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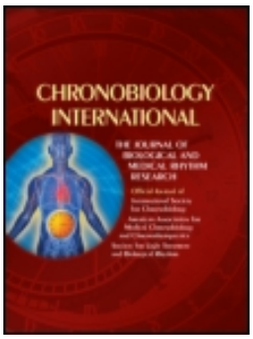

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# Cyclic Presence and Absence of Conspecifics Alters Circadian Clock Phase But Does Not Entrain the Locomotor Activity Rhythm of the Fruit Fly *Drosophila melanogaster*

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Circadian clocks use a wide range of environmental cues, including cycles of light, temperature, food, and social interactions, to fine-tune rhythms in behavior and physiology. Although social cues have been shown to influence circadian clocks of a variety of organisms including the fruit fly *Drosophila melanogaster*, their mechanism of action is still unclear. Here, the authors report the results of their study aimed at investigating if daily cycles of presence and absence (PA) of conspecific male visitors are able to entrain the circadian locomotor activity rhythm of male hosts living under constant darkness (DD). The results suggest that PA cycles may not be able to entrain circadian locomotor activity rhythms of *Drosophila*. The outcome does not change when male hosts are presented with female visitors, suggesting that PA cycles of either sex may not be effective in bringing about stable entrainment of circadian clocks in *D. melanogaster*. However, in hosts whose clock phase has already been set by light/dark (LD) cycles, daily PA cycles of visitors can cause measurable change in the phase of subsequent free-running rhythms, provided that their circadian clocks are labile. Thus, the findings of this study suggest that *D. melanogaster* males may not be using cyclic social cues as their primary zeitgeber (time cue) for entrainment of circadian clocks, although social cues are capable of altering the phase of their circadian rhythms. (Author correspondence: vsharma@jncasr.ac.in, vksharmas@gmail.com)

**Keywords:** Circadian, *Drosophila*, Social cycles, Synchronization, Synchronizer, Zeitgeber

## INTRODUCTION

Circadian clocks generate oscillations with near 24-h periodicity and keep track of local time by entraining to external time cues (zeitgebers) in a way that the period of rhythms they control becomes indistinguishably close to 24 h (Daan & Aschoff, 2001; Dunlap et al., 2004). These clocks make use of well-developed signal transduction systems, dedicated to the perception of external time cues that can convey temporal signals to circadian clocks in a way that they can be translated to effectively modulate overall behavior and physiology (Duguay & Cermakian, 2009). Such mechanisms for the perception of light signals have been quite extensively studied in mice and fruit flies *Drosophila melanogaster* (Dunlap et al., 2004; Emery et al., 1998, 2000; Helfrich-Förster et al., 2001; Stanewsky et al., 1998). Although light is the primary, and perhaps most reliable, zeitgeber for circadian clocks in a wide variety of organisms, there is sufficient evidence to suggest that nonphotic cues, such as temperature, food, and social cycles, may also serve as zeitgeber (Dunlap et al., 2004; Saunders, 2002).

Many organisms use the presence of other individuals from the same or other species as time cues to the extent that the cyclic presence and absence (PA) entrains their free-running rhythms. For example, in organisms such as mice, bats, and birds, PA cycles of conspecifics entrain circadian locomotor activity rhythm (Gwinner, 1966; Marimuthu et al., 1981; Menaker & Eskin, 1966; Viswanathan & Chandrashekaran, 1985). In the bat species *Hipposideros speoris*, PA cycles of free-living conspecifics entrain circadian activity rhythm of individuals maintained in captivity inside a cave (Marimuthu et al., 1981), whereas rhythms of a different species of an emballonurid bat (*Taphozous nudiventris kachhensis*), also kept captive in the same cave, remain unaffected, suggesting that social communication of temporal information in bats is species specific (Marimuthu & Chandrashekaran, 1983). Similarly in mice, PA cycles of

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the mother entrain the circadian locomotor activity rhythm of pups (Viswanathan & Chandrashekaran, 1985), for at least 23–26 days of postnatal development (Viswanathan, 1999). Furthermore, in the Syrian hamster *Phodopus sungoru*, foster mothers were shown to be able to synchronize rhythms of pups that had previously been entrained 12 h out of phase by their biological mothers (Duffield & Ebling, 1998), suggesting that PA cycles can re-entrain circadian rhythms in hamsters. In the European rabbit *Oryctolagus cuniculus*, nursing by mother just for few minutes every day was able to entrain the oscillation of clock-gene expression in the hypothalamus (Caldelas et al., 2007). The above studies suggest that PA cycles serve as zeitgeber for the circadian clocks of a variety of mammalian species. Although the precise nature of social signals still remains elusive, some likely candidates for signal transduction are via vision, olfaction, sound, and physical interactions (Davidson & Menaker, 2003).

Social cues are found to entrain circadian clocks of many insect species, including eusocial honeybees. Honeybees foragers trained to feed at a particular time of the day in one hive and then transferred to another hive in which the training schedule was different were found to forage at both times, suggesting that there is an influence of social interactions on the time of foraging (Medugorac & Lindauer, 1967). Honeybee queens caused greater shift in the phase of circadian rhythm of workers compared to a control group presented with a single worker (Moritz & Sakofski, 1991), suggesting that honeybee queens can modulate circadian clocks of workers. In fruit flies *D. melanogaster*, synchronization of circadian clocks by social cues was demonstrated in an elegant study by Levine and coworkers (2002). Wild-type flies maintained in groups showed considerable degree of phase coherence in their circadian locomotor activity rhythm, suggesting synchronizing effect of social interactions on circadian clocks. When wild-type flies were co-housed with $per^{0}$ flies in 4:1 ratio, they showed relatively less phase coherence than when maintained in homogenous groups. It was, therefore, suggested that synchrony among individuals living in homogeneous groups is achieved because of strong positive interactions among group members (Krupp et al., 2008; Levine et al., 2002). Olfaction plays a key role in such social interactions, because the phase coherence of paralytic olfactory mutant hosts, such as $para^{Sbl\text{-}1}$ and $para^{Sbl\text{-}2}$, is not affected by the presence of arrhythmic *period*-null ( $per^{01}$) visitors (Levine et al., 2002). Peripheral clocks located in the olfactory neurons (Krishnan et al., 1999) have also been implicated in such social interactions. Expression of clock genes in the fly head and oenocytes of wild-type flies was altered when wild-type flies were maintained with $per^{01}$ flies in 4:1 ratio (Krupp et al., 2008). Rescue of circadian oscillation in the lateral and dorsal neurons in otherwise arrhythmic $per^{01}$ mutant flies had little or no effect on the phase coherence of the group, suggesting that peripheral oscillators regulate social synchrony among hosts (Levine et al., 2002). Social interactions among flies involve pheromones released from oenocytes (Wigglesworth, 1970), the fly abdomen (Demerec, 1994), which have recently been shown to have functional circadian clocks with cycling clock genes (Krupp et al., 2008). Although previous studies did suggest that social interactions influence the phase of circadian rhythms in *D. melanogaster*, whether social cycles of PA can serve as a zeitgeber for the circadian clocks of *Drosophila* remains an open question.

Here we present the results of our study that examined the effect of PA cycles of conspecifics on the circadian locomotor activity rhythms in fruit flies *D. melanogaster*. Wild-type *Canton S* (*CS*) virgin host males in constant darkness (DD) were made to interact one-on-one for 12 h daily with male or female visitors coming from 12:12 h light/dark (LD) cycles. The results suggest that circadian clocks of fruit flies *D. melanogaster* may not be able to entrain to social cycles, although PA cycles can cause significant change in the phase of the circadian locomotor activity rhythm.

## MATERIALS AND METHODS

### Fly Strains

Freshly emerged virgin flies of the following strains were used in our study: *Canton S* (*CS*), white eye (*w*), loss of function mutants of *period* ( $per^{0}$) and *cryptochrome* genes ($cry^{02}$ and $cry^{03}$), and flies in which the temperature-sensitive $shibire^{ts}$ gene is ectopically expressed in the pigment-dispersing factor (PDF)-positive clock neurons ( $pdfGAL4/UASshibire^{ts}$, henceforth referred to as *shibire* flies). Transgenic *shibire* flies have circadian clocks with an extremely labile circadian period that is known to change as a function of environmental temperature (Kilman et al., 2009). Freshly emerged flies were collected and maintained under 12:12 h LD cycles in glass vials (95 mm × 10 mm) as same-sex groups of 30 individuals/vial before starting the experiments.

