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# On the significance of circadian clocks for insects*

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**Abstract**

Circadian clocks are ubiquitous and are believed to provide adaptive advantage to their owners in two different ways: (i) by synchronizing behavioral and physiological processes to periodic factors of the environment, and/or (ii) by coordinating various metabolic processes within the organism. The molecular mechanisms constituting circadian clocks are conserved across a wide range of taxa, and are relatively better understood for the fruit fly *Drosophila melanogaster*. There is growing evidence to show that the circadian architecture of *Drosophila* is multi-oscillatory and in some insects it shows developmental plasticity, i.e. differential response to light regimes experienced during pre-adult stages. It is argued that plasticity of circadian clocks is advantageous to insects living in fluctuating environments. Recent studies in social insects suggest that such plasticity in circadian systems may be crucial to the overwhelming success of social insects.

**Keywords:** Fruit fly, circadian rhythm, genes, adaptation, multi-oscillator, social, honeybee, ant.

## 1. Introduction

Life on earth is exposed to highly predictable daily rhythms of light and temperature. Availability of food, mates and activities of predators are also affected by such periodic changes. It is, therefore, not surprising that many of our behaviors follow a daily periodicity. It is common to see some plants opening their leaves during the day and closing them during the night and others moving them up and down, honeybees visiting food sources precisely at the same time every day, fruit flies eclosing from pupal case close to dawn, and exhibiting daily rhythms of activity, mating, and egg laying. Daily rhythms of behavior and physiology are also common in several species of birds, fishes and mammals. The most obvious explanation given for these rhythms is that organisms mimic passively the environmental periodicity. However, when these organisms are isolated from the periodic environmental factors–by maintaining them under constant laboratory conditions with light, temperature, humidity and sound constant–a large majority of these daily rhythms persists with $\tau$ normally close to but seldom exactly 24 h (such rhythms are called circadian rhythms; *circa* = approximately, *dies* = day). Until recently it was believed that circadian rhythms are characteristics of complex cellular organization and therefore might not be displayed by prokaryotic organisms. This, however, is no longer true; circadian rhythms of photosynthesis and nitrogen fixation have now been reported in a species of cyanobacteria [1], [2]. This ubiquity of occurrence suggests that circadian clocks are adaptive [3]–[8]. Furthermore, some key characteristics of circadian clocks such as the ability to maintain a constant period at different

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temperatures within physiological limit, and the ability to synchronize (entrain) to periodic environmental factors, qualify them as biological chronometers.

Individual values of $\tau$ are often normally distributed around the species mean, usually with a fairly small variance [9], [10]. However, there is enough evidence to show that $\tau$ undergoes modification, according to prior environmental conditions, a phenomenon known as 'after-effects'[11]–[23]. In a recent study, we too have reported the extent of after-effects in the circadian locomotor activity rhythm of four independent populations of *D. melanogaster* [16]. Figure 1 shows representative locomotor activity records of adult flies reared as pre-adults in constant light (LL), light/dark cycle (LD; 12:12 h), and constant darkness (DD) and subsequently assayed in DD regime. The locomotor activity of these flies was recorded individually using an indigenously developed computerised activity recording device [17]. The activity graph (referred commonly as actogram) is obtained by plotting (over a period of 24 h) chronologically daily activity data of each individual one below another. Actograms are often double plotted to facilitate easier visualisation of activity patterns. We found that the average $\tau$ in DD, in the flies that were raised in LL, LD cycles [12:12 h] and in DD regimes were significantly different from each other, suggesting effect of pre-adult light regimes on the circadian organisation. Similar results have also been reported in a few studies on *Drosophila* [20], cockroach [21] and crickets [22], [23]. It appears that circadian clocks of several insect species are labile and may undergo changes due to light regimes experienced during pre-adult development. In addition, the $\tau$ of an individual is also known to undergo spontaneous changes with age [24], [25].

Circadian rhythms are one of the most tractable models to study cellular and molecular mechanisms connecting genes and behavior. The essential rhythm-generating mechanism is cell-autonomous, i.e. isolated cells of many organisms continue to show self-sustained circadian oscillation [26], [27]. The *period* (*per*) locus in *Drosophila* was identified as critical to circadian rhythms as early as 1971, and the molecular mechanisms regulating them have now started to become clear due to fairly recent advances in molecular biology techniques [28]–[34]. It is becoming clear that several genes working in tandem in feedback loops control circadian rhythms at various levels of complexity.

The purpose of this review is to describe our present understanding of the molecular basis of circadian clocks and then discuss their significance for insects. I have also discussed briefly the multi-oscillatory architecture of circadian clocks in *Drosophila* to emphasise the complexity in circadian system and finally have touched upon circadian aspects of social organisation. Although some findings in bacteria, plants and mammals are mentioned, I have mostly limited myself to circadian clocks of fruit fly *D. melanogaster*.

## 2. How do molecular mechanisms generate circadian timings?

The fact that circadian clocks have a genetic basis became clear way back in 1932, when Bünning [35] demonstrated that plants of intermediate periodicity in the so-called 'sleep movements' of their leaves can be produced in the first filial generation by crossing plants of short and long periodicity. However, the major breakthrough came in 1971 in terms of the identification of the *period* locus in *D. melanogaster* [32]. Perhaps the reason behind the delay was the prevalent

