EVIDENCE

Study of adaptation usually begins with conjecture(s) about functional significance of an organism’s trait(s) under its biotic/abiotic environment. Therefore, the evidence suggesting evolution of trait(s) by the process of natural selection for its proposed function is essential to demonstrate adaptation. Naturally, comprehensive understanding of adaptation requires evidence for (1) genetic basis of trait variation, (2) consequences of variation in trait for reproductive success, (3) mechanistic explanation of link between trait and reproductive success, and (4) ecological/environmental factors acting as natural selection (Endler, 1986; Larson and Losos, 1996; Rose et al., 1996; Sinervo and Basolo, 1996). Although available evidence creates a general impression that circadian rhythms confer advantage to their owners through their proposed functions, critical examination shows that there are several gaps in our understanding of circadian rhythms as adaptation.

(1) Extrinsic and intrinsic advantages are integral components of adaptive hypotheses about circadian rhythms; therefore, test of intrinsic as well as extrinsic advantage is essential to draw inference. However, in case of many studies, experimental protocol allowed testing of either (a) intrinsic advantage or (b) intrinsic and extrinsic advantages. In most of the studies on nonphototrophic organisms, it was possible to test only intrinsic advantage of circadian rhythms, leaving the contribution of extrinsic advantage untested (Table 1).

(2) In case of studies testing the effect of disruption of circadian rhythms on fitness, non-clock effects of gene mutations or SCN ablations on fitness cannot...
TABLE 1. Evaluation of studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Intrinsic (I) or Extrinsic (E)</th>
<th>Fitness attribute due to clock</th>
<th>Measures of fitness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pittendrigh &amp; Minis, 1972</td>
<td>I, E</td>
<td>Yes</td>
<td>Longevity/survivorship</td>
</tr>
<tr>
<td>von Saint Paul &amp; Aschoff, 1978</td>
<td>I, E</td>
<td>Yes</td>
<td>Longevity/survivorship</td>
</tr>
<tr>
<td>Ouyang et al., 1998</td>
<td>I, E</td>
<td>No</td>
<td>Growth rate</td>
</tr>
<tr>
<td>Woelfle et al., 2004</td>
<td>I, E</td>
<td>No</td>
<td>Growth rate</td>
</tr>
<tr>
<td>Dodd et al., 2004</td>
<td>I, E</td>
<td>No</td>
<td>Growth rate, biomass, chlorophyll</td>
</tr>
<tr>
<td>Yerushalmi et al., 2011</td>
<td>I, E</td>
<td>No</td>
<td>Genotype frequency</td>
</tr>
<tr>
<td>Klarsfeld &amp; Rouyer, 1998</td>
<td>I, E</td>
<td>No</td>
<td>Longevity/survivorship</td>
</tr>
<tr>
<td>Saunders, 1972</td>
<td>I, E</td>
<td>Yes</td>
<td>Development time</td>
</tr>
<tr>
<td>Lone et al., 2010</td>
<td>I, E</td>
<td>Yes</td>
<td>Development time</td>
</tr>
<tr>
<td>Hurd &amp; Ralph, 1998</td>
<td>I, E</td>
<td>No</td>
<td>Longevity/survivorship</td>
</tr>
<tr>
<td>Kumar et al., 2005</td>
<td>I, E</td>
<td>No</td>
<td>Longevity/survivorship</td>
</tr>
<tr>
<td>Allemand et al., 1973</td>
<td>I, E</td>
<td>Yes</td>
<td>Longevity/survivorship</td>
</tr>
<tr>
<td>Sheeba et al., 2000</td>
<td>I</td>
<td>Yes</td>
<td>Longevity/survivorship, fecundity</td>
</tr>
<tr>
<td>Beaver et al., 2002</td>
<td>I</td>
<td>Yes</td>
<td>Survival inside host between host transmission potential</td>
</tr>
<tr>
<td>O’Donnell et al., 2011</td>
<td>I, E</td>
<td>Yes</td>
<td>Longevity/survivorship</td>
</tr>
<tr>
<td>DeCoursey et al., 1997</td>
<td>I, E</td>
<td>No</td>
<td>Longevity/survivorship</td>
</tr>
<tr>
<td>DeCoursey &amp; Krulas, 1998</td>
<td>I, E</td>
<td>No</td>
<td>Longevity/survivorship</td>
</tr>
<tr>
<td>DeCoursey et al., 2000</td>
<td>I, E</td>
<td>No</td>
<td>Longevity/survivorship</td>
</tr>
<tr>
<td>Daan et al., 2011</td>
<td>I, E</td>
<td>No</td>
<td>Longevity/survivorship</td>
</tr>
</tbody>
</table>