### Presence/Absence (PA) Cycles

In all our experiments, virgin flies of same age group were introduced individually into 5 × 65-mm glass tubes and maintained under 12:12 h LD cycles for 4 days. This is the only stage when flies were subjected to $CO_2$ anesthesia. Following this, both host and control flies were transferred to DD (dim red light of wavelength >650 nm) for the rest of experiment (unless specified otherwise), whereas those used as visitors continued to be maintained under 12:12 h LD cycles (Figure 1). In DD, those flies designated as hosts were presented with visitors (henceforth, such host flies are referred to as flies with "cyclic social interactions" or CSI flies). This was accomplished by manual transfer of individual flies from the visitor tube to host tube by removing the plugs from the activity tubes and swiftly placing the mouths of two tubes in apposition. The visitor fly was tapped gently into the host tube. At the end of the social interaction

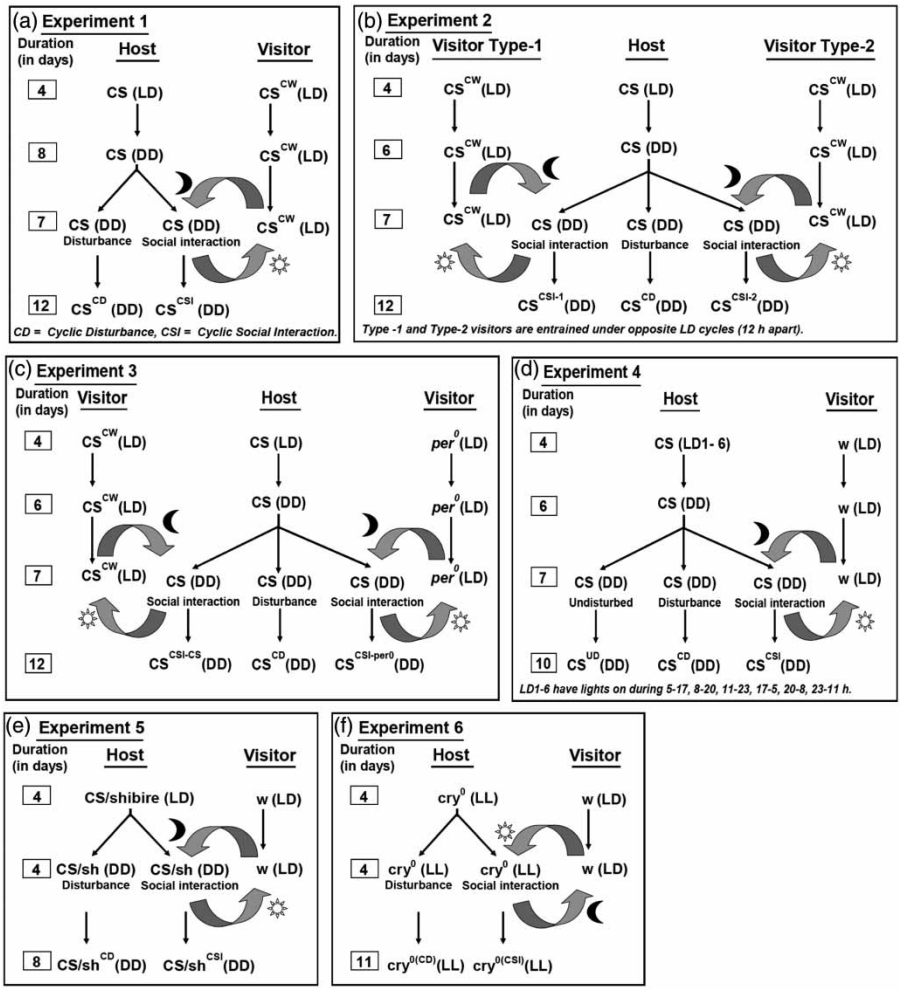


FIGURE 1. Flowchart of the six experiments. The boxed numbers on extreme left indicate the duration of each stage or treatment. The regime being experienced by each type of fly is shown in parentheses at each stage, whereas superscripts indicate treatment experienced by the fly. (a) In Experiment 1, for the first 4 days, *Canton S* (*CS*; designated as hosts) and *CS* with cut wings ($CS^{CW}$; designated as visitors) were entrained singly under LD. For the next 8 days, *CS* flies were introduced to DD, whereas $CS^{CW}$ continued to be in LD. For the next 7 days, treatments consisting of either social interaction or mechanical disturbance were administered. *CS* hosts interacted with $CS^{CW}$ visitors for the 12 h corresponding to the dark phase of the visitors. *CS* flies that received $CS^{CW}$ are called CSI hosts. The other group of *CS* flies were handled in a similar way and subjected to mechanical disturbance twice daily at two time points (corresponding to entry and exit of visitor in CSI regime), but did not receive any visitors themselves. These *CS* flies were designated $CS^{CD}$. For the following 12 days, both *CS* groups ($CS^{CD}$ and $CS^{CSI}$) were left undisturbed in DD for 12 days. (b) In Experiment 2, the visitors were of two types (1 and 2), each of them entrained under exactly opposite phases (12 h apart). The hosts received the visitors during the night phase of the visitor's clock, so one group of hosts ($CS^{CSI-1}$) received visitors between 20:00 and 08:00 h, whereas the second group, $CS^{CSI-2}$, received visitors between 08:00 and 20:00 h. All other steps were similar to Experiment 1. (c) Experiment 3 also involved two types of visitors, whereas one was similar to Experiment 1, the other type of visitors was clockless ( $per^0$). All other steps were similar to Experiment 2. (d) Experiment 4 hosts were highly phase desynchronous and first maintained under six different LD 12:12 h regimes (LD1–6). After 4 days, the pooled highly phase-desynchronous set received visitors similar to Experiment 2. These visitors were differentiated based on white eye color (*w*). An additional control host, which was undisturbed, was also present under DD ($CS^{UD}$). All other steps were similar to Experiment 2. (e) In Experiment 5, two types of hosts were used, one of which was *CS*, similar to that in previously described experiments, and a second host that had a labile circadian clock due to the expression of temperature-sensitive *shibire* in the lateral ventral neurons using the *pdfGAL4* driver (*shibire*). All other steps were similar to Experiment 2. (f) In Experiment 6, host flies were *cryptochrome*-null flies ($cry^0$). Since they free-run in LL, all steps in this experiment were carried out in LL, except for the initial 4 days of entrainment in LD 12:12 h (not shown here). Visitors were *w* genotype, and they experienced the light phase of the LD cycle in the company of the hosts, in contrast to the other experiments described here. All other procedures were similar to those of Experiment 2.

duration, the two flies were separated in the same way. Control flies were treated with a similar procedure of handling except they did not receive visitors. Cyclic mechanical disturbance was given by tapping activity tubes without entry of any guest flies, and this procedure was repeated after 12 h. Thus, controls experienced similar mechanical disturbances as CSI flies (henceforth referred to as flies with "cyclic disturbance" or CD flies). In some experiments, we used another set of controls that was left undisturbed for the entire duration of the experiment (henceforth referred to as "undisturbed" control flies or UD flies).

### Locomotor Activity Recording

For the entire duration of the experiment, locomotor activity of the individual host and control flies was recorded with help of the Drosophila Activity Monitors (DAM) system of Trikinetics (USA). For the most part of our study, activity of flies was recorded individually, except for the 12-h periods daily when host flies were co-housed with visitors. After treatments, we continued to record the locomotor activity behavior of hosts and controls under DD to estimate the phase of locomotor activity rhythm on the last day of cyclic treatment. Activity data were collected in 15-min bins and analyzed with the help of CLOCKLAB software from Actimetrics (USA).

### Experiment 1: Effect of PA Cycles on Phase Synchrony of Hosts

To study the effect of PA cycles on the phase synchrony of hosts, two sets of *CS* flies—hosts (n = 59) and controls (n = 31)—were introduced individually into activity tubes and maintained under 12:12 h LD cycles (lights-on between 04:00 and 16:00 h, local time) for 4 days, and then transferred to DD (Figure 1a). Another set of *CS* flies (n = 59) with cut marks on their wings (visitors, $CS^{CW}$) were introduced individually into activity tubes and kept under 12:12 h LD cycles (lights-on from 08:00 to 20:00 h) for the entire duration of the experiment. After 8 days in DD, host flies experienced cyclic social interactions (CSI) by being co-housed in pairs daily for 7 successive days with visitors for 12 h starting at 20:00 h, after which visitors were placed back in their original regime for the subsequent 12-h light phase such that they experienced the dark phase of their LD cycle in company of the hosts and the light phase in isolation in their original regime. The tubes containing control-disturbed (CD) flies were handled twice daily, at 08:00 h and 20:00 h, for 7 successive days to match the timing of mechanical disturbance experienced by the experimental CSI flies due to introduction and removal of visitors. After cyclic treatments, locomotor activity behavior of CSI and CD flies continued to be recorded under DD for a minimum of 12 days.