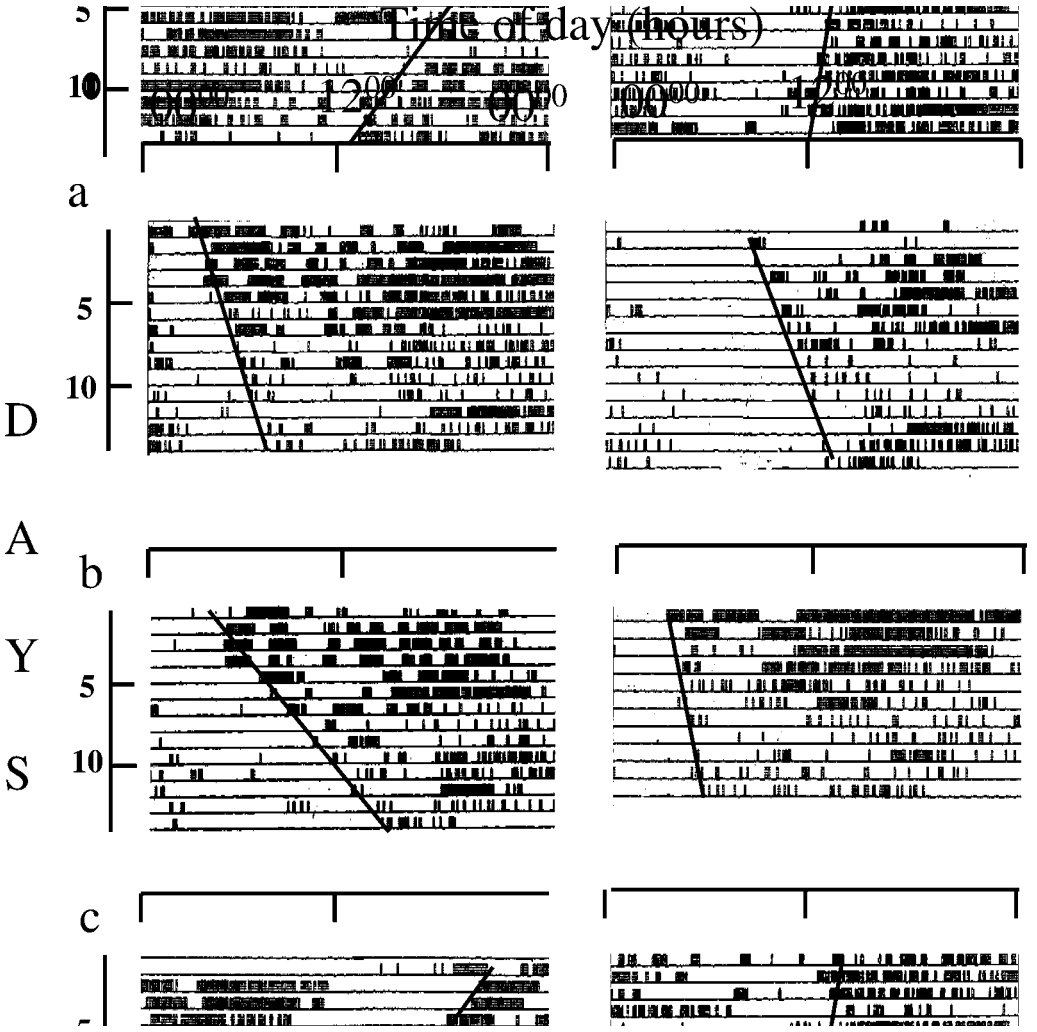


FIG. 1(a–c). Representative locomotor activity records of individual flies. The *t* of individuals reared in LL, LD and DD regimes as pre-adults were (a) 24.33 h, (b) 24.78 h, and (c) 23.24 h, respectively. The abscissa represents time of day while ordinate represents the number of days. Thick bars indicate activity while horizontal lines indicate rest. The lines across the onsets of locomotor activity are indicative of the trend and were not used to calculate the $\tau$ (modified after [16]).

belief among some circadian rhythm researchers that multiple genes control circadian clocks. Since mid-80s, when the first clock gene *period* (*per*) was cloned, thousands of papers have been published describing newer genes and their role in the time-keeping mechanism. In the

recent past, several molecular models have been proposed, most of which are based on *in vitro* observations. The surge in *in-vitro* analyses has led to frequent revisions of these models. Despite the recent rush in molecular genetic approach to understand regulation of circadian clocks we have very little idea about how the self-sustained oscillations are generated, how they are translated into behavioral rhythms and how these molecular oscillations are fine-tuned by various Zeitgebers [time cues] [33], [34]. The latest molecular model postulated while this paper was being written is described in Fig. 2.

The *Drosophila* molecular circadian clocks consist of a series of precisely timed steps that are kept deliberately slow. These steps are part of three interlinked transcription–translation (TTL) feedback loops that function in tandem [29], [31], [34]. Such loops have also been described for cyanobacteria [36], [37], *Neurospora* [28], [38], mammals [30], [39] and recently for plants [40]. Different sets of genes regulate circadian rhythmicity in a more or less conserved mechanism in a wide range of organisms. The TTLs generate self-sustained oscillations in behaviour and physiology even in constant conditions [41]–[43] that can be entrained by LD cycles [44]–[48].

In *Drosophila*, mRNA and protein levels of two clock genes, *period* (*per*) and *timeless* (*tim*) (*per*: 33, 41; *tim*: 49-52) show rhythmic abundance. The mRNAs peak during the early part of the night (ZT13–ZT16) [53], [54], whereas proteins peak late in the night (ZT18–ZT24) [55]–[58]. The proteins PER and TIM enter the nucleus as dimer complex formed by protein–protein interaction and negatively regulate their own transcription [59]. Although PER and TIM do not have DNA-binding domains, they influence their own transcription by physically associating with other transcription factors [60]–[63]. Recent experiments have demonstrated that PER alone may be sufficient to negatively regulate its own transcription [64], [65].

In flies with *clock* (*clk*) [66] and/or *cycle* (*cyc*) [67] mutation, *per* and *tim* expression is low and not cycling, suggesting that these genes act as positive regulators for *per* and *tim* transcription. In addition to PAS, a protein–protein interaction domain [66]–[68], CLK and CYC also contain a basic helix-loop-helix (bHLH) region which allows further protein–protein interaction and most importantly binding to DNA [66], [67]. The CLK/CYC heterodimer binds to the E-box in *tim* and *per* promoter region, which subsequently leads to production of high levels of PER/TIM complex, which then moves into the nucleus and interacts with CLK/CYC. Since PER persists in the nucleus longer than TIM during early part of the day [55]–[57], and even as a monomer is an efficient transcriptional repressor of *per* and *tim* genes [64], the role of TIM in the negative feedback loop is arguable [69].

The *clk* gene is rhythmically expressed 12 h out of phase compared to *per* and *tim* expression, i.e. *clk* mRNA peaks late in the night (ZT23–ZT4) [60], [69]. The *Drosophila cyc*, on the other hand, does not cycle and is constitutively expressed in the cytoplasm. It is believed that PER/TIM dimer activates *clk* transcription because *clk* mRNA is high at times when PER/TIM levels are high, and in $per^{01}$ and $tim^{01}$ mutants *clk* mRNA is expressed at very low levels [70]. Mutants lacking functional *clk* and *cyc* express *clk* mRNA at constitutively high levels, suggesting that PER/TIM acts as a derepressor of *clk* transcription, and that CLK/CYC may be the repressors [31], [71]. Recently it was shown that the CLK/CYC activates the transcription of the gene *vrille* [*vri*] and *Par domain protein 1* (*Pdp1*). *clk* transcription is first repressed by VRI and then