Evaluation of studies was based on three criteria: (1) intrinsic (I) or extrinsic (E) hypothesis tested (column 2); (2) whether effects on fitness could be attributed to clock function alone (column 3); and (3) measures of reproductive fitness used (column 4).

(1) In many of the studies testing the adaptive value of circadian rhythms by assessing fitness consequences of effects of manipulation of circadian phenotype or ecology, fitness estimates have been made on the basis of measurements of individual fitness components such as longevity, fecundity behavioral, or morphological traits such as growth rate/development time and body size (mass) that are thought to influence fitness in isolation (Table 1). Fitness is a representation of genotype in future generations (Pekkala et al., 2011) and therefore comprises cumulative influence of all behavioral, physiological, morphological peculiarities of organisms over its life history on the number of offspring contributed to future generations. Thus, inferences about the adaptive value of a trait based on individual measures of life history traits such as longevity and fecundity could be misleading (Prout, 1971).

Apart from issues regarding proxies used for fitness estimation, all these studies (except DeCoursey et al., 1997; Emerson et al., 2008) have been performed on highly inbred laboratory strains of sexually reproducing organisms (e.g., iso-female Drosophila melanogaster strains Canton S, Oregon R) or specific laboratory strains of asexually reproducing organisms (cyanobacteria) that lack segregating genetic variation. However, natural populations harbor segregating genetic variation for a variety of traits, including those related to fitness. Apart from segregating genetic variation, genotype to fitness relationship becomes more complex due to occurrence of dominance, epistasis, and genotype by environment interactions (Wade, 2000). In such a scenario, effects of mutations or any other manipulations on fitness tested on inbred lines represent fitness consequences on a very specific genetic background. Fitness consequences of phenotype or ecology manipulations in inbred lines thus may not be generalized.

(2) In experiments involving manipulation of circadian phenotypes using different light regimes (such as non-24-h LD cycles), non-clock-mediated effects of unnatural light regimes are not separable from clock-mediated effects on fitness. Since light intensities used in laboratory conditions are relatively low, contribution of non-clock-mediated effects on fitness could be considered negligible; hence, fitness consequences of light regime manipulation may be solely attributed to altered circadian phenotype (Table 1).

(3) Studies on latitudinal clines in circadian phenotypes have been successful in providing evidence that suggests adaptive significance of circadian clocks. Since latitudinal clines are purely based on correlation between latitudes and circadian phenotypes or clock allele frequencies, they merely suggest the possible action of natural selection on circadian clocks. Clock genes are known to regulate processes such as development and life span through non-clock function, thus it is hard to distinguish whether the observed clinal variations in clock properties or allele frequencies reflect selection acting on circadian rhythms or they are correlated response to selection on non-clock functions. In addition, in the above studies, populations were collected from distant locations across different geographical locations.
a range of latitudes, whereas there are no data available to test whether the populations have diverged from a common ancestral population or there has been a mixing of genetic variation between populations, which raises the possibility of differences in genetic background between the populations. Therefore, lack of knowledge about selection pressures acting on the populations, and their intensities, and knowledge about the genetic background makes it difficult to conclude that the observed clinal variation in clock properties is due to the action of natural selection on clock function.

(5) Although evolution of circadian clocks in response to rhythmic selection pressure in recent systematic laboratory selection experiments clearly establish the role of rhythmic selection pressure as potential natural selection, mechanistic links between circadian clocks and fitness are yet to be ascertained.