### Experiment 2: Effect of PA Cycles of Conspecifics on Hosts With Different Visiting Hours

To study the effect of PA cycles on hosts that receive visitors at different phases of their circadian clocks, three sets of *CS* flies were introduced individually into activity tubes and maintained under 12:12 h LD cycles (lights-on during 14:00 to 02:00 h) for 4 days and then transferred to DD (Figure 1b). After 6 days in DD, the first set (n = 50; CSI-1) was paired daily between 20:00 and 08:00 h with visitors from LD cycles in which lights were on between 08:00 and 20:00 h (Type 1 visitors), for 7 successive days, whereas the second set (n = 28; CSI-2) was paired during 08:00 to 20:00 h with visitors from LD cycles in which lights were on between 20:00 and 08:00 h (Type 2 visitors). The third set (n = 30; CD) was handled similarly to the CSI flies but were not presented with visitors. After cyclic treatments, locomotor activity behavior of CSI and CD flies continued to be recorded in DD for a minimum of 12 days.

### Experiment 3: Effect of PA Cycles of Clockless Visitors

To study the effect of PA cycles of clockless visitors on hosts, wild-type *CS* flies were introduced individually in activity tubes and maintained under 12:12 h LD cycles (lights-on between 14:00 and 02:00 h) for 4 days and then transferred to DD (Figure 1c). The visitors ( $per^0$ and *CS* flies) were kept individually in activity tubes and maintained under LD cycles in which lights were on from 08:00 to 20:00 h. After 6 days in DD, these flies were divided into three sets, of which one set (n = 55; CSI) was paired daily for the entire night with $per^0$ visitors for 7 successive days, whereas the other set (n = 50; CSI) was paired daily for the entire night with *CS* visitors. The third set (n = 28) was handled twice daily (CD) in a manner similar to the CSI flies but were not presented with visitors. After 7 days of treatment, locomotor activity behavior of CSI and CD flies continued to be recorded under DD for a minimum of 12 days.

### Experiment 4: Effect of PA Cycles on Highly Phase-Desynchronized Hosts

To study the effect of PA cycles on the phase synchrony of highly phase-desynchronized hosts, we created a set of flies with a high degree of phase desynchrony by placing groups of *CS* flies first under six different LD cycles (lights-on during 05:00–17:00 h, 08:00–20:00 h, 11:00–23:00 h, 17:00–05:00 h, 20:00–08:00 h, and 23:00–11:00 h) for 4 days, and then pooling them into composite groups of 50–60 flies/vial, taking equal number of flies from each regime. The magnitude of the phase coherence vector of these flies prior to PA cycles was as low as 0.0004 (on a scale of 0–1). Flies from this highly phase-desynchronous group were divided into three sets and introduced individually into activity tubes and placed in DD to study the effect of PA cycles (Figure 1d). After 6 days in DD, the first set (n = 38) was paired daily during 08:00 to 20:00 h for 7 days with *w* male visitors (entrained under LD cycles with lights-on between 20:00 and 08:00 h), whereas the second and third sets were treated as CD (n = 42) and UD controls (n = 30). After 7 days of treatment, locomotor activity behavior of CSI, CD, and UD flies continued to be recorded under DD for a minimum of 10 days.

To ascertain sex-related effects of PA cycles on the phase synchrony of hosts, the above experiment was carried out with female visitors. Flies from this highly phase-desynchronous group were divided into four sets and introduced individually into activity tubes and placed in DD to study the effect of PA cycles (Figure 1d). The following treatments were given daily for 7 successive days between 08:00 and 20:00 h: the first set (n = 26) was paired daily with *w* females from LD cycles in which lights were on from 20:00 to 08:00 h, the second set (n = 22) with *w* males from the same LD

cycles as hosts, the third set (n = 24) was handled twice daily at 08:00 h and 20:00 h (CD controls), and the fourth group (n = 26) was left unperturbed (UD controls). After 7 days of treatment, locomotor activity behavior of CSI, CD, and UD flies continued to be recorded under DD for a minimum of 10 days.

### Experiment 5: Effect of PA Cycles on Phase-Synchronized Hosts

To study the effect of PA cycles on the phase synchrony of flies whose phase has already been set by LD cycles, two sets of *CS* flies (n = 17 and n = 18) were introduced individually into activity tubes and maintained under LD cycles (lights-on during 08:00 to 20:00 h) for 4 days, and then transferred to DD (Figure 1e). In contrast to Experiment 1, from the first day onwards the first set (CSI) was paired daily between 08:00 and 20:00 h with *w* visitors (maintained under LD cycle with lights-on from 20:00 to 08:00 h) for 4 successive days, whereas the second set (CD) was disturbed twice daily in the morning (at 08:00 h) and evening (at 22:00 h). After 4 days of treatment, locomotor activity behavior of CSI and CD flies continued to be recorded under DD for a minimum of 8 days.

To study the effect of PA cycles on the phase synchrony of flies with labile circadian clocks whose phase has already been set by LD cycles, we took *shibire* flies and subjected them to the PA cycle of *w* visitors, in the same way as the *CS* flies described above (Figure 1e). The first set of *shibire* flies (n = 21) was subjected to cyclic social interaction (CSI), whereas the second set (n = 18) was subjected to cyclic disturbance twice daily (CD). After 4 days of treatment, locomotor activity behavior of CSI and CD flies continued to be recorded under DD for a minimum of 8 days.

### Experiment 6: Effect of PA Cycles on Light-Dependent Splitting of $cry^0$ Hosts

In this experiment, we asked whether PA cycles can delay light-dependent splitting of the activity rhythm in $cry^0$ flies (Figure 1f). We introduced two sets each of $cry^{02}$ and $cry^{03}$ males individually into activity tubes and maintained them for 4 days under a 12:12 h LD cycle (lights-on between 20:00 h and 08:00 h), and then transferred them to LL. In contrast to wild-type flies, $cry^0$ flies are rhythmic in LL (Dolozelova et al., 2007). From the 5th day onwards, one set each of $cry^0$ hosts (n = 32 from each of the $cry^{02}$ and $cry^{03}$ strains) were paired daily for the duration of the day (08:00 to 20:00 h) with *w* visitors coming from LD cycles (lights-on from 08:00 to 20:00 h) for 4 successive days (CSI), whereas the second set (n = 32 from each of the $cry^{02}$ and $cry^{03}$ strains) of $cry^0$ flies were disturbed twice daily at 08:00 and 20:00 h (CD). After treatments, locomotor activity of CSI and CD flies continued to be recorded under LL for a minimum of 11 days.

### Analyses of Locomotor Activity Data

Locomotor activity data were used to determine the phase of the locomotor activity rhythm prior to and after conclusion of PA cycles. To do so, regression lines were drawn through the daily offsets of activity prior to or following treatments, which were extrapolated to the first or last day of cyclic treatment. The phase values of the locomotor activity rhythm (offset of activity) were used to draw the circular diagrams. For statistical analyses, empirically obtained phase data were subjected to bootstrapping, where data were resampled with replacement to generate replicate sets of phase value data (Good, 2005). For example, from a set of $x$ empirical data points, we first generate a pool of $5x$ data points, where each of the original data points is represented 5 times, and then from this pool each bootstrap sample of $x$ values is randomly sampled 5 times, thus generating (n = 5) replicates. These replicate phase values were subjected to circular vector analysis (Batschelet, 1981) to obtain the magnitude ($r$, on a scale 0 to 1), and direction ($a^0$, on a scale of 1–360° or 1–24 h) of the phase-coherence vectors. A magnitude of 1 would mean that all individuals in a given set have exactly the same phase, whereas a magnitude of 0 would mean that individuals in the group are highly phase desynchronized. These data were used for analysis of variance (ANOVA), where $r$ and $a^o$ values were treated as fixed factors. ANOVA was followed by post hoc multiple comparisons using Tukey's test. In addition, we subjected phase distributions of experimental and control treatments to the Watson-Williams test to determine if the phase distribution of CSI flies was significantly different from CD and/or UD controls. All statistical analyses were implemented using STATISTICA (StatSoft, 1995) and circular statistics methods (Batschelet, 1981). The study described in this article conforms to international ethical standards (Portaluppi et al., 2010).

## RESULTS

### Experiment 1: Phase Synchrony of Host Males Is Reduced by PA Cycles of Conspecifics

Whereas phase synchrony of CD flies remained unaltered, that of CSI flies was reduced significantly. This indicates that host flies that experienced cyclic social interactions are less synchronous than controls that were merely disturbed periodically (Figure 2). This suggests that PA cycles reduce phase synchrony among hosts.