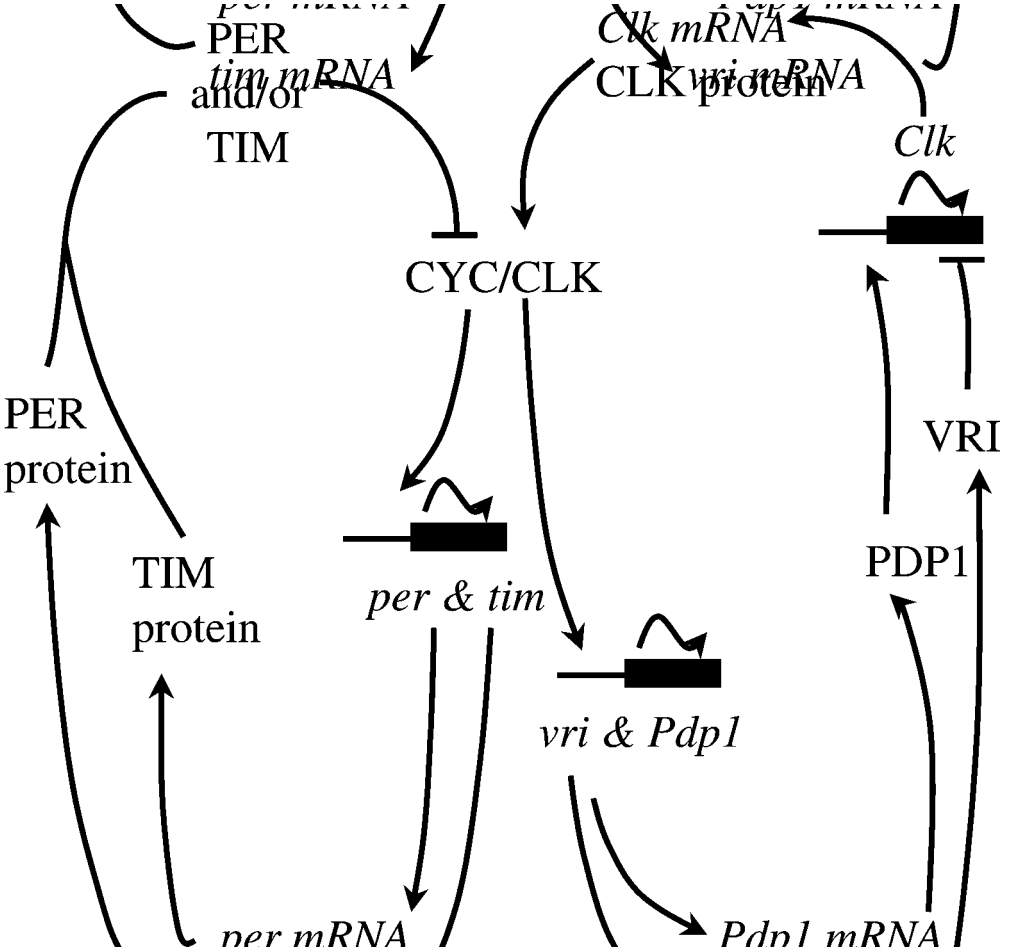


FIG. 2. The model of the *Drosophila* molecular circadian clock involving the genes *period* (*per*), *timeless* (*tim*), *Drosophila Clock* (*clk*), and *Cycle* (*cyc*); the mRNA and protein levels of the two clock genes, *per* and *tim* show rhythms in abundance. The mRNAs peak during the early part of the night (ZT13–ZT16), whereas the proteins peak late in the night (ZT18–ZT24). PER and TIM enter the nucleus as a complex formed by protein–protein interaction. The gene *clk* is rhythmically expressed 180° out of phase compared to *tim* and *per* expression, i.e. *clk* mRNA peaks late in the night (ZT23–ZT4) while *Drosophila cycle* doesn't cycle, and is constitutively expressed in the cytoplasm. It is believed that PER/TIM activates *clk* transcription, and CLK activates the transcription of the genes *vrille* (*vri*), and *Pdp1* whose protein inhibits transcription of *clk* as well as other output genes.

activated by PDP1 [31], [72]. Repression and activation of *clk* are separated by the different phases of VRI and PDP1.

According to the molecular model of *Drosophila* circadian clocks, high levels of PER/TIM within the nucleus at night block the action of positive transcription factors CLK/CYC on *per*

and *tim*, but derepress *clk*. Therefore, *clk* mRNA level is high when *per* and *tim* mRNA levels go down. PER/TIM levels fall early in the morning, first TIM gets degraded and then the protein double time (DBT) degrades PER [71], releasing the CLK/CYC dimers to repress *clk* transcription. After a delay, due to DBT-mediated degradation of PER, PER and TIM form dimer and enter the nucleus to derepress *clk*, and thus the cycle continues [31], [71]. DBT promotes the progressive phosphorylation of PER, leading to rapid degradation of hyper-phosphorylated isoforms by the ubiquitin-proteasome pathway [73]. *SLIMB*, an F-box/WD 40-repeat protein, functioning in the ubiquitin-proteasome pathway, interacts preferentially with phosphorylated PER and stimulates its degradation [73]. Overexpression of *slimb* or expression in clock cells of a dominant-negative version of *slimb* disrupts normal rhythmic activity in flies.

The rhythmically expressed gene *cryptochrome* (*cry*) appears to act both as a photopigment as well as a core clock component [47], [74]. CRY protein is a flavoprotein belonging to the family of 6,4-photolyases, and is able to absorb light and act as a photopigment [47], [75], [76]. The *cry* mutant ($cry^b$), with modified flavin-binding sites due to one amino-acid substitution, exhibits an altered circadian phenotype [75]. Experiments using S2 cell lines demonstrated physical interaction between CRY and TIM, and the ability of CRY to inhibit PER/TIM repression of dCLK/CYC-mediated E-box transcription [77]. However, the effect of CRY on transcription requires constant light (in nucleus), while in the cytoplasm CRY is able to bind to TIM in DD.

The gene *vri* [78], [79] is rhythmically transcribed in the lateral neurons (LNs) in phase with *tim* and *per.* The LNs are the sites of putative circadian pacemakers for locomotor activity rhythm in *Drosophila*; overexpression of *vri* mimics blocking of *per* and *tim* expression. In addition, *vri* is under the control of the same loops that regulate *per* and *tim* mRNA levels [31], [78].

Some of the genes are awaiting confirmation of their roles in the circadian molecular mechanisms. The gene *lark*, whose product encodes an RNA-binding protein, is involved in eclosion but not locomotor activity rhythm [80], [81]. The protein LARK cycles in abundance in a restricted number of neurons in the central nervous system and the ventral lateral neurons LNs of late stage pupae, with higher levels in the day, suggesting its role in determining the gate of eclosion. The protein co-localizes with a neuropeptide CCAP which plays a role in insect ecdysis [81 and reference therein]. In *per*-null mutants, the cycling of LARK disappears.