Critical analysis of evidence thus shows that our understanding of circadian clocks as adaptation is incomplete in one or more respects in each of the studies. This suggests that there is a long way to go before we understand the adaptive value of circadian rhythms comprehensively. In this review, we tried to cover the results published in a large number of papers. To keep the list of references to a reasonable length, we have not made citations to the literature exhaustive. Instead, we have tried to be representative and have preferred, as far as possible, to cite reviews or relatively recent papers. Thus, omission of papers from the list of references does not imply that we felt that they are unimportant, rather it merely reflects our ignorance or space constraints.

This article meets the ethical standards of the journal as outlined in Portaluppi et al (2010).

DIRECTIONS FOR FUTURE STUDIES

(1) The general impression that emerges out of our critical analysis of literature is the lack of comprehensive understanding of the adaptive value of circadian rhythms in any of the systems studied so far. This sense of incomplete understanding appears to be a result of absence of one or more key pieces of evidence discussed earlier: (a) genetic basis of trait variation, (b) consequences of variation in trait for fitness, (c) mechanistic explanation of link between trait and fitness, and (d) ecological/environmental factors acting as natural selection. As no single empirical method can provide all the essential evidence, a combination of methods is recommended (Endler, 1986).

(2) Daily cycles of various biotic and abiotic environmental factors make each cycle a complex temporal pattern of challenges and opportunities. Circadian rhythms are believed to confer advantage to living beings by scheduling behaviors and/or physiological processes at appropriate time of the environmental cycle (extrinsic advantage). Many studies tested the adaptive value of circadian clocks by studying the effects of experimental manipulation of circadian phenotypes on the reproductive fitness or its component(s) under mild laboratory conditions such as relatively low light intensities (compared with those in nature), moderate ambient temperature, and ad libitum food. Such conditions do not represent rhythmic challenges faced by organisms under natural conditions; therefore, the extrinsic advantage hypothesis is not testable. The understanding of adaptive value of circadian rhythms is incomplete without test of extrinsic advantage; therefore, testing the effects of such manipulation under natural conditions or at least laboratory conditions mimicking natural challenges is recommended.

(3) Consequence of manipulation of trait/organism’s ecology on fitness is one of the most commonly used approaches to test the adaptive value of a trait. Measuring true fitness is usually practically not feasible. Therefore, surrogate measures that are thought to represent true fitness are used as fitness estimates primarily due to the ease of measurement. Longevity, fecundity, development time/growth rate, and body size (mass) are some of the most commonly used surrogate measures of fitness (Hunt & Hodgson, 2010). Most of the time fitness is estimated by measuring only one surrogate. Moreover, the best measure of fitness (representing true fitness) may vary with the organism’s ecology (Falconer & Mackay, 1996) and hence also with experimental conditions. Therefore, arbitrarily chosen fitness surrogate may lead to erroneous conclusions. Most of the studies that have tested the effects of manipulation of circadian rhythms/their rhythmic ecology have used one or the other arbitrarily chosen fitness surrogates and therefore may not be taken as measures of true fitness. Therefore, composite measures of fitness as used in Emerson et al. (2008) or lifetime reproductive successes (Pekkala et al., 2011) that include several surrogate measures are recommended. More studies on natural populations harboring segregating genetic variation (like Emerson et al., 2008) are required in order to make generalized conclusions.

(4) Recent rigorous selection studies in fruit fly D. melanogaster have demonstrated the plausibility of bringing about change in circadian clocks as a correlated response in populations adapted to selection for the phase of rhythmic process. Although these studies provide strong evidence for evolution of circadian clocks in real time, the causal role of clocks in adaptive change in these populations is still suggestive. With hindsight gained from these studies, the approach of laboratory selection can be exploited further by investigating such populations for genetic and molecular bases of
adaptations and the underlying circadian clocks. Such studies can help elucidate mechanistic link between adaptation and circadian clocks and can thereby help establish the causal role of clocks.

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Declaration of Interest

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Adaptive Significance of Circadian Clocks