The $r$ and $a^o$ values of the phase-coherence vector in CSI flies before the imposition of PA cycles were 0.82 and +0.49, whereas after PA cycles they were 0.74 and −4.08, respectively. The $r$ and $a^o$ values of the phase-coherence vector in CD flies before treatment were 0.79 and +1.27, whereas after treatment they were 0.78 and −3.65, respectively. ANOVA on the $r$ values revealed a significant effect of treatment (CSI/CD) ($p < .0001$) and stage (before/after treatment) ($p < .01$); however,

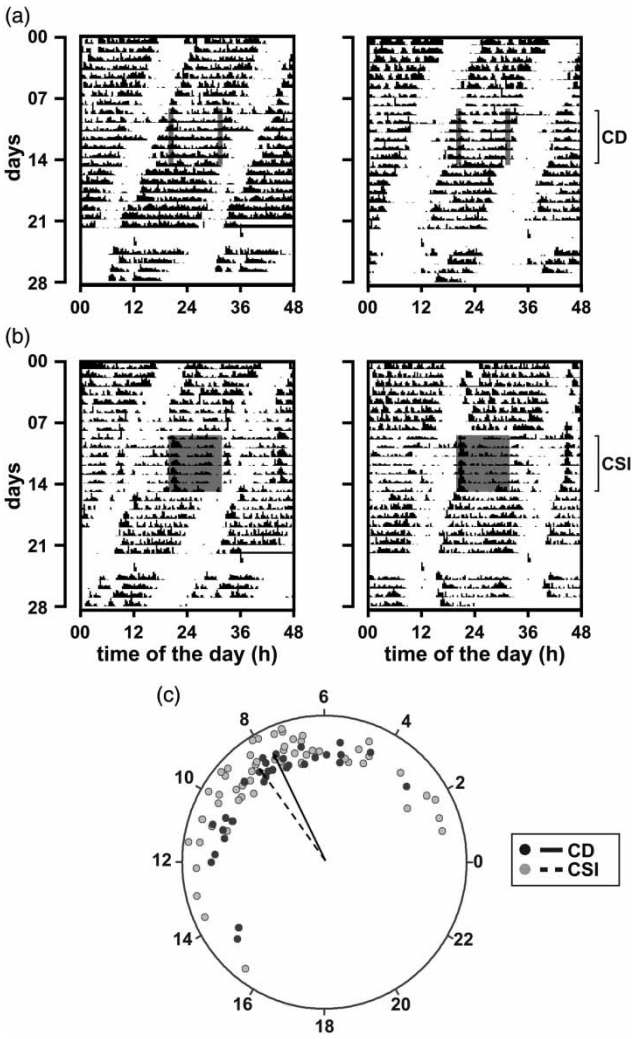


FIGURE 2. Phase synchrony of males is not altered by cyclic social interaction with conspecifics. Actograms of two representative host flies, each from a group of flies subjected to mechanical disturbance twice daily at 20:00 and 08:00 h (local time) (CD controls) (a), or to daily 12-h social interaction with *CS* visitors between 20:00 and 08:00 h (local time) (CSI flies) (b). Cyclic treatments were continued for 7 successive days, after which both CSI and CD flies were subjected to constant darkness (DD). Rectangular gray shade in (a) indicates timings of mechanical disturbance, whereas 12-h rectangular gray shades in (b) indicates timings of presence of visitor flies. Except for the rectangular dark gray areas, at all other times host flies were left undisturbed and solitary under DD. In the circular plot (c), gray circles and broken black line represent phases and phase-coherence vector of CSI flies subjected to presence and absence (PA) cycles of visitors, whereas black circles and black unbroken line represent phases and phase-coherence vector of CD controls. The phase-coherence vector of CSI flies is significantly different in magnitude ( $p < .0005$) and direction ( $p < .001$) compared to CD flies; however, the two phase distributions do not differ ( $p > .25$) as per Watson-Williams test. Due to technical faults, activity recording was interrupted for ~2 days towards the end of the recording in DD, as represented by the blank spaces near the bottom of the actograms.

treatment × stage interaction was not statistically significant ( $p = .08$). Post hoc multiple comparisons using Tukey's test showed that whereas $r$ values of CD flies did differ before and after treatment ( $p = .82$), those of CSI flies subjected to PA cycles were decreased significantly ( $p < .05$). ANOVA on $a^o$ values revealed significant effect of treatment (CSI/CD) ( $p < .0005$) and stage (before/after treatment) ( $p < .0001$); however, treatment × stage interaction was not statistically significant ( $p = .09$). Post hoc comparisons showed that $a^o$ values of CD flies and CSI flies differed significantly before and after treatment ( $p < .0005$ for both comparisons).

The $r$ value of the phase-coherence vector of CSI flies was significantly smaller than that of CD controls ( $p < .0005$), and the $a^o$ values of CSI flies were significantly different from CD controls ( $p < .001$) (Figure 2). However, phase distributions of CSI and CD flies did not differ statistically as per the Watson-Williams test ( $p > .25$) (Figure 2c).

### Experiment 2: PA Cycles of Conspecifics With Different Visiting Hours Influence the Extent of Phase Synchrony Among Hosts

Since it is possible the level of synchrony achieved depends upon phase of circadian clocks of host flies at the beginning of social interactions (visiting hours), we conducted another experiment in which the hosts received visitors at one of two phases that were 12 h apart (Figure 1b). The results showed that the phase synchrony of host flies presented with Type 1 or Type 2 visitors was lower than controls; hosts who interacted with Type 1 visitors had higher phase synchrony than hosts that interacted with Type 2 visitors, suggesting that host's visiting hours do influence outcome of PA cycles of conspecifics.

ANOVA on $r$ data showed a significant effect of visiting hours ( $p < .0001$). Post hoc multiple comparisons revealed that although the $r$ value of CSI flies presented with Type 1 ( $p < .005$) or Type 2 ( $p < .0005$) visitors was lower than CD controls, hosts that interacted with visitors during subjective evening phase (Type 1) had greater $r$ values than hosts that interacted with visitors during subjective morning phase (Type 2) ( $p < .0005$). ANOVA on $a^o$ data also showed significant effect of visiting hours ( $p < .0001$). Post hoc comparisons revealed that the $a^o$ value of hosts presented with flies from either Type 1 ( $p < .01$) or Type 2 ( $p < .0005$) visitors was significantly different from CD controls (Figure 3a, b). Furthermore, $a^o$ values of the two groups of hosts also differed significantly from each other ( $p < .0005$; Figure 4a, b). However, the Watson-Williams test revealed that the phase distribution of the two groups of hosts did not differ statistically ( $p > .20$) (Figure 4a–c).

### Experiment 3: Phase Synchrony of Hosts Interacting With Clockless Visitors Is Comparable to That of Controls

Having seen that visiting hours have significant effect on the phase synchrony of hosts, we asked whether robustness of the circadian clock of visitors may alter the outcome of cyclic social interactions. Whereas wild-type conspecifics continued to show lower ability to synchronize the host's circadian clocks, clockless $per^0$ visitors were able to evoke the same level of phase

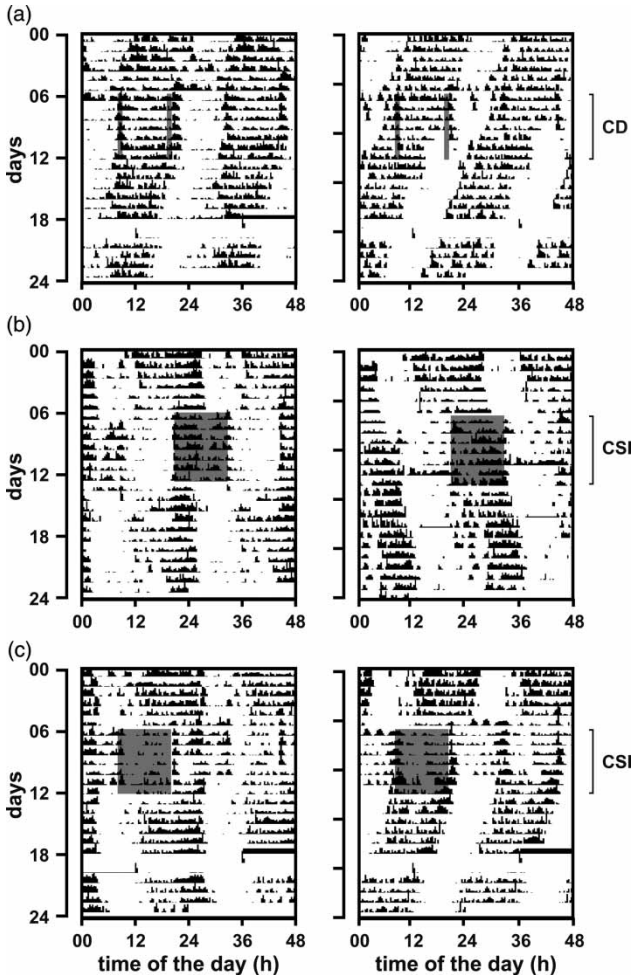


FIGURE 3. Extent of phase synchrony among hosts depends upon visiting hours of guests. Actograms of two representative control flies (CD controls), each from group of flies that were either disturbed twice daily at 08:00 or 20:00 h (local time) (a), or presented daily with *CS* visitors for 12 h (CSI flies) either between 20:00 and 08:00 h (Type 1) (b) or between 08:00 and 20:00 h (Type 2) (c). Cyclic treatments were continued for 7 successive days, after which both CSI and CD flies were subjected to constant darkness (DD). Rectangular gray shades in (a) indicate timings of mechanical disturbance, whereas 12-h rectangular gray shades in (b) and (c) indicate cyclic presence of visitor flies. Except for the rectangular dark gray areas, host flies were left undisturbed and solitary under DD.