The gene *pigment dispersing factor* (*pdf*), probably the primary factor for transmitting information about circadian time to the effector organs, responsible for executing circadian locomotor activity rhythm [82], [83], is expressed in a subset of the larval and adult brain pacemaker cells. Although the transcript of the gene does not cycle, its protein does at specific nerve terminals, in a manner dependent on functional *per* and *tim*. The circadian behavior of *pdf* mutant is similar to transgenic with ablated circadian pacemaker cells.

A number of output genes has also been identified and their role in the circadian regulatory mechanisms has been described [84]. The transcript and the protein of one of the recently identified genes *take out*, localize to corpora cardiaca, crop, and antennae and is found to oscillate [85]. The expression of this gene is induced by starvation and has a sequence similarity with the juvenile hormone-binding protein as well as the product of the gene adjacent to *per* in *Drosophila* [86].

## 3. Why do we have circadian clocks?

Circadian clocks are believed to confer adaptive value in two ways: (i) for coordinating various cyclic processes within an organism (intrinsic adaptive value) and (ii) enabling organisms to keep track of local time by maintaining a stable phase relationship with cyclic environmental factors (extrinsic adaptive value).

Though some studies on organisms living in the deep sea and subterranean caves reported either no overt circadian rhythms or rhythmic processes with periodicities very deviant from 24 h [87], [88], more recent studies on cave-dwelling organisms have reported several instances of intact circadian clocks [89], [90]. We, in our laboratory, used four populations of *D. melanogaster* that have been reared under constant conditions inside incubators where light (of about 100 lux intensity), temperature (24 ± 1ºC) and relative humidity (85%) were kept constant for over 600 generations, to investigate whether circadian clocks confer intrinsic adaptive value. We studied eclosion (act of emergence of adult flies from pupal case), locomotor activity and oviposition (act of egg laying) rhythms to see whether these flies have retained functional circadian clocks after living in an aperiodic environment for several hundreds of generations. Time series data obtained for eclosion and oviposition rhythms were subjected to Fourier spectral analysis using STATISTICA™ (Statsoft) [91] and statistical significance of observed patterns in the periodogram was tested using the technique suggested by Siegel [92] (Fig. 3). Locomotor activity data was analyzed using linear regression lines drawn across onsets of activity [7]. All three rhythms free-ran in constant conditions and interestingly showed stable entrainment to a wide range of laboratory LD cycles (10:10 h, 12:12 h and 14:14 h) [5]–[7], [93]. The light regime in which the locomotor activity rhythm was assayed also influenced the fraction of flies exhibiting a circadian rhythmicity (Fig. 4). The fraction was 26.4% and 77% when assayed in LL and DD, respectively. In LL, the fraction of rhythmic flies (experiment 2) increased significantly after an exposure to dark pulse during the pre-adult stage (experiment 1) (Fig. 4). The results thus suggest that a large fraction of flies across all four populations (LL1, LL2, LL3, LL4) that lived in an aperiodic environment for several hundred generations has retained the capacity to exhibit circadian rhythms in DD, and even in bright LL (Fig. 4). Fitness studies on *D. melanogaster* populations suggest that traits which do not confer any fitness advantage under a given environment are rapidly (within 100–150 generations) affected adversely by mutation accumulation [94], and if the trait involved bears some cost, the decline can be even faster (within 20–50 generations) [95], [96]. The fact that the flies used in our experiments exhibit circadian rhythms of locomotor activity [7], oviposition [6] and eclosion [5], which can entrain stably to LD cycles of wide range of periodicities [93], suggests that circadian rhythms do have some intrinsic adaptive value.

Some attempts have also been made to demonstrate extrinsic adaptive significance of circadian clocks in periodic environments, by estimating longevity of *D. melanogaster* [97] and blow fly *Phormia terraenovae* [98] under LD cycles of several periodicities and in LL. Longevity was used as a measure of fitness in most previous studies on adaptive significance of circadian rhythms. Hence it was speculated that organisms with periodicity close to those of the environmental periodicity have greater fitness advantage compared to other periodic regimes deviating from the 24 h framework. However, in a separate study Klarsfeld and Rouyer [99] reported contrasting results with *per* mutants of *D. melanogaster*. The advantage of possessing

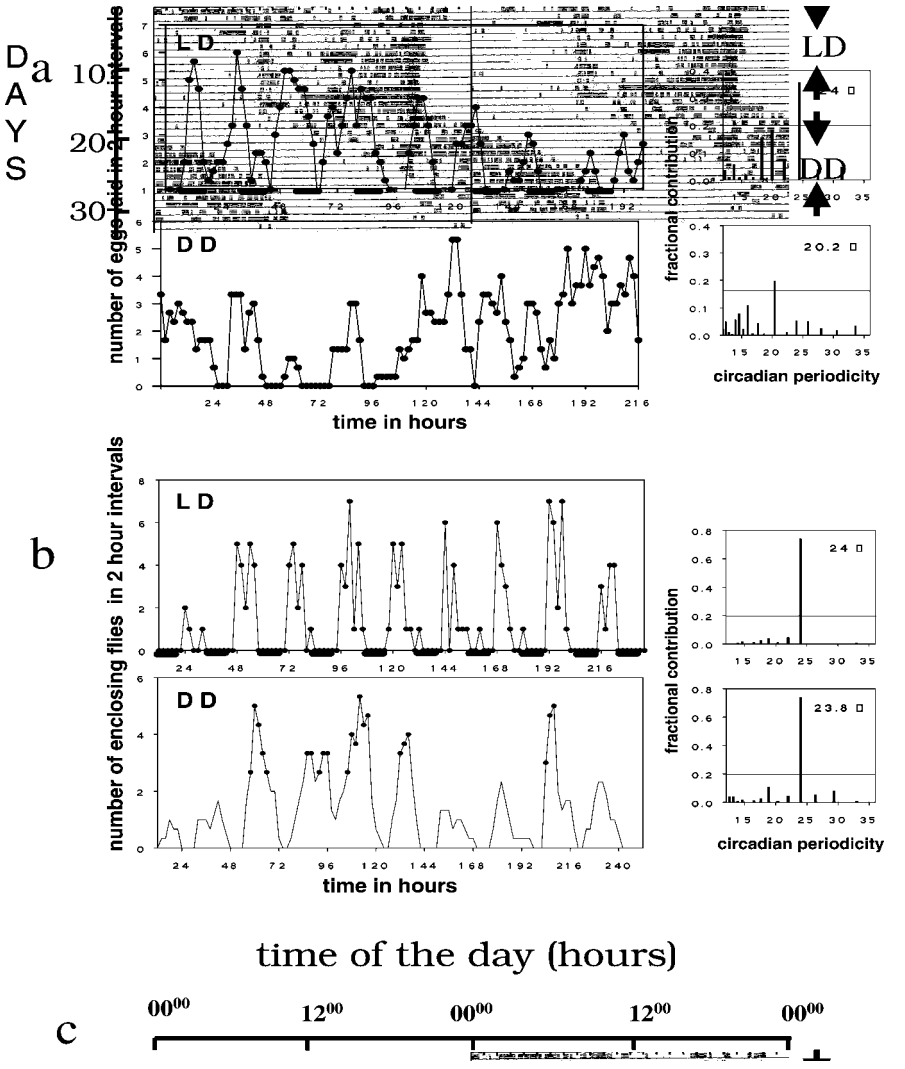


FIG. 3. One representative figure each from the (a) eclosion (act of emergence of adult flies from pupal case), (b) locomotor activity, and (c) the oviposition (number of eggs laid by a female in 2 h interval) rhythm of four populations (LL1, LL2, LL3, LL4) of *D. melanogaster* which lived in an aperiodic environment for more than 600 generations. The three rhythms free-ran in constant conditions and showed stable entrainment to laboratory LD cycles (12:12 h; modified after [5]–[8]).

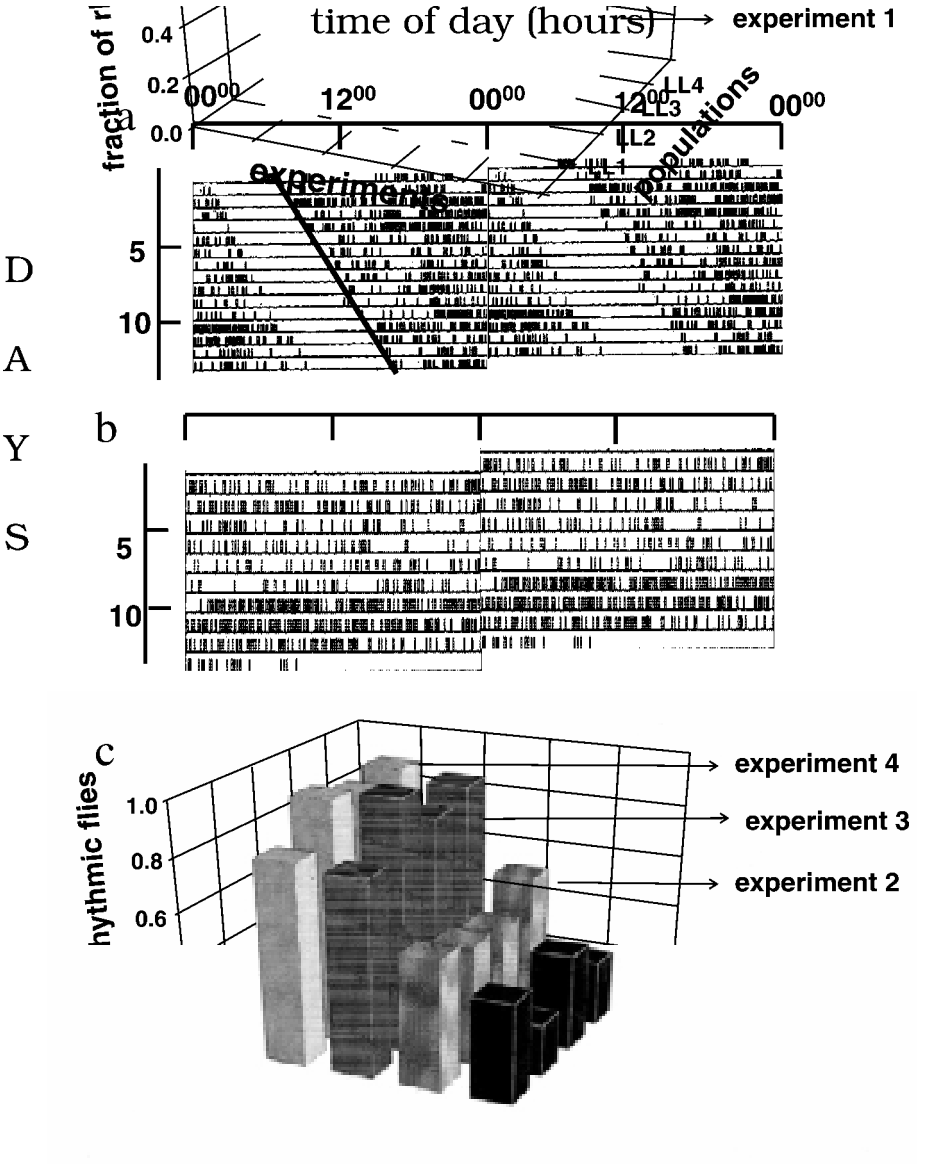


FIG. 4. Representative locomotor activity records of flies assayed in LL regime exhibiting (a) circadian rhythm and (b) arrhythmicity. Abscissa shows time of day while ordinate shows number of days. Thick bars indicate activity, while horizontal lines indicate rest (modified after [7]). (c) Fraction of flies that exhibited circadian locomotor activity rhythm when assayed after subjecting them to four different experimental protocols. In all four experiments, the flies were maintained in LL during the pre-adult stage. In experiment 1, the locomotor activity of adults was monitored in LL (intensity of about 100 lux) for 30 days immediately after eclosion. In experiment 2, freshly laid eggs were subjected to 12 h of darkness after which the same protocol as in experiment 1 was followed. In experiment 3, the locomotor activity of adults was first monitored under LD 12:12 h for 15 days and then in DD for the next 15 days, while in experiment 4 the adult locomotor activity was monitored in DD for the first 15 days after eclosion. In experiments 1, 3 and 4 both males and females were assayed, while in experiment 2 only male flies were assayed (modified after [7]).

circadian clocks was also assessed in golden-mantled ground squirrels *Spermophilus lateralis* [100]–[102] and Siberian chipmunks *Eutamias sibiricus* [103]. Removal of the suprachiasmatic nucleus (SCN, which is the site of circadian pacemakers of mammals) did not cause any significant reduction in longevity under laboratory conditions. However, the longevity of SCN-lesioned animals was reduced in field conditions, possibly due to impairment of the circadian rhythm of activity and consequent predation [104], [105]. Longevity was used as a measure of fitness in most of the previous studies on adaptive significance of circadian rhythms. It is well documented in *Drosophila* that fecundity and longevity trade off [106], and therefore using longevity as the sole indicator of fitness could be erroneous.