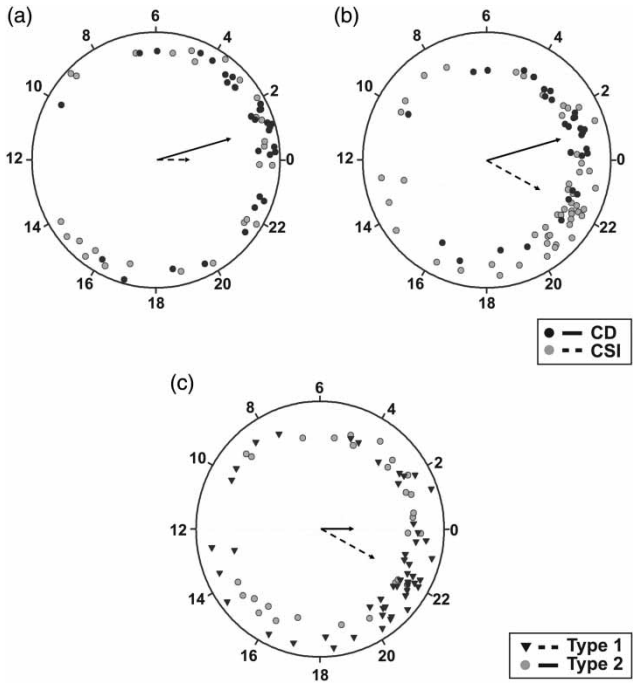


FIGURE 4. Phase distribution of hosts receiving guests at different visiting hours. In the circular plots (a–c), gray circles and broken black line show the phase and phase-coherence vector of CSI flies subjected to presence and absence (PA) cycles of visitors, whereas black circles and unbroken black line represent phases and phase-coherence vector of CD controls. The magnitude ( $p < .0005$) and direction ( $p < .0005$) of phase-coherence vector of hosts receiving Type 1 visitors (broken black line) are significantly different than those with Type 2 visitors (unbroken black line) (a–c). The phase-coherence vector of hosts receiving Type 1 visitors is significantly different in magnitude ( $p < .005$) and direction ( $p < .01$) compared to CD controls. The phase-coherence vector of hosts receiving Type 2 visitors is significantly different in magnitude ( $p < .0005$) and direction ( $p < .005$) compared to CD controls. However, phase distributions of CSI and CD flies and CSI flies with Type 1 and Type 2 visitors do not differ statistically ( $p > .20$) as per Watson-Williams test. All other details are the same as in Figure 2.

synchrony among hosts as cyclic mechanical disturbance (Figure 5).

ANOVA on *r* data showed significant effect of visitor's strain ( $p < .001$). Post hoc comparisons revealed the *r* value of CSI flies receiving arrhythmic $per^0$ visitors did not differ statistically from CD controls ( $p = .42$; Figure 5c); however, the *r* value of CSI flies receiving $per^0$ visitors was significantly greater than it was for those that received rhythmic *CS* visitors ( $p < .01$; Figure 5d). ANOVA on $a^o$ data showed significant effect of visitor's strain ( $p < .0001$). Post hoc comparisons revealed that the $a^o$ value of CSI flies that received either type of visitors ( $per^0$ or *CS*) was significantly different from CD controls ( $p < .0001$), whereas the $a^o$ of CSI flies that received $per^0$ or *CS* visitors did not differ from each other ( $p = .82$). The phase distribution of CSI flies that received *CS* visitors did not differ statistically from the phase distribution CSI of flies that received $per^0$ visitors (Watson-Williams test, $p = .25$; Figure 5d).

### Experiment 4: PA Cycles of Male or Female Visitors Fail to Synchronize Phase of Highly Desynchronized Hosts

We tested whether the fact that hosts used in our experiments thus far always had well-synchronized clocks to start with may have an overriding effect on the outcome of PA cycles (see Materials and Methods and Figure 1d). Our studies showed that when hosts had highly desynchronous phases to begin with, cyclic social interactions with visitors failed to synchronize host rhythms.

ANOVA of *r* data showed significant effect of treatment (CSI/CD/UD) ( $p < .05$). Yet, post hoc comparisons revealed that *r* values of the phase-coherence vector of

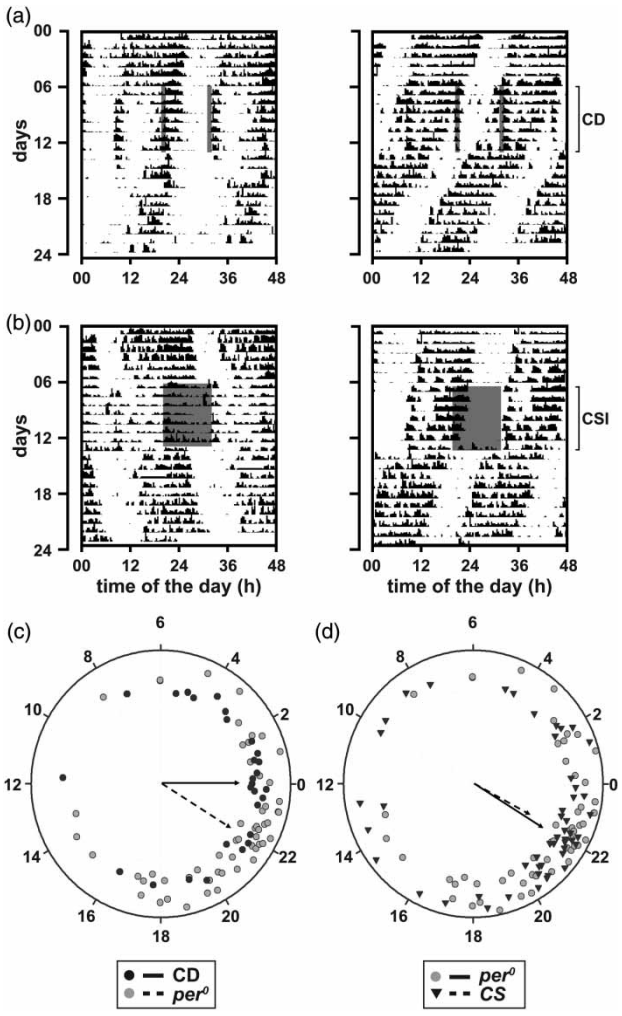


FIGURE 5. Effect of presence and absence (PA) cycles of visitors with and without functional circadian clocks on phase synchrony of hosts. Actograms of two representative host flies (CD controls), each from group of flies that were either disturbed twice daily at 08:00 or 20:00 h (local time) (a), or presented daily with $per^0$ visitors (b) for 12 h (CSI flies) between 20:00 and 08:00 h. The phase-coherence vector of hosts receiving $per^0$ visitors does not differ in magnitude ( $p = .42$) but differs in direction ( $p < .0005$) compared to CD controls (c). Furthermore, the phase-coherence vector of hosts receiving CS visitors is significantly different in magnitude ( $p < .001$), but not in direction, compared to hosts with $per^0$ visitors ( $p = .82$) (d). The phase distributions of hosts exposed to PA cycles of CS and $per^0$ visitors do not differ statistically ( $p > .25$) as per Watson-Williams test. All other details are the same as in Figure 2.

CSI flies did not differ statistically from CD and UD controls ( $p > .05$), but there was significant difference between $r$ values of CD and UD controls ( $p < .01$). The effect of treatments on $a^o$ was statistically significant ( $p < .01$). Post hoc comparisons revealed that the $a^o$ value of CSI flies did not differ from CD controls ( $p = .13$) but was significantly different from UD controls ( $p < .005$). The Watson-Williams test revealed phase distributions of CSI and CD controls ( $p > .20$), CSI and UD controls ( $p = 1.00$), or CD and UD controls ( $p > .20$) did not differ statistically (Figure 6a).

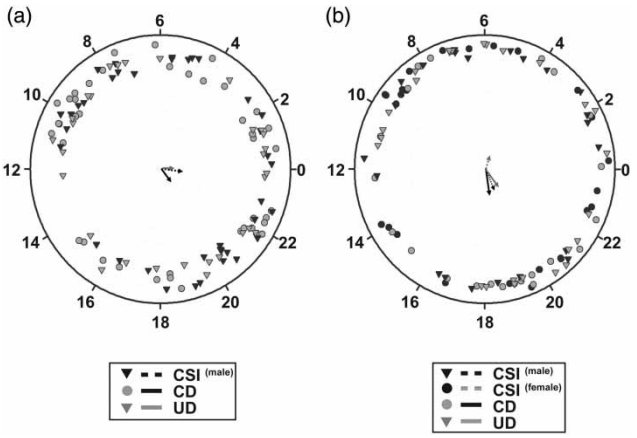


FIGURE 6. Effect of presence and absence (PA) cycles on the phase synchrony of highly phase-desynchronized hosts. To create maximum degree of phase desynchrony among host flies, *CS* flies were subjected to six different LD cycles and then pooled to form three host groups. First group of flies was subjected daily to 12-h presence and absence (PA) cycles; the second group was subjected to daily cyclic disturbance in the morning and evening (CD controls); and the third group were left undisturbed (UD controls). (a) Phase and phase-coherence vector of CSI flies exposed to PA cycles are shown as black triangles and black broken line, CD flies as gray circles and unbroken line, and of UD flies as gray triangles and gray unbroken line. The magnitude of the phase-coherence vector of CSI flies does not differ from CD or UD controls ( $p > .05$). The direction of the phase-coherence vector of CSI flies does not differ from CD ( $p = .13$) but is significantly different from UD controls ( $p < .005$). (b) Phase and phase-coherence vector of CSI flies exposed to PA cycles of males are shown as black triangles and broken black line, and those exposed to PA cycles of females are shown as black circles and broken gray line. The phase and phase-coherence vector of CD/UD controls are shown as gray circles/triangles and black and gray unbroken lines. The magnitude and direction of the phase-coherence vector of CSI flies do not differ from CD and UD controls ( $p > .05$).

Next we asked whether visitors of the opposite sex are better able to cause phase synchrony among hosts. Comparison of $r$ and $a^o$ values of the phase-coherence vector revealed that phase and phase synchrony of CSI flies that received male or female visitors did not differ from CD controls ( $p > .05$). The Watson-Williams test revealed that phase distributions of hosts receiving male or female visitors and of CD controls did not differ statistically ( $p > .50$) (Figure 6b). Thus, even PA cycles of female visitors were unable to cause phase synchrony among highly phase-desynchronized male hosts.

### Experiment 5: PA Cycles Modulate Phase of Circadian Clocks of Hosts With Labile Period

Next we asked whether the phase of hosts with labile circadian clock can be altered by PA cycles. PA cycles were able to alter phase of the locomotor activity rhythm in mutant *shibire* flies, whereas that of wild-type *CS* hosts remained unaffected. The phase of the locomotor activity rhythm of *CS* hosts exposed to PA cycles drifted as much as disturbance controls, whereas that of *shibire* flies drifted relatively less than disturbance controls (Figure 7).

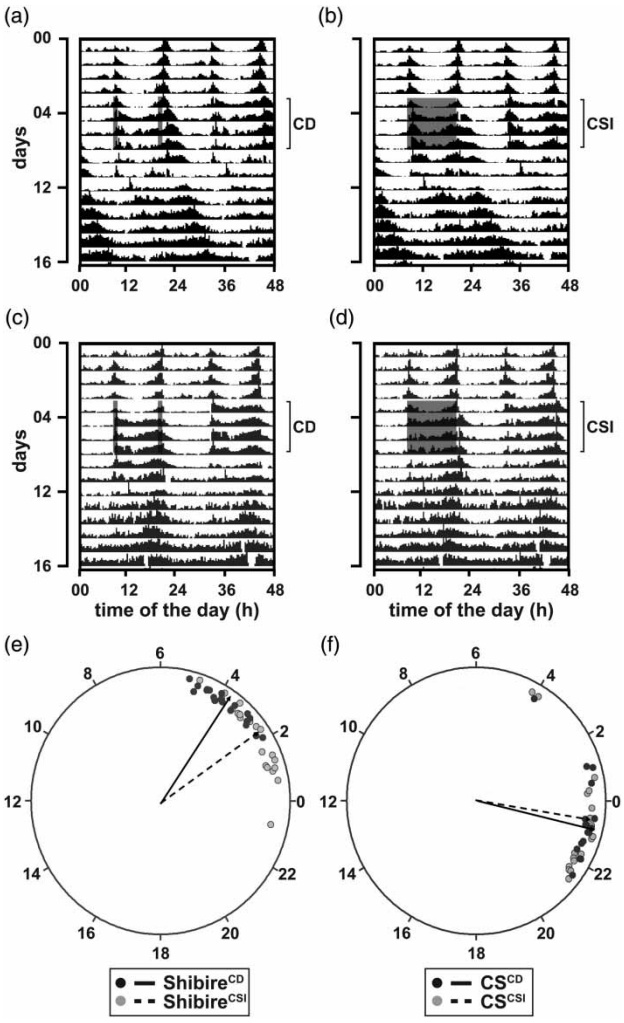


FIGURE 7. Effect of presence and absence (PA) cycles on the phase synchrony of phase-synchronized hosts with labile circadian clocks. Average actogram of host CS fly (CD controls) from groups of flies that were either disturbed twice daily at 08:00 or 20:00 h (local time) (a), or presented daily *white eyed* (*w*) (b) visitors for 12 h (CSI flies) between 08:00 and 20:00 h. Average actogram of host *shibire* fly (CD controls) from group of flies that were either disturbed twice daily at 08:00 or 20:00 h (local time) (c), or presented daily *white eyed* (*w*) (d) visitors for 12 h (CSI flies) between 08:00 and 20:00 h. In CS (f) flies, presence and absence (PA) cycles of 4 days does not have any significant effect on the magnitude and direction of phase-coherence vector ( $p > .05$). In *shibire* flies (e), PA cycles of 4 days have significant effect on the direction of phase-coherence vector ( $p < .001$). Solid black circles and line indicate phase and phase-coherence vector of CD controls, and gray circles and broken black line those of CSI flies.

ANOVA of *r* data showed significant effect of host strain (*shibire*/*CS*) ( $p < .005$) and treatment (CSI/CD) ( $p < .01$); however, the effect of host strain × treatment interaction was not statistically significant ( $p = .60$). Post hoc comparisons revealed that *r* values of *shibire* and *CS* hosts (CSI) did not differ from those of CD controls ( $p > .05$) (Figure 7). ANOVA of $a^o$ data showed significant effect of host's strain ( $p < .0001$), treatment ( $p < .001$), and host's strain × treatment interaction ( $p < .005$). Post hoc comparisons revealed that $a^o$ of socially interacting *shibire* flies was significantly less than CD controls ( $p < .001$), whereas that of *CS* flies did not differ from CD controls ( $p = .98$) (Figure 7).

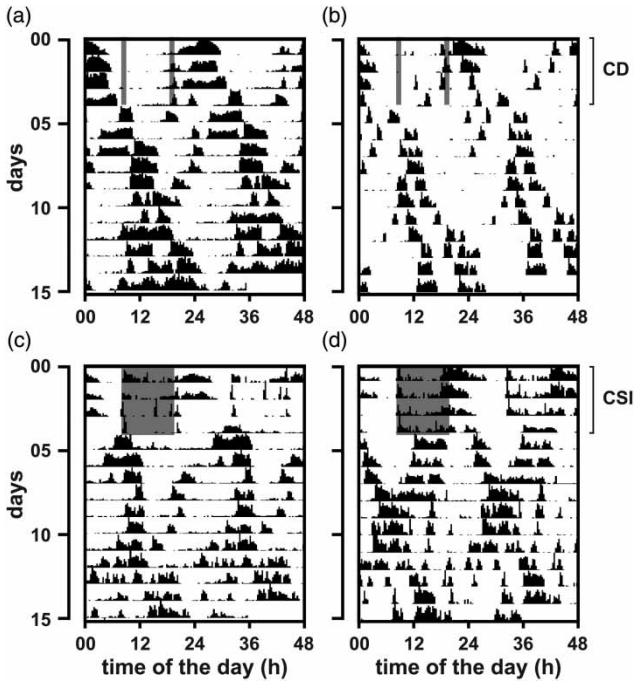


FIGURE 8. Social cycles have no impact on light-dependent splitting of $cry^0$ flies. Actograms of one representative fly each from control flies: $cry^{02}$ (a); $cry^{03}$ (b), and flies subjected to PA cycles: $cry^{02}$ (c); $cry^{03}$ (d), of *w* males for 4 days between 08:00 and 20:00 h. PA cycles do not alter light-dependent splitting behavior of these flies. All other details are the same as in Figure 2.

### Experiment 6: PA Cycles Do Not Alter Light-Dependent Splitting in $cry^0$ Hosts

Having seen that hosts with extremely labile circadian clocks can be phase shifted by PA cycles, we asked whether PA cycles can delay light-dependent splitting in $cry^0$ hosts. Visual inspection of activity data revealed absence of effect of PA cycles on light-dependent splitting of $cry^0$ hosts; splitting in CSI flies was as prevalent as in CD controls (Figure 8).