The cyanobacterium *Synecchococcus* sp. was used to study the adaptive significance of circadian rhythms [4]. Three mutant strains with distinct $\tau$ of bioluminescence rhythm were competed against one another in various combinations under several LD cycles (LD 12:12 h, 11:11 h, LD 15:15 h) (Fig. 5). The results showed that the strain whose periodicity closely matched those of the LD cycles, out-competed others [4], thus suggesting that circadian rhythms do confer extrinsic adaptive value.

Several studies on *Drosophila* reported latitudinal clines, with the higher northern latitude populations showing a difference in timing of eclosion, locomotor activity and oviposition rhythms in LD cycles than the ones from lower latitude [107]–[109]. The observed latitudinal clines were taken as evidence for the adaptive significance of circadian rhythms [69]. However, the existence of latitudinal clines for circadian rhythms can only be considered as indirect evidence for adaptation as there may be many factors that are uncontrolled in these studies such as the ancestry and other geographical factors that may be different in different regions. Moreover, the fact that these studies have been mostly done on inbred cultures which have been raised in the laboratory from isofemale lines further complicates the issue [8].

## 4. How many circadian clocks do we have?

Multiple circadian clocks (oscillators) have been suggested to be operational in some organisms. The marine dinoflagellate *Gonyaulax polyedra*, exhibits circadian rhythms in photosynthesis, cell aggregation, superoxide dismutase production, phototaxis, bioluminescence and cell division [110], which are under the control of two oscillatory subsystems [111]. In LL, the locomotor activity rhythm of the Arctic ground squirrel and Syrian hamsters broke up into two components, and each component free-ran with a different period [11], [112]. The Saturnid moths possess two independently light entrainable circadian clocks, one in the forebrain and another in the prothoracic gland [113]. At least three different clocks are believed to regulate initiation of larval wandering, diapause induction and pupal eclosion rhythms in the flesh fly *Sarcophaga argyrostoma*; adult locomotor activity, the deposition of cuticular growth layers on thoracic apodemes and duration of larval wandering, are also likely to be controlled by different circadian clocks [114]. Therefore the circadian architecture in several organisms appears to be multi-oscillatory [110], [115], [116].

In *D. melanogaster*, two physiological processes, vitellogenesis and egg retention regulate oviposition [117]. Vitellogenesis, which is controlled by the circadian clocks, could play a major

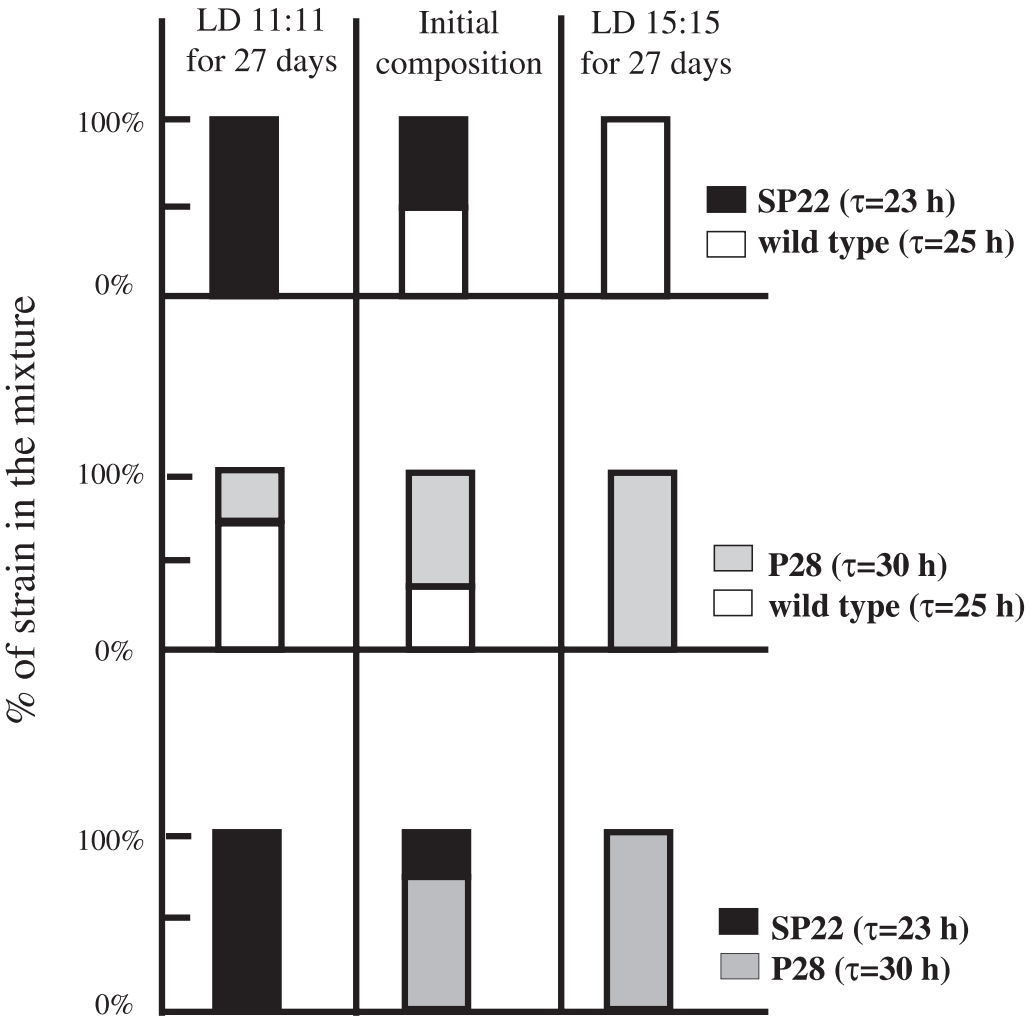


FIG. 5. Three mutant strains of the cyanobacterium *Synecchococcus* sp. with distinct $\tau$ were competed against one another in various combinations under several LD cycles (LD 12:12 h; 11:11h, LD 15:15 h). The results showed that the strain whose periodicity most closely matched the LD cycles, out-competed others (modified after [4]).

role in the expression of free-running oviposition rhythms under constant conditions [117]. At the molecular level, *per* mRNA levels cycle in tissues such as the LNs, the corpora cardiaca which regulates the eclosion rhythm, gut, malphigian tubules, and the male reproductive system [118]–[121], but not in ovaries [27], [122] although oviposition is known to follow a circadian pattern [6], [123]. These results suggest that the oviposition rhythm in *Drosophila* may not be under the control of circadian clock mechanisms involving *per* gene.