## DISCUSSION

In this report we showed that the presence and absence (PA) cycles of 12:12 h does not cause entrainment of the fruit fly *D. melanogaster* circadian locomotor activity rhythm. The phase of the circadian rhythm of experimental flies remains by and large unaffected by PA cycles, irrespective of visitors' sex or strain. However, phase synchrony of hosts that received visitors at one phase (Type 1, roughly corresponding to evening) was significantly greater than hosts that received visitors at another phase 12 h apart (Type 2, roughly corresponding to morning). Whereas phase synchrony of CD controls remained unaltered after daily cyclic treatment, that of

CSI host flies exposed to PA cycles was reduced significantly. The poor phase synchrony among host flies was primarily due to cyclic social interactions, because CD controls that were handled similarly to experimental CSI flies, but were not presented with visitors, showed significantly greater phase synchrony than flies exposed to PA cycles. Furthermore, phase synchrony among hosts was significantly greater when they received arrhythmic, clockless ( $per^0$) visitors than when they received rhythmic wild-type (*CS* or *w*) visitors, suggesting that PA cycles of visitors with functional clocks reduced phase synchrony among host flies. Ineffectiveness of PA cycles in synchronizing the circadian locomotor activity rhythm of hosts became quite apparent when host males with highly heterogeneous phases were made to interact with rhythmic visitor males or females. The phase of the host's rhythm underwent change due to PA cycles, but this was purely due to physical disturbance and not social interaction with visitors, because the magnitude of the phase-coherence vector was similar when CSI flies were compared with CD or UD flies; however, differences were seen between CD and UD flies. Further, the direction of the phase-coherence vector of CSI flies did not differ from CD controls, whereas that of CSI differed significantly from UD controls. Moreover, flies with labile circadian period ( *pdfGAL4/UASshibire*) showed some influence of cyclic presence of visitors, suggesting that social cues may serve as a weak zeitgeber for the *Drosophila* circadian timing system. What appears to be masking effects in some actograms (Figure 2b) was activity due to introduction and exit of the visitor fly, because during the 12-h period of social interaction activities of the host and visitor flies were recorded together. However, given that we estimated the phase of entrainment by extrapolating the phase of the steady state (post-PA cycles) back to the last day of treatment cycles, this should take care of masking effects, if any. Nevertheless, we acknowledge that there is some masking effect that is independent of the presence of visitors, for example, those present in the actograms of the disturbance controls (Figure 3a).

Social cues have been shown to entrain circadian rhythms of a wide variety of organisms, including mice, rats, bats, birds, and fish (Crowley & Bovet, 1980; Duffield & Ebling, 1998; Goel & Lee, 1997; Halberg et al., 1954; Handelmann et al., 1980; Reebs, 2000; Reppert & Schwartz, 1986; Viswanathan & Chandrashekaran, 1985). Social cues in the form of song cycles were reported to entrain the circadian locomotor activity rhythm of birds (Gwinner, 1966; Menaker & Eskin, 1966); however, it was subsequently discovered that in some cases entrainment was due to physical disturbance and not social cues; for example, in birds, cyclic white noise is also found to be equally effective in bringing about entrainment as the songs of fellow birds (Reebs, 1989). In fruit flies *D. melanogaster*, social interactions have been shown to influence the phase and period of circadian rhythms when flies are kept together in groups for several days (Levine et al., 2002). But until now, the question remains as to whether social cues can entrain circadian rhythms of *Drosophila*. The results of our present study suggest that social cues may have very little influence on the circadian clocks of *Drosophila*. In fact, daily social interaction with rhythmic visitors reduced phase synchrony of hosts to a relatively lower level than controls that were disturbed twice a day, once in the morning and once in the evening (Figures 1, 4, 5). However, hosts that received visitors at a certain phase showed greater phase synchrony than those that received visitors at another phase (Figures 3, 4). This suggests that interactions with conspecifics at certain phases are critical in determining phase synchrony among hosts. This is consistent with the finding of a previous study in which "late visitors" did not have any effect on the circadian rhythm of "early hosts," whereas "early visitors" were able to alter the phase of "late hosts" (Levine et al., 2002).

Other studies have indicated that phase of host's circadian rhythm is likely to influence the outcome of cyclic social interactions. In rats, fetuses born to suprachiasmatic nucleus (SCN)-lesioned mothers display desynchronized glucose utilization and disrupted *N*-acetyltransferase rhythm compared to those born to intact mothers (Reppert & Schwartz, 1986). Similarly in Syrian hamsters, pups born to SCN-lesioned mothers were found to have greater phase desynchrony than those born to intact mothers (Davis & Gorski, 1988), suggesting a role for the mother's circadian clock in socially synchronizing pup's circadian clock. In this study daily PA cycles of clockless $per^0$ visitors evoke similar response in hosts as do cyclic physical disturbances. PA cycles of visitors with intact clocks result in reduced phase synchrony among hosts compared to CD controls (Figure 5), probably because of mismatch between the phase of host and visitor rhythms. The $per^0$ visitors do not influence phase of hosts probably because they have no phase identity of their own, and they do not have rhythmicity in olfactory ability (Krishnan et al., 1999; Krupp et al., 2008). In a previous study, presence of $per^0$ flies amidst a group of wild-type flies was found to result in decreased phase synchrony among group members (Levine et al., 2002), and it was argued this may be due to lack of effective communication between CS flies in the presence of $per^0$ visitors. Further, social interaction with visitors from different time zones had profound effect on the circadian phase of hosts (Levine et al., 2002), suggesting that reduced phase synchrony among hosts in our study may be due to differences in phases of host and visitor clocks. Our study offers a different interpretation of the results. We propose that the better phase synchrony among host flies interacting with $per^0$ visitors may be due to lack of effective communication between hosts and $per^0$ visitors, whereas decrease in phase synchrony due to visitors with intact clocks may be because of the mismatch between the phase of circadian rhythms of visitors and hosts.

The results of our study show that phase coherence in socially interacting CSI flies is often comparable to CD control flies that experience cyclic disturbance (Figure 2), suggesting that cyclic disturbance can cause phase synchrony; however, visual examination of individual actograms indicates that there is no appreciable change in the phase of the rhythm following cyclic treatments, indicating that phase synchrony among host flies is not newly achieved. Furthermore, daily PA cycles are even less effective in bringing about phase synchrony in highly phase-desynchronized flies ($r = 0.0004$), suggesting that the phase coherence observed in previous studies may be a carryover effect from previous entrainment regimes and is not due to cyclic social cues (Figure 6a). Given that cyclic physical disturbance is as effective as PA cycles, results of our study can be taken to suggest that social cues, at least in the form of PA cycles, do not act as a primary zeitgeber for the circadian timing system of *Drosophila*. However, the indication of a possibly weak zeitgeber (lack of entrainment but modulation of phase in a phase- or period-dependent manner) also suggests that exposing hosts to PA cycles for longer than 7 days might be effective in entraining their circadian rhythms. This seems likely, because in the study by Levine and coworkers (2002) flies maintained together for 14 days in group were more synchronous compared to isolates.

Previous studies have shown that pairwise interactions between males and females have significant effect on the activity rhythm of *Drosophila* (Fujii & Amrein, 2010; Fujii et al., 2007; Hamasaka et al., 2010). Our study tested whether such sociosexual interactions provided daily can bring about phase synchrony and modulate the phase of highly desynchronized flies. The outcome remains unchanged, cyclic social interaction with females was unable to synchronize circadian clocks of male hosts (Figure 6b). Furthermore, social cues were ineffective in delaying LL-mediated splitting of activity bouts in $cry^0$ flies, suggesting lack of effect of PA cycles on the circadian rhythm of hosts (Figure 8). These results further suggest that social cues in the form of PA cycles do not serve as zeitgeber in fruit flies *D. melanogaster*. If they do exert an effect, they may act in collaboration with other time cues, such as LD, temperature, and food availability cycles, to bring added stability to the entrained rhythm.

To examine if social cues can help in retaining the phase of already set rhythm from previous entrainment schedule, we subjected CS males to PA cycles. We found that the phase of the activity rhythm in DD drifted from the phase previously set by entrainment to LD cycles, indicating that PA cycles are unable to retain the already set phase of entrainment. To further test this, we used *shibire* males with mean circadian period of $25.61 \pm 0.15$ h at 25°C. The results revealed that phase synchrony of CSI and CD flies were similar; however, phase shifts after 4 days of free-running in DD were significantly less in CSI flies than CD controls, indicating social cycles have significant impact on the circadian rhythms of *shibire* flies (Figure 7). The period of *pdfGAL4* and *UASshibire* flies is close to 24 h, and the fact that these flies do not respond to PA cycles (data not shown) suggests that the phase stability displayed by *shibire* flies is not an artifact of genetic background. Further, this indicates that PA cycles are capable of retaining the phase of circadian rhythm close to the phase determined by previous cyclic experience, provided that the host flies have a labile circadian timing system. Our study suggests that PA cycles are unable to entrain the circadian locomotor activity rhythm of *D. melanogaster*; however, consistent effect of social interaction is observed in terms of phase synchrony among socially interacting flies, confirming the findings of previous studies that circadian clocks are responsive to social cues (Levine et al., 2002), and are likely to fine-tune circadian rhythms at the group level.