In *D. pseudoobscura* the $\tau$ and the PRC of eclosion and locomotor activity rhythms were reported to be different, which suggest that separate circadian clocks may control these rhythms

[124]. In *D. melanogaster* we reported that the *t* and the *y* of eclosion, locomotor activity and oviposition rhythms were significantly different from each other (Fig. 6) [125]. In a separate study, Helfrich [126] had reported that in *D. melanogaster* the $\tau$ of eclosion rhythm and those of the locomotor activity rhythm were different. These results suggest that different circadian clocks control eclosion, locomotor activity and oviposition rhythms.

In the $per^{01}$, $tim^{01}$, and *disco* mutants of *D. melanogaster*, locomotor activity and eclosion were arrhythmic [127]. In another study, the $\tau$ of oviposition and activity rhythm of the *per* mutants and wild type flies were found to be significantly correlated. It was concluded that the *per* gene influences the circadian rhythmicity of both activity and oviposition in a similar manner [123]. The fact that these rhythms can be modified proportionally in *per* mutants rules out the possible role of completely independent circadian pacemakers controlling these three rhythms. Several mutations, like *ebony* and *lark* affect, however, either eclosion or locomotor activity rhythm [128]. These results suggest that different circadian oscillators, if not clocks, control different circadian rhythms in *D. melanogaster* [125].

**5. What are the consequences of social organization on circadian clocks?**

The influence of social organization on circadian rhythms can be best understood in social insects and a good amount of work has already gone into understanding this in honeybees. For the survival of a social insect colony a number of tasks need to be performed simultaneously [129]. Changes in colony sizes, age structures associated with colony development, inter-individual interactions, time of year, food availability, predation pressure, and climatic conditions, present the individuals of a social insect colony with additional challenges [129]. Circadian rhythms in social insects are believed to have played an important role in the development and maintenance of social structure [130]. The activities of various castes are integrated, enabling social insect colonies to develop and produce reproductives [131].

The activity of an entire colony of the Asian Cape honeybee *Apis mellifera capensis* followed a circadian pattern in DD, suggesting the presence of endogenous circadian clocks and their synchronization by social cues [132]–[136]. The timing of activity of an isolated bee drifted from that of the colony, and the $\tau$ of activity rhythm of the individuals were either longer or shorter than the $\tau$ of their parent colonies, suggesting mutual synchronization of individuals while in colony [137]. Honeybees are known to synchronize their foraging activity in both field and laboratory conditions to periodic availability of food [134], [135], [138]–[149]. A possible mechanism for this synchronization could be physical contacts [150], [151], similar to the social synchronization reported in humans [152], [153], beavers [154], bats [155], some other vertebrates (fish [156]; mouse [157], [158]; hamsters [159]) and recently in *Drosophila*, a species which was considered to be the least social amongst insects [160].

The honeybee *Apis mellifera capensis* has evolved age-dependent polyethism to coordinate tasks in its colony; younger bees perform in hive tasks such as taking care of the queen and the broods and cleaning the hive while older bees perform outdoor tasks such as collecting nectar and pollen and guarding the nest [129]. The state of circadian clocks under such age-dependent polyethism has been extensively investigated in honeybees by monitoring daily patterns of task

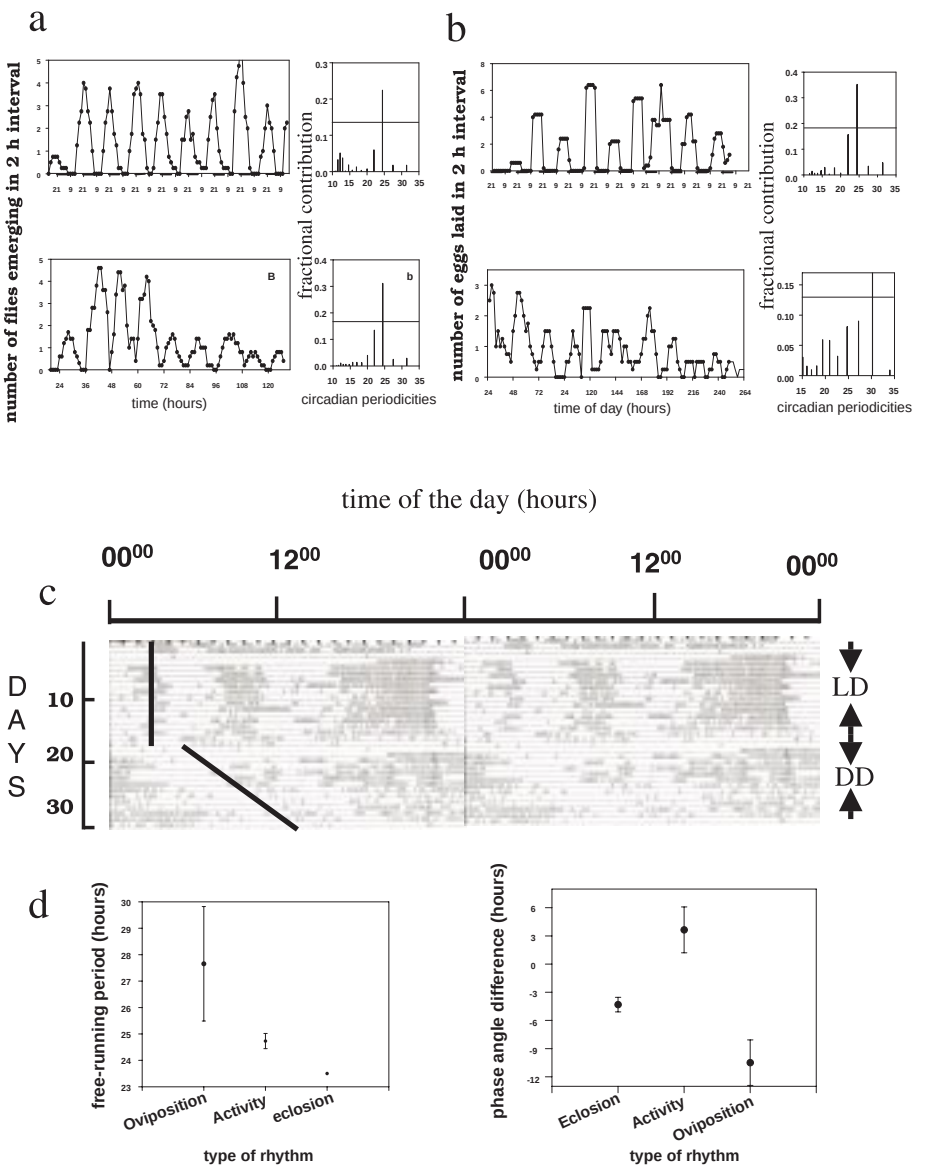


FIG. 6. (a) Time series data of number of eclosing flies in one representative vials in light/dark (LD) cycles (upper panel) and in continuous darkness (DD) (lower panel). The corresponding periodograms show a significant contribution of 24 and 23.6 h periodicities. (b) Time series data of number of eggs laid by a female fly in light/dark (LD) cycles (upper panel) and in continuous darkness (DD) (lower panel). The corresponding periodograms show a significant contribution of 24 h and 30 h periodicities. (c) Locomotor activity records of a single fly for the first 15 days in light/dark (LD) cycles, followed by the next 15 days in continuous darkness (DD). The free-running period ($\tau$) of the fly was 24.59 h. (d) Plot of free-running periods ($\tau$) and the phase relationship ($\psi$) of eclosion, locomotor activity and oviposition rhythms with the error bars representing 95% confidence interval around the mean for visual hypothesis testing. The values of the free-running period of the eclosion, locomotor activity and the oviposition rhythms were 23.64 h, 24.73 ± 0.29 h (mean ± 95% CI), 27.66 ± 2.16 h (mean ± 95% CI), respectively (modified after [125]).

performance within the colony [161], [162]. The younger bees (for the first 3 weeks of adult life) that perform hive tasks round the clock, do so without any daily pattern of activity, while the older bees that collect nectar and pollen exhibit circadian rhythms of activity [129]. The younger honeybees (during the first 3–5 days after eclosion) do not show any rhythmicity of activity [133], oxygen consumption [163] and temperature regulation [164]. Furthermore, in honeybees the *per* mRNA levels also appear to be correlated with age [139], older bees having higher levels of oscillating *per* mRNA than the younger ones.

Honeybee workers have the unique ability to accelerate/decelerate development depending upon the colony needs [130]. Under situations where the colony has a deficit of foragers, the younger bees accelerate development and start foraging precociously; by contrast, foragers revert to performing nursing when the colony has fewer nurses [130]. Such plasticity in the behavioral development of honeybees could present a compelling challenge to their circadian clocks in these workers, and it appears that honeybees have evolved mechanisms to deal with it; the precocious foragers develop circadian rhythmicity and the overage nurses become arrhythmic [131].

Several species of ants occupy distinct temporal niche in the natural environment [165], most probably for temporal partitioning of resources. Species like *Myrmecia, Rhytidoponera*, *Docryon* and *Iridomyrmex humilis* start activity during mid-day and continue to be active throughout the day, whereas other species like *Colobostruma* and *Camponotus*, make their appearance in a regularly staggered succession during the night [166]. Ant species such as *Pogonomyrmex californicus*, *Pogonomyrmex rugous*, *Messor andrei*, *Messor pergandrei*, *Formica pilicornis* and *Myrmecocystus mimicus* exhibit circadian activity/rest rhythm under constant conditions [167]–[170]. The activity/rest rhythm remains unchanged when some fragments of the ant colony were kept under LD cycles [171]. In a separate study the activity/rest rhythm of several species of *Formica* were found to entrain to 12:12 h LD and temperature cycles [172], [173]. Furthermore, in ants, the virgin queens exhibited circadian rhythm of activity in DD, which apparently disappeared after mating [174].

We, in our laboratory, have extensively studied the locomotor activity rhythm of several castes of the social ant *Camponotus compressus* under constant laboratory conditions (Sharma VK, Lone S, Goel A, Chandrashekaran MK, unpublished manuscript). The results suggest that different castes of workers follow different strategies to maintain coordination in the colony. Our results along with those obtained for honeybees suggest that social insects maintain flexibility in their circadian clocks perhaps to increase the efficiency of the day-to-day functioning of the colony.

## 6. Conclusions

Circadian clocks in the fruit fly *D. melanogaster* are developmentally plastic; adult flies show altered circadian patterns depending upon the light regimes experienced during pre-adult stages or during early adulthood. Such plasticity is viewed as an adaptive strategy of organisms living in fluctuating environments.

The molecular mechanisms regulating circadian clocks in the fruit fly *D. melanogaster* has been worked out in detail and it is believed to consist of interlocked feedback loops based on transcription and translation of the genes *per*, *tim* and *clk*. The CLK and CYC activate *per* and *tim* transcription and repress transcription of *clk* via VRI, and PDP1 perhaps by activating the transcription of the gene *vri* and *Pdp1*. PER and TIM, on the other hand, either acting independently or in a combined manner, inhibit its own transcription by inactivating CLK/CYC [31].

Empirical studies on organisms living in periodic and constant environments suggest that circadian clocks confer adaptive value by coordinating various cyclic metabolic processes within the organism to external environmental cycles. The evidence ranges from persistence of circadian rhythms in organisms living in constant environments to those having fitness advantage in periodic environments due to circadian clocks. Although the results of most of these experiments suggest that circadian clocks are adaptive none of these evidences rigorously demonstrate their adaptive significance. In order to prove convincingly that circadian clocks are adaptive, one needs to demonstrate that organisms can evolve circadian rhythmicity and therefore have superior fitness in their parental environment compared to any other environment. Laboratory selection studies (both artificial and natural) need to be carried out in various periodic and aperiodic environments, preferably in organisms with short generation times.

Several studies suggest that the circadian architecture in *D. melanogaster* is multi-oscillatory. However, at the molecular level the control of various behavioral rhythms is not clearly understood. Extensive studies are required at behavioral, physiological and molecular levels to understand the multi-oscillatory control of circadian clocks.

The role of circadian clocks in social organization has been investigated extensively in honeybees. Circadian clocks are believed to have played an important role in the development and maintenance of social colonies. Changes in colony size, forager to nurse ratio and colony age structures result in correlated changes in circadian rhythms. It also appears that the plasticity of circadian clocks could be crucial in maintaining coordination among various castes, enabling social insect colonies to develop and reproduce. Extensive studies, similar to the ones in honeybees, need to be carried out on other social organisms to explore the role of circadian clocks in social organization.

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### Nomenclature

**Zeitgeber time (ZT)**–the time in the external environment, ZT0 coincides ‘light-on’ and ZT12 ‘lights-off’ of 24 h (12:12 h) LD cycles.

**Free-running period ($\tau$)**–time interval between recurrences of a given phase of circadian rhythm under constant conditions.

**Phase relationship between the rhythm and the external environment ($\psi$)**–time interval between a well-defined phase of the rhythm (e.g. onset of activity or peak of eclosion) and a phase of the LD cycle (e.g. 'light-on' of laboratory LD cycle).

**Phase response curve (PRC)**–a plot of phase shifts in the circadian rhythm evoked by exposure to brief stimuli at various phases as a function of time of exposure.

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