Our study suggests that the fruit fly *Drosophila melanogaster* cannot be entrained by the cyclic presence and absence (PA) of male or female visitors. However, there is consistent modulation of phase of the host's rhythm, which indicates that *Drosophila* is sensitive to social cues. Synchrony among hosts depends on the time when they interact with the visitors. Although clocks of *Drosophila* are sensitive to social cycles in a time-dependent manner, they are also able to distinguish between clockless and intact visitors. Furthermore, social cycles are able to synchronize the host's clocks better when the host's circadian system is labile. These findings are especially relevant for host flies that were highly phase synchronized to start with. The visitors are, however, able to influence the phase of the host's rhythm that has been already set by LD cycles, though they are unable to synchronize clocks of highly desynchronized hosts. These results indicate that daily visits by male or female visitors are ineffective in causing phase synchrony, suggesting that social cues in the form of PA cycles are very weak zeitgeber for fruit flies *D. melanogaster*, and may be additionally useful in fine-tuning of circadian clocks to allow stability of entrainment.

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## REFERENCES

Batschelet E. (1981). *Circular statistics in biology*. London: Academic Press.

Caldelas I, Tejadilla D, Gonzalez B, Montufar R, Hudson R. (2007). Diurnal pattern of clock gene expression in the hypothalamus of the newborn rabbit. *Neuroscience* 144:395–401.

Crowley M, Bovet J. (1980). Social synchronization of circadian rhythms in deer mice (*Peromyscus maniculatus*). *Behav. Ecol. Sociobiol.* 7:99–105.

Daan S, Aschoff J. (2001). The entrainment of the circadian clocks. In Takahashi J, Moore RY (eds.). *Handbook of behavioral neurobiology*. New York: Kluwer Academic/Plenum Publishers.

Davidson AJ, Menaker M. (2003). Birds of a feather clock together-sometimes: social synchronization of circadian rhythms. *Curr. Opin. Neurobiol.* 13:765–769.

Davis FC, Gorski RA. (1988). Development of hamster circadian rhythms—role of the maternal suprachiasmatic nucleus. *J. Comp. Physiol. A* 162:601–610.

Demerec M. (1994). *The biology* of Drosophila fascism. New York: Cold Spring Harbor Laboratory Press.

Dolezelova E, Dolezel D, Hall JC. (2007). Rhythm defects caused by newly engineered null mutations in *Drosophila*'s *cryptochrome* gene. *Genetics* 177:329–345.

Duffield GE, Ebling FJ. (1998). Maternal entrainment of the developing circadian system in the Siberian hamster (*Phodopus sungorus*). *J. Biol. Rhythms* 13:315–329.

Duguay D, Cermakian N. (2009). The crosstalk between physiology and circadian clock proteins. *Chronobiol. Int.* 26:1479–1513.

Dunlap JC, Loros JJ, De Coursey PJ. (2004). *Chronobiology: biological timekeeping*. Sunderland, MA: Sinauer Associates.

Emery P, So WV, Kaneko M, Hall JC, Rosbash M. (1998). CRY, a *Drosophila* clock and light-regulated *cryptochrome*, is a major contributor to circadian rhythm resetting and photosensitivity. *Cell* 95:669–679.

Emery P, Stanewsky R, Helfrich-Förster C, Emery-Le M, Hall JC, Rosbash M. (2000). Drosophila *CRY is a deep brain circadian photoreceptor*. *Neuron* 26:493–504.

Fujii S, Amrein H. (2010). Ventral lateral and DN1 clock neurons mediate distinct properties of male sex drive rhythm in *Drosophila*. *Proc. Natl. Acad. Sci. U. S. A.* 107:10590–10595.

Fujii S, Krishnan P, Hardin P, Amrein H. (2007). Nocturnal male sex drive in *Drosophila*. *Curr. Biol.* 17:244–251.

Goel N, Lee TM. (1997). Olfactory bulbectomy impedes social but not photic reentrainment of circadian rhythms in female *Octodon degus. J. Biol. Rhythms* 12:362–370.

Good P. (2005). *Introduction to statistics through resampling methods and R/S-PLUS*. New York: Wiley.

Gwinner E. (1966). Periodicity of a circadian rhythm in birds by species–specific song cycles (Aves, Fringillidae: *Carduelis spinus*, *Serinus serinus*). *Cell. Mol. Life Sci.* 22:765–766.

Halberg F, Visscher MB, Bittner JJ. (1954). Relation of visual factors to eosinophil rhythm in mice. *Am. J. Physiol.* 179:229–235.

Hamasaka Y, Suzuki T, Hanai S, Ishida N. (2010). Evening circadian oscillator as the primary determinant of rhythmic motivation for *Drosophila* courtship behavior. *Genes Cells* 15:1240–1248.

Handelmann G, Ravizza R, Ray WJ. (1980). Social dominance determines estrous entrainment among female hamsters. *Horm. Behav.* 14:107–115.

Helfrich-Förster C, Winter C, Höfbauer A, Hall JC, Stanewsky R. (2001). The circadian clock of fruit flies is blind after elimination of all known photoreceptors. *Neuron* 30:249–261.

Kilman VL, Zhang L, Meissner RA, Burg E, Allada R. (2009). Perturbing dynamin reveals potent effects on the *Drosophila* circadian clock. *PLoS One* 4:e5235.

Konopka RJ, Pittendrigh C, Orr D. (1989). Reciprocal behaviour associated with altered homeostasis and photosensitivity of *Drosophila* clock mutants. *J. Neurogenet.* 6:1–10.

Krishnan B, Dryer SE, Hardin PE. (1999). Circadian rhythms in olfactory responses of *Drosophila melanogaster*. *Nature* 400:375–378.

Krupp JJ, Kent C, Billeter JC, Azanchi R, So AK, Schonfeld JA, Smith BP, Lucas C, Levine JD. (2008). Social experience modifies pheromone expression and mating behavior in male *Drosophila melanogaster*. *Curr. Biol.* 18:1373–1383.

Levine JD, Funes P, Dowse HB, Hall JC. (2002). Resetting the circadian clock by social experience in *Drosophila melanogaster*. *Science* 298:2010–2012.

Marimuthu G, Chandrashekaran MK. (1983). Social cues of a hipposiderid bat inside a cave fail to entrain the circadian rhythm of an emballonurid bat *Naturwissenschaften* 70:620–621.

Marimuthu G, Rajan S, Chandrashekaran MK. (1981). Social entrainment of the circadian rhythm in the flight activity of the microchiropteran bat *Hipposideros speoris*. *Behav. Ecol. Sociobiol.* 8:147–150.

Medugorac L, Lindauer M. (1967). Das Zeitgedächtnis der bienen unter dem Einfluss von Narkose und von sozialen Zeitgebern. *Z. Vergl. Physiol.* 55:450–474.

Menaker M, Eskin A. (1966). Entrainment of circadian rhythms by sound in *Passer domesticus*. *Science* 154:1579–1581.

Moritz RFA, Sakofski F. (1991). The role of the queen in circadian rhythms of honeybees (*Apis mellifera* L.). *Behav. Ecol. Sociobiol.* 29:361–365.

Portaluppi F, Smolensky MH, Touitou Y. (2010). Ethics and methods for biological rhythm research on animals and human beings. *Chronobiol. Int.* 27:1911–1929.

Reebs SG. (1989). Acoustical entrainment of circadian activity rhythms in house sparrows: constant light is not necessary. *Ethology* 80:172–181.

Reebs SG. (2000). Can a minority of informed leaders determine the foraging movements of a fish shoal? *Anim. Behav.* 59:403–409.

Reppert SM, Schwartz WJ. (1986). Maternal suprachiasmatic nuclei are necessary for maternal coordination of the developing circadian system. *J. Neurosci.* 6:2724–2729.

Saunders DS. (2002). *Insect clocks*. Amsterdam: Elsevier.

Stanewsky R, Kaneko M, Emery P, Beretta B, Wager-Smith K, Kay SA, Rosbash M, Hall JC. (1998). The $cry^b$ mutation identifies *cryptochrome* as a circadian photoreceptor in *Drosophila*. *Cell* 95:681–692.

StatSoft. (1995). *Statistica Vol. 1: general conventions and statistics 1*. Tulsa, OK: StatSoft Inc.

Viswanathan N. (1999). Maternal entrainment in the circadian activity rhythm of laboratory mouse (C57BL/6J). *Physiol. Behav.* 68:157–162.

Viswanathan N, Chandrashekaran MK. (1985). Cycles of presence and absence of mother mouse entrain the circadian clock of pups. *Nature* 317:530–531.

Wigglesworth VB. (1970). Structural lipids in the insect cuticle and the function of the oenocytes. *Tissue. Cell* 2:155–